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Rational development of anti-*Leishmania* vaccines for humans focusing on both the vector and the parasite

Ph.D. thesis of the 3rd cycle of studies for the award of the Doctor Degree in Pharmaceutical Sciences – Microbiology Specialty

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March 2020

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTA TESE APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE A DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.

A PRESENTE TESE É DE MINHA AUTORIA E NÃO FOI UTILIZADA PREVIAMENTE NOUTRO CURSO OU UNIDADE CURRICULAR, DESTA OU DE OUTRA INSTITUIÇÃO. AS REFERÊNCIAS A OUTROS AUTORES (AFIRMAÇÕES, IDEIAS, PENSAMENTOS) RESPEITAM ESCRUPULOSAMENTE AS REGRAS DE ATRIBUIÇÃO, E ENCONTRAM-SE DEVIDAMENTE INDICADAS NO TEXTO E NAS REFERÊNCIAS BIBLIOGRÁFICAS, DE ACORDO COM AS NORMAS DE REFERENCIAÇÃO. TENHO CONSCIÊNCIA DE QUE A PRÁTICA DE PLÁGIO E AUTO-PLÁGIO CONSTITUI UM ILÍCITO ACADÉMICO.

This work was performed at the Parasite Disease Group from the Institute for Molecular and Cell Biology (IBMC) in Porto, Portugal, now part of the Institute for Research and Innovation in Health Sciences (i3S), also in Porto, Portugal; and at the Vector Molecular Biology Section of the Laboratory of Malaria and Vector Research, part of the National Institute of Allergy and Infectious Diseases - NIAID - one of the National Institutes of Health – NIH in Rockville, MD, USA.

The author has received financial support through i) a fellowship from the European Community's Seventh Framework Programme under grant agreements No. 603181 (Project MuLeVaClin); and an individual doctoral fellowship awarded by the Portuguese Foundation for Science and Technology (FCT, Portugal) with the reference SFRH/BD/121252/2016. Additionally the author was also funded by Fulbright Portugal.

The experimental research performed in this work has was funded by i) the project NORTE-01-0145-FEDER-000012, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF); ii) the European Community's Seventh Framework Programme under grant agreement No.603181 [Clinical Studies on a Multivalent Vaccine for Human Visceral Leishmaniasis (MuLeVaClin)]; and iii) the Intramural Research Program of the NIH, National Institute of Allergy and Infectious Diseases.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscripts this dissertation focuses on.



AUTHOR'S DECLARATION

Under the terms of the "Decreto-lei no 216/92, de 13 de Outubro", it is hereby declared that the author afforded a major contribution to the conceptual design and technical execution of the work, interpretation of the results and manuscript preparation of the published (or on process of publication) works included in this dissertation.

Under the terms of the "Decreto-lei no 216/92, de 13 de Outubro", it is hereby declared that the following original articles/communications were prepared in the scope of this dissertation.

SCIENTIFIC PUBLICATIONS

Articles in international peer-reviewed journals

In the scope of this dissertation:

Cecílio, P, Oristian J, Meneses C, Serafim TD, Valenzuela JG, Cordeiro-da-Silva A, Oliveira F. Engineering a vector-based pan-*Leishmania* vaccine for humans: proof of principle. Submitted for publication.

Cecílio P, Oliveira F, Cordeiro da Silva A. *Vaccines for Human Leishmaniasis: Where Do We Stand and What Is Still Missing?*. Leishmaniases as Re-emerging Diseases. Farhat Afrin and Hassan Hemeg, IntechOpen. 2018; 59-93. doi:10.5772/intechopen.75000.

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Costa DM, **Cecílio P**, Santarém N, Cordeiro da Silva A, Tavares J. *Murine infection with bioluminescent Leishmania infantum axenic amastigotes applied to drug discovery.* Sci Reports. 2019; 9:18989. doi:10.1038/s41598-019-55474-3.

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COMMUNICATIONS

Oral communications in the scope of this dissertation

Cecílio P*, Pérez-Cabezas B, Fernández L, Moreno J, Carrillo E, Requena JM, Fichera E, Reed S, Coler R, Kamhawi S, Oliveira F, Valenzuela JG, Gradoni L, Glueck R, Gupta G, Cordeiro-da-Silva A. Exploring the effect of a sand fly salivary protein "prime" on the immunogenicity of a complex vaccine formulation containing both vector and parasite-derived antigens. Leishmaniasis 2018 (Caparica, Portugal, Oct 2018).

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Cecílio P, Pérez-Cabezas B, Fernández L, Moreno J, Carrillo E, Requena JM, Fichera E, Reed S, Coler R, Kamhawi S, Oliveira F, Valenzuela JG, Gradoni L, Glueck R, Gupta G, Cordeiro-da-Silva A*. Priming with a salivary protein from visceral leishmaniasis vector enhances antigenicity of parasite antigens in the context of vaccination. 7th International SOVE Conference (Palma de Mallorca, Spain, Oct 2017).

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Poster communications in the scope of this dissertation

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*** presenting author**

“Para ser grande, sê inteiro: nada
Teu exagera ou exclui.
Sê todo em cada coisa. Põe quanto és
No mínimo que fazes.
Assim em cada lago a lua toda
Brilha, porque alta vive.”

- Fernando Pessoa *in* Odes de Ricardo Reis (notas de João Gaspar Simões e Luiz de Montalvor). Lisboa: Ática, 1946(imp.1994):148.

Aos meus pais e irmã

por tudo; e mais alguma coisa!

ACKNOWLEDGEMENTS

Já dizia Virgílio: “Enquanto os rios correrem para o mar, os montes fizerem sombra aos vales e as estrelas fulgirem no firmamento, deve durar a recordação do benefício recebido na mente do homem reconhecido”. No final desta etapa de vida, merecem então, todas as pessoas que a partilharam comigo, tanto na esfera profissional, como na pessoal, devido reconhecimento. Para o fazer, aqui deixarei breves palavras (sentidas), que depois complementarei, pessoalmente, com todas as merecidas.

À Professora Anabela Cordeiro da Silva, agradeço, em primeiro lugar, ter-me apresentado ao “mundo das leishmanioses”. Obrigado pela oportunidade ofereceida de fazer parte da equipa de um projeto Europeu, e pelo desafio proposto de desenvolver um trabalho de Doutoramento. A confiança que depositou em mim, bem como as ótimas condições que me ofereceu para levar avante todos os projetos, ficarão para sempre na minha memória.

Ao Fabiano tenho que agradecer primeiro que tenha acreditado na minha ideia do antigénio de fusão, e que tenha aceitado desenvolvê-la connosco. É sempre melhor ter mais cabeças a pensar e mais mãos a guiar. Por isso, muito obrigado por teres aceitado ser o meu co-orientador! É tão fácil trabalhar contigo, que até dá gosto. Mais do que um mentor, guardo-te como um amigo.

À Begoña agradeço, primeiro os ensinamentos que me ajudaram a crescer como cientista, e depois todo o trabalho partilhado, que por isso foi mais suportável. Não me podia ter calhado parceira melhor! Também a ti te tenho como amiga, que acho que mais ou menos tem uma visão de vida semelhante à minha. Que o trabalho tenho como objectivo a vivência de uma vida cheia, porque o contrário certamente não será tão saboroso. Só se vive uma vez!

To James I have to thank all the help and effort put into the development of the Chimera project. Without you we would not have gotten this far. It is comforting to get to know people like you that are only beginning but appear to have been doing science for quite a while. No pressure... but I have high expectations of you!

To Jesus I have to thank the opportunity given to be part of your “work family” in two different occasions. As I told you personally, I really appreciate the way you lead your lab! The conditions to work are exceptional. But what I will cherish the most is the healthy environment that makes working so much easier! I really admire you.

To Shaden I just want to express my admiration! Scientifically (and I suppose it is not an exclusive conviction of mine), you are one of my role models. To all of my NIH mates, I have to thank the fostering of the best work environment, all the shared knowledge and work, but most importantly, all the shared moments that made my life in America something worth living. Obrigado Anderson, Djonê, Iliano, Tiago, Thiago, Cláudio e Tales! Thank you Maha, Maria, Eva, Josh, Joe and Tim!

Também os meus colegas e amigos Portugueses merecem um agradecimento. Também cá acabámos por ser uma família. Também por cá vocês me proporcionaram um bom ambiente de trabalho, e ainda melhor ambiente fora do trabalho. Foi com tristeza (mas muito orgulho) que fui vendo os meus companheiros formarem-se, e abraçar novos desafios. Vasco, Ricardo, Inês, Joana, Diana, Daniela e Carla; fizeram-me falta no dia a dia. Mas lá nos fomos encontrando nalgumas ocasiões daquelas que pedem um fininho ou dois (Mariana e Rosalina não me esqueci de vocês). Será também com orgulho que verei os mais novos chegarem a bom porto (que é certo que aconteça). David, Ana Rafaela, Ana, Mónica, Inês, Sofia e Clara; força nisso! O trabalho recompensa! Para o fim deixo o Nuno, presença constante em todos estes anos, e companhia do melhor que se pode ter no trabalho e fora dele; e a Joana Tavares, a quem agradeço pelo apoio e pelo reconhecimento (também tu és um modelo científico que eu considero, e que merecia um reconhecimento diferente - fora de todo o grupo PD, que acho que partilha a minha ideia).

Como passamos a maioria do tempo a trabalhar, é normal que as pessoas com quem partilhamos a maioria do nosso tempo acabem por se tornar importantes (e que reconheci, ainda que brevemente, acima). O tempo não pára e as vidas evoluem. No entanto, há pessoas que vão ficando nas nossas vidas e que queremos re-encontrar o mais frequentemente possível. A todos os meus amigos agradeço a presença, a lembrança na ausência, e o apoio que tornaram todo este percurso mais agradável. Obrigado Luís, Paulo, Beto, Nicole, Didi, Cátia, Diana, Rita, Sandra, Tamára, Helena, Marta, Ana Luísa, Luísa, Rita, Ricardo, Ernesto e André. És o mais recente, e tens sido o mais presente. Agradeço-te também a ti, Tiago, o companheirismo e todos os momentos partilhados. Temos muitos mais sítios para ver, e muito mais aventuras para viver. Venham elas, o mais depressa possível!

É bom poder dizer que, se a família se escolhesse, eu escolhia a que tenho. Grande, calorosa, preocupada! A todos eles tenho que agradecer uma infância feliz e uma vida adulta saudável. Agradeço também o suporte e as palavras de incentivo, a convivência e

partilha de momentos, em Portugal ou no Canadá, e a presença, nem que seja na forma de uma chamada. Obrigado avós Padeira e Fátima (e aos avôs que já não souberam que eu ía continuar a estudar). “Vivemos sobre os ombros de gigantes”. Vocês foram os meus! Desculpa, avó Padeira, por não ter lá estado! Obrigado Maria, Nanda, Luís, Gina e Ratico, Lena, Teresa e Derek, Ângela e Pedro, Paulo, tia Lurdes e ti Matateu, tia Tita e Toino Zé, tia Judite e ti Pedro, ti Fernando e tia Céu, ti Artur e tia Belica, Pedro Filipe, Ana Filipa, Bárbara, Rafael, Zé Pedro e Ricardinho, Juliano, Ana, Miguel e Sofia, Patrícia, Hugo, Ricardo e Isabel, Tiago, Sandra e Clarinha, Pedro Alexandre, Alexandra e Beatrice, Katelina e Brian, Gémeos (o vosso nome vem duas vezes), Rita e Micael e Diogo!

Finalmente tenho que agradecer ao núcleo duro, a quem dedico esta tese. Aos meus pais tenho primeiro que agradecer o meu ser. Não só a minha existência, mas também a minha maneira de existir! Obrigado pelo amor, o apoio incondicional, os conselhos e ensinamentos e a presença constante! Não podia pedir melhor! E num aparte, podias pensar que tavas a ser chata quando me perguntavas todos os dias como corriam as coisas (e a escrita, nestes últimos tempos), mãe. Mas nem sabes o bem que essas mensagens faziam! Obrigado também por me terem dado uma irmã. A partilha a crescer, a companhia a viver e a presença, mesmo de longe, foram e são muito importantes. Sabes que tenho muito orgulho em ti; na mulher que te tornaste! Fico à espera das próximas conquistas que hão-de ser muitas! Para além de agradecer, tenho também que vos pedir desculpa. Desculpa por estar pouco; desculpa por ir para longe; desculpa por quase nunca dizer que vos amo!

ABSTRACT

Leishmaniasis is a spectrum of diseases caused by more than 20 different species of *Leishmania* parasites, transmitted to humans *via* bites of infected phlebotomine sand flies. Cumulatively, considering the more than 90 countries endemic for at least one of the disease forms (most of them developing nations), every year, around one million new cases are estimated to occur, together with 20.000 deaths. Importantly, most of the parasite species that cause human disease are zoonotic agents, making the traditional infectious-diseases' control measures (treatment of active cases), mostly irrelevant for the decrease of leishmaniasis incidence. In fact, the experts agree that the only feasible long-term potential for elimination of human disease is dependent on the development of effective anti-*Leishmania* vaccines.

Although a vaccine for leishmaniasis is considered an achievable goal, supported by the notion that previous infection (either natural or man-made – leishmanization) leads to a robust immunity against the parasite, today we still don't have a vaccine available for humans. This is however not a consequence of the lack of research in the subject; many vaccines have been tested along the years, with reported good pre-clinical potentials, but most of the few that reached the clinical trials' stage failed. This "low translatability" potential is probably justified by a combination of different factors, related, not only with vaccine design, but also with vaccine testing, that require more rational approaches (allowed by modern vaccinology).

Here we applied the rational-based vaccine design and propose two vaccine strategies with a probably better translation potential to the clinical context, since the target population, humans, was accounted for in every development phase. Importantly, our approaches more than to focus on the parasite, consider the vector, not only from the perspective of vaccine-testing (using natural transmission to get closer to the ecological context), but also as a source of antigens with the potential to impact *Leishmania* infection establishment. We show pre-clinically that both candidates have a good immunogenic potential, and additionally that vector-derived antigens may impact the immunogenic potential of the parasite-derived ones, in the context of a prime-boost vaccination scheme. Based on our work, we propose that the future vaccine for human leishmaniasis should be constituted of both vector- and parasite-derived antigens to elicit two different responses that negatively impact *Leishmania* establishment in vaccinated humans.

Keywords: *Leishmania*; sand fly; human leishmaniasis; vector-borne disease; vaccines.

RESUMO

As leishmanioses são um espectro de doenças causadas por mais de 20 espécies diferentes de parasitas do género *Leishmania*, transmitidos pela picada de flebotomíneos. Cumulativamente, considerando os mais de 90 países endémicos de pelo menos uma leishmaniose (a maioria deles, nações em vias de desenvolvimento), todos os anos estima-se que ocorram cerca de um milhão de novos casos, e 20.000 mortes. Devido ao facto de a maioria das espécies de *Leishmania* patogénicas para humanos serem agentes zoonóticos, as medidas de controlo, associadas tradicionalmente a doenças infeciosas (tratamento de casos ativos), são pouco relevantes para a diminuição da incidência das leishmanioses. De facto, os especialistas concordam que a unica maneira de se conseguir controlar as leishmanioses humanas, a longo prazo, é através do desenvolvimento de vacinas eficazes.

Embora uma vacina para as leishmanioses seja considerada uma opção viável, justificada pelo facto de que uma infeção prévia (natural ou causada pelo homem – leishmanização), leva ao desenvolvimento de uma imunidade robusta contra os parasitas, hoje ainda não existe uma vacina para humanos. Isto não é, no entanto, consequência de falta de investigação na área; muitas vacinas foram testadas ao longo dos anos, e definidas como eficazes no contexto pré-clínico, mas a maioria das poucas que foram testadas em ensaios clínicos, falharam. Este baixo potencial de translação é provavelmente justificado por uma combinação de fatores, relacionados, não só com a fase de desenvolvimento, mas também com a fase de teste destas vacinas, que requerem abordagens mais racionais (que a vacinologia moderna hoje permite).

Esta dissertação debruça-se no desenvolvimento racional de vacinas, e propõe duas estratégias com maior potencial de translação para o contexto clínico, uma vez que a população alvo, os humanos, foi considerada em todas as fases de desenvolvimento. Igualmente relevante, o foco destas estratégias foi, mais do que o parasita, também o vetor, não só considerando os ensaios pré-clínicos (usar a transmissão natural numa aproximação do contexto ecológico), mas também pensando nele como uma fonte de antigénios com potencial para afetar o estabelecimento de infeções por *Leishmania*. Recorrendo a ensaios pré-clínicos mostramos que os dois candidatos têm um bom potencial imunogénico, e que adicionalmente, os antigénios do vetor influenciam a imunogenicidade dos antigénios do parasita no contexte de um esquema de imunização não tradicional. Com base nos

trabalhos aqui apresentados, propomos que uma futura vacina contra as leishmanioses humanas deve ser constituída por antigénios provenientes, tanto do vetor, como do parasita, de forma a induzir, simultaneamente, duas respostas imunes diferentes, que, pelo menos de uma forma aditiva, vão causar um impacto negativo no estabelecimento de infeções causadas por parasitas do género Leishmania, em humanos vacinados.

Palavras chave: *Leishmania*; flebotomíneo; leishmanioses humanas; doenças transmitidas por vetores; vacinas.

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ABBREVIATIONS

A2	Amastigote specific protein 2
ADCC	Antibody-dependent Cell-mediated Cytotoxicity
ADP	Adenosine Diphosphate
Akt	Protein Kinase B
ALM	Autoclaved *Leishmania major*
ALO	Arabino-1, 4-Lactone Oxidase
AMPs	Antimicrobial peptides
APCs	Antigen-Presenting Cells
BCG	Bacillus Calmette–Guérin
BMDCs	Bone Marrow-derived DCs
BMM	Bone Marrow-derived Macrophages
BT1	Biopterin Transporter 1
CCL	C-C Chemokine Ligand
CCR	C-C Chemokine Receptor
Cen	Centrine
CL	Cutaneous Leishmaniasis
CoV	Coronavirus
CPA/B	Cysteine Peptidase A/B
CR3	Complement Receptor 3
CTLA-4	Cytotoxic T Lymphocytes Antigen 4
CXCL	C-X-C Chemokine Ligand
CystProt	Cysteine Proteinase
DALYs	Disability-Adjusted Life Years
DCs	Dendritic Cells
DCL	Diffuse Cutaneous Leishmaniasis
dhfr-ts	Dihydrofolate Reductase-Thymidylate Synthase
DsCL	Disseminated Cutaneous Leishmaniasis
DTH	Delayed-Type Hypersensitivity

eIF	Elongation Factor
ERK	Extracellular signal-Regulated Kinases
ESP	Excreted/Secreted Proteins
FML	Fucose-Mannose Ligand
GCS	Glutamylcysteine Synthetase
GILP	Glycosylinositolphospholipid
GLA	Glucopyranosyl Lipid A
GP	Glycoprotein
GP63	*Leishmania* metalloprotease (“leishmanolisin”)
HASP	Hydrophilic Acylated Surface Protein
HbR	Haemoglobin Receptor
HSP	Heat Shock Protein
IFN	Interferon
IL	Interleukin
IL-4Rα	IL-4 Receptor alpha
HBV	Hepatitis B Virus
HCV	Hepatitis B Virus
HPV	Human Papillomavirus
ITAM	Immunoreceptor Tyrosine-based Activation Motif
iNOS	Inducible NOS
IRFs	Interferon Regulatory Factors
JAK	Janus kinase
KMP	Kinetoplastid Membrane Protein
KO	Knock-Out
LACK	*Leishmania* Homolog of Receptors for Activated C-Kinase
LeIF	*Leishmania* Elongation Initiation Factor
Ld	*L. donovani*
Li	*L. infantum*
LJL	*Lutzomyia longipalpis* Jacobina Large
LJM	*Lutzomyia longipalpis* Jacobina Medium

LmSTI1	*Leishmania major* Stress-inducible Protein 1
Lmaj	*L. major*
Lmex	*L. mexicana*
LPG	Lipophosphoglycan
LR	Leishmaniasis Recidivans
MCL	Mucocutaneous Leishmaniasis
MCP	Monocyte Chemoattractant Protein
MERS	Middle East Respiratory Syndrome
MHC	Major Histocompatibility Complex
MIDGE	Minimalistic Immunogenically Defined Gene Expression
MIF	Macrophage Inhibiting Factor
MIP	Macrophage Inflammatory Protein
MPL	Monophosphoryl Lipid A
NdK	Nucleoside diphosphate Kinase
NF-kB	Nuclear Factor-kB
NH	Nucleoside Hydrolase
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NOX	Membrane Bound NADPH-Dependent Oxidases
NW	New World
NTDs	Neglected Tropical Diseases
ODN	Oligodeoxynucleotides
OW	Old World
ORFF	Open Reading Frame Fragment
P0	Acidic Ribosomal Protein P0
PAMPs	Pathogen-Associated Molecular Patterns
PBMCs	Peripheral Blood Mononuclear Cells
PBS	Phosphate-Buffered Saline
PD1	Programed Death Protein 1
PDI	Protein Disulphide Isomerase

PdSP	*Phlebotomus duboscqi* Salivary Protein
PEPCK	Phosphoenolpyruvate Carboxykinase
PHB	Prohibitin
PGE2	Prostaglandin E2
PI3K	Phosphatidylinositol 3'-Kinase
PKDL	Post Kala Azar Dermal Leishmaniasis
PMN	Polymorphonuclear
PPM	Phosphomannomutase
PpSP	*Phlebotomus papatasi* Salivary Protein;
PS	Phosphatidylserine
PSA	Promastigote Surface Antigen
PSG	Promastigote Secretory Gel
SE	Stable Emulsion
SGH	Salivary Gland Homogenates
RANTES	Regulated on Activation, Normal T-Cell Expressed and Secreted
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
Ser/Thr	Serine/Threonine
SHP	Sarcoma Homology 2-Domain Phosphatase-1
SIR	Silent Information Regulator
SMT	Sterol 24-c-methyltransferase
SOCS	Suppressors of Cytokine Signaling
Spp	Species
STAT	Signal Transducer and Activator of Transcription
TDR	Thiol-Dependent Reductase
TGF-β	Transforming Growth Factor Beta
Th	T helper
TK-CD	Thymidine Kinase (herpes simplex) - Cytosine Deaminase (*S. cerevisiae*)
TLRs	Toll-Like Receptors
TNF-a	Tumor Necrosis Factor Alpha

TPI	Triose Phosphate Isomerase
TPR	Trypanothione Reductase
Tregs	Regulatory T cells
TRYP	Tryparedoxin Peroxidase
TSA	Thiol-specific Antioxidant
UN	United Nations
Unk	Unknown
VL	Visceral Leishmaniasis
WHO	World Health Organization

I. INTRODUCTION

1. Leishmaniasis, a complex spectrum of diseases

Leishmaniasis is the name given to diseases caused by *Leishmania* spp. parasites that in nature are transmitted mainly by phlebotomine sand flies, being therefore part of the very relevant vector-borne infectious diseases family. Infection and/or disease is described for a wide range of animals, from reptiles (Belova, 1971; Chen *et al*, 2019) to mammals (Bates, 2007; Molina *et al*, 2012), including dogs - being canine leishmaniosis of great veterinary significance (Otranto and Dantas-Torres, 2013) - and humans. Not because animal disease is irrelevant, which is far from being true, but to align with the scope of this thesis, the main focus will be given to human disease.

Leishmania donovani parasites were identified retrospectively in Egyptian and Nubian mummies, making human *Leishmania* infection, and probably human leishmaniasis at least 4000 years old (Zink *et al*, 2006; Steverding, 2017). Today, around twenty *Leishmania* species are identified as human pathogens (Table 1), causing a wide range of disease manifestations that go from localized lesions in the skin to the fatal affliction of internal organs (Bates, 2007; Akhoundi *et al*, 2016). This is why, rather than being a single disease, leishmaniasis is considered as a spectrum of diseases, that in a broader clinical context can be grouped in two main groups: Cutaneous Leishmaniasis (CL) and Visceral Leishmaniasis (VL) (Burza *et al*, 2018). Complex enough, while different *Leishmania* species are associated with similar clinical manifestations (Bates, 2007; Akhoundi *et al*, 2016; Burza *et al*, 2018), not unfrequently the same parasite species is linked to distinct clinical pictures (Weiss *et al*, 2009; Ranasinghe *et al*, 2012; Cunha *et al*, 2013), suggesting that parasite tropism and/or virulence may not be the only pathogenesis determinants. This is supported by the many times repeated statement saying that infection and disease progression depend on “complex interactions between the parasite and the host immune response” (Cecilio *et al*, 2014). Still, while the “atypical leishmaniasis” presentations described for immunocompromised individuals (e.g. malnourished, HIV positive and pharmacologically immunosuppressed) are easier to explain (Belli *et al*, 1999; Clemente *et al*, 2007; van Griensven *et al*, 2014; Diro *et al*, 2015), the same is not true considering disease in immunocompetent individuals, meaning that both the parasite and the host determinants that condition infection and/or disease are yet majorly unknown. This said, each *Leishmania*

species is still mainly associated with one type of disease, and frequently qualified as either dermotropic or viscerotropic (Table 1).

Cutaneous leishmaniasis *lato sensu* are by far the most relevant forms of leishmaniasis considering total cases (global disease incidence), and number of human-pathogenic *Leishmania* species to them associated (Table 1). Reports from 2018 confirm cutaneous disease is endemic in 89 countries around the world, with the majority of reported cases occurring in 11 countries: Afghanistan, Algeria, Bolivia, Brazil, Colombia, Iraq, the Islamic Republic of Iran, Pakistan, Peru, the Syrian Arabic Republic (account more than 30% of all cases) and Tunisia (Figure 1) (WHO, 2018a). Disease manifestations include the classic localized skin lesions, usually self-healing, with various presentations that can vary from small ulcerations, to granulomatous nodules, to larger erythematous wounds – CL *stricto sensu* (Hartley *et al*, 2014) – but also the affliction of mucosal tissues (particularly, but not only the nose, mouth and throat) than can lead to their partial or total destruction – Mucocutaneous Leishmaniasis (MCL) (Hartley *et al*, 2014; Kevric *et al*, 2015) – or even the diffuse forms (metastatic disease) that can affect virtually the entirety of the skin, either result of primo-infections – Diffuse Cutaneous Leishmaniasis (DCL) (Hartley *et al*, 2014; Kevric *et al*, 2015) – or secondary to successfully treated visceral disease – Post Kala Azar Dermal Leishmaniasis (PKDL) (Hartley *et al*, 2014; Mukhopadhyay *et al*, 2014). Although estimations point to 600.000 to one million new cases occurring yearly worldwide, the disease incidence is probably higher (Karimkhani *et al*, 2016; WHO, 2019a). Importantly, disease incidence continues to increase (Alvar *et al*, 2012; WHO, 2018a). Even if CL are not fatal conditions, they are also not innocuous. The estimated global mean age-standardized DALYs (Disability-Adjusted Life Years) for CL were an estimated 0.58 per 100.000 people in 2013 (Karimkhani *et al*, 2016). The ultimate consequences of CL are permanent scarring of even disfiguration that may result on social stigmatization and/or life-long consequences from both the economical and psychological standpoints (Kassi *et al*, 2008; Okwor and Uzonna, 2016; Bailey *et al*, 2019).

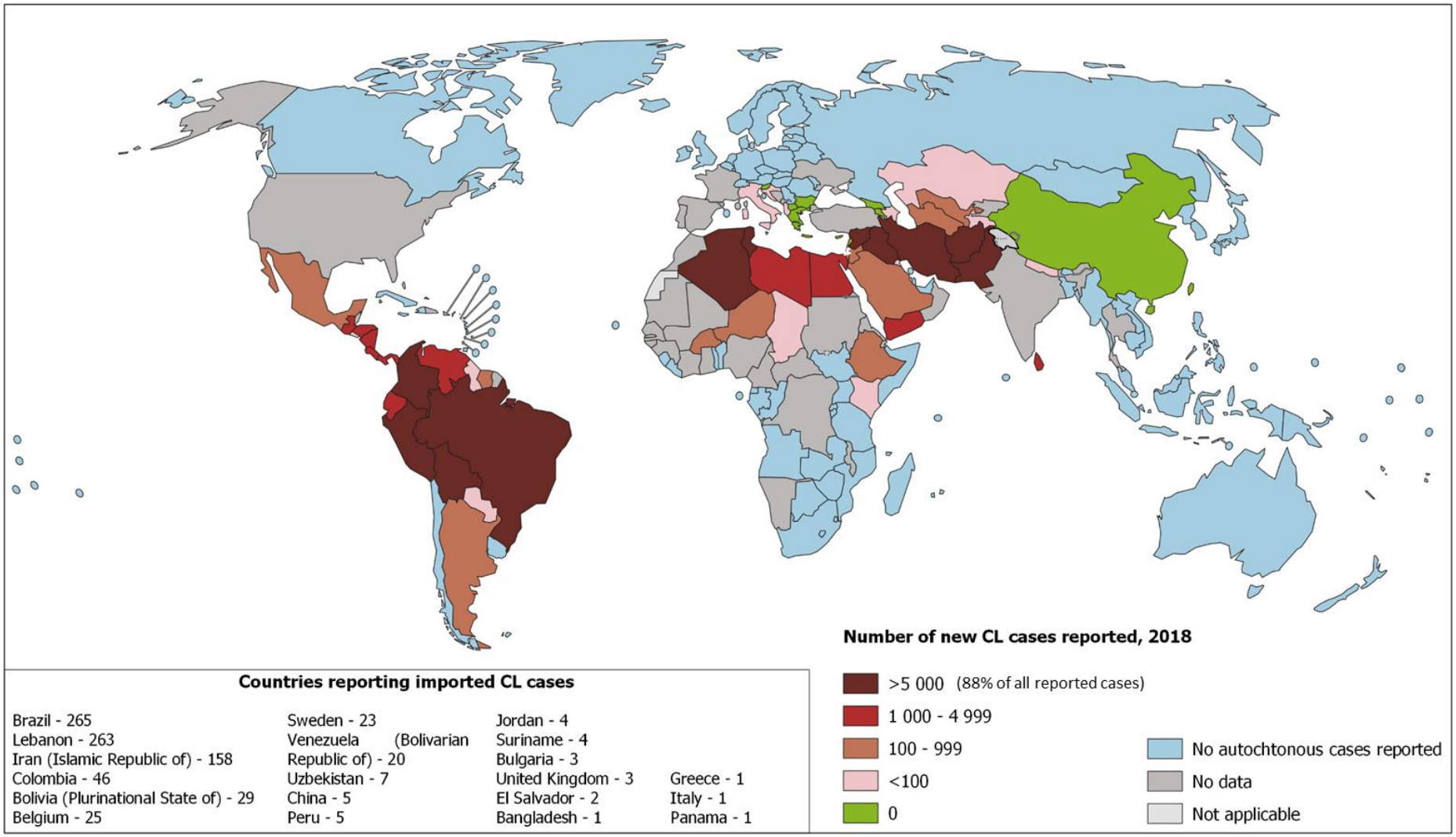


Figure 1. Worldwide distribution of Cutaneous Leishmaniasis reported cases, 2018. Adapted from WHO, 2019b.

Visceral Leishmaniasis (also known as Assam Fever, Dumdum Fever, or Kala Azar) is however not less relevant, although for different reasons. The major one is the disease outcome. In the absence of treatment, the case fatality rate is reported to be over 95%; and even with potential access to treatment, VL is reported to result in case-fatality rates as high as 20% (Alvar *et al*, 2012; WHO, 2019a). Contrarily to CL (*lato sensu*), VL is mainly associated with two *Leishmania* species, *L. infantum* and *L. donovani* (Table 1). These parasite species are able to leave the skin and subsist within the reticuloendothelial system, accumulating mainly in the bone marrow, the liver and the spleen (Kamink *et al*, 2017). Symptoms include persistent irregular fever and splenomegaly. Hepatomegaly, hypergammaglobulinemia and weight loss, are also common symptoms, particularly in the more severe clinical presentations, where pancytopenia is also generally observed (Burza *et al*, 2018). These lower cell counts, result of both splenic sequestration and bone marrow function impairment, result in hemorrhages, severe anemia and secondary bacterial infections, the frequent causes of death (Rodrigues *et al*, 2016; Burza *et al*, 2018). In 2018, VL was reported as an endemic disease in 76 countries around the globe (Figure 2) (WHO, 2018a). Every year 50.000 to 90.000 new cases and 26.000 to 65.000 deaths are estimated to occur (WHO, 2019a). Around 83% of the reported cases in 2018 occurred in 5 countries: Brazil, Ethiopia, India, South Sudan, and Sudan (Figure 2) (WHO, 2018a). Overall, VL incidence has been decreasing in the past 10 years, a potential consequence of elimination efforts in the Indian subcontinent (Burza *et al*, 2018). Still, the epidemiological patterns of VL, particularly in South Asia, are known to be cyclical, meaning that this decline of incidence might just be a reflection of the natural context (Burza *et al*, 2018).

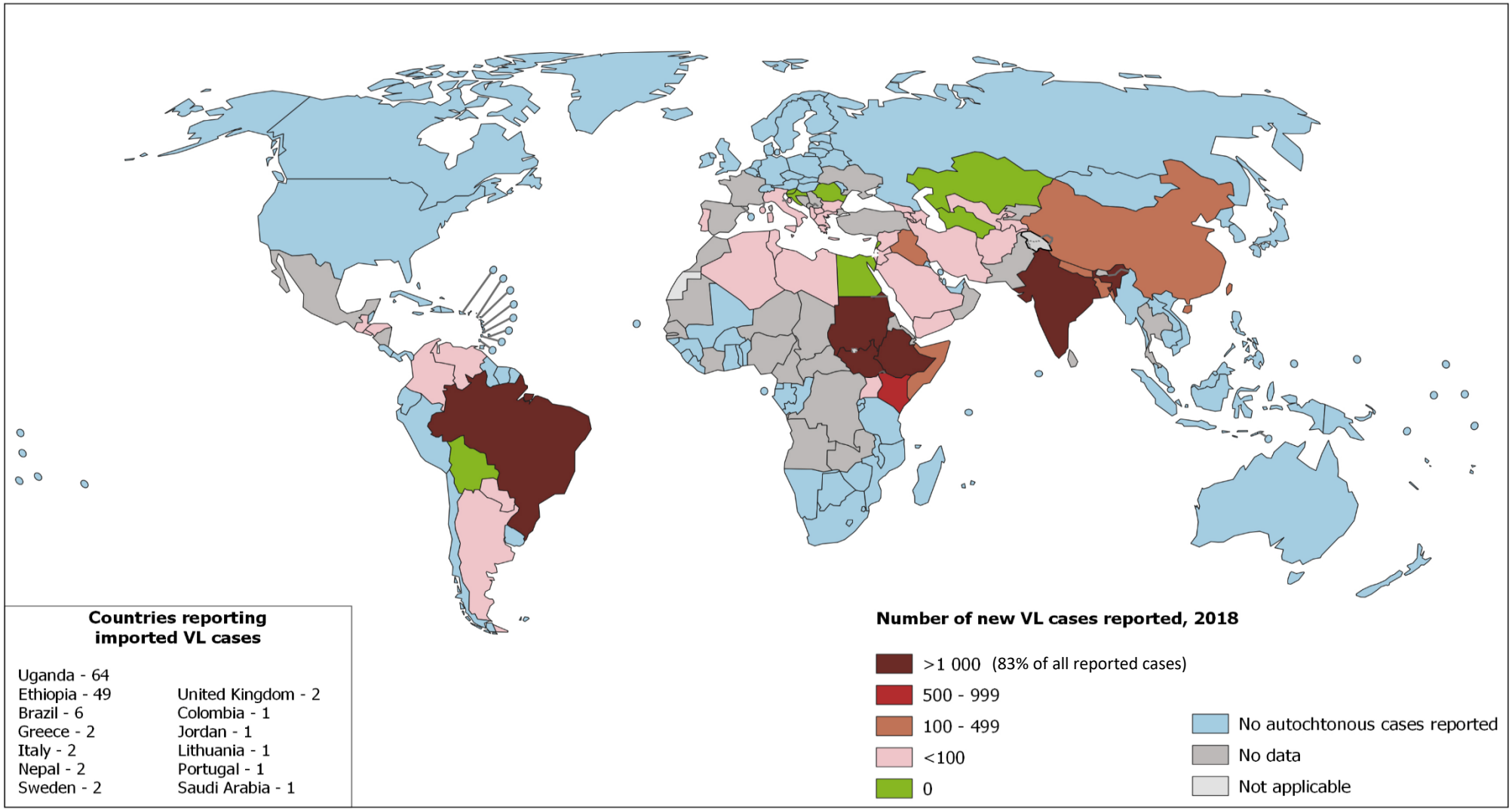


Figure 2. Worldwide distribution of Visceral Leishmaniasis reported cases, 2018. Adapted from WHO, 2019c.

Globally, near one billion people live in high burden disease areas, and are therefore at risk of developing leishmaniasis: more than half a billion for VL and almost 400 million for CL, estimated from 2014 epidemiological data (WHO, 2016). Importantly, many of these areas are categorized as “least developed” or “poor”. Indeed, one of the major underlying determinants of leishmaniasis is reported to be poverty (Alvar *et al*, 2006). On one way, the consequences of low-incomes overlap with both infection and disease development risk factors, while on the other leishmaniasis consequences contribute to poverty reinforcement (and the closing one of the most unfair vicious cycles) (Alvar *et al*, 2006). This fact by itself justifies the classification of leishmaniasis as one of the Neglected Tropical Diseases (NTDs), diseases that affect the poorest of the poor, for which the total Development Assistance for Health values expended (average of 0.6% of the total for the years of 2003 to 2007) are completely disproportionate considering their global burden: currently 1/6 of the human population suffers from at least one (Liese and Schubert, 2009; Mitra and Mawson, 2017).

Leishmaniasis endemicity depends on active and sustained parasite transmission, and therefore, on the geographical overlap of parasites and competent vectors. Neither parasite nor vector species are ubiquitous. Specific parasite species are associated with specific areas of the globe, and consequently with particular vector species of the same defined areas, that are permissive to infection (Table 1) (Bates, 2007; Akhoundi *et al*, 2016). Adding up to these notions, most of the human *Leishmania* pathogenic species are associated with zoonotic transmission. Only for *L. donovani* parasites, no animal other than man has been incriminated as a reservoir; for all of the remaining *Leishmania* species that cause disease in humans at least one animal reservoir (frequently sylvatic) is recognized (Table 1) (Bates, 2007). All of the abovementioned justify the epidemiological complexity of leishmaniasis. As a logical consequence, disease control is extremely difficult to accomplish (Kamhawi, 2017). Additionally, also going towards this epidemiological complexity, leishmaniasis cannot ever be looked at from a static perspective. There is a real risk of disease expansion (considering non-endemic regions), a consequence of the arrival of parasites and/or vector species to new areas, which can be driven by weather patterns alterations and/or migrations (Pavli and Maltezou, 2010; Oryan and Akbari, 2016). It is therefore not unreasonable to qualify global warming, globalization and war/conflicts as major potential risk factors of leishmaniasis emergence (Dujardin *et al*, 2008; Berry and Berrang-Ford, 2016; Kholoud *et al*, 2018).

Table 1. Leishmaniasis: etiological, clinical and epidemiological aspects. Adapted from Bates, 2007; Roque and Jansen, 2014; Akhoundi *et al*, 2016; Cotton, 2017; Burza *et al*, 2018 and Olivo Freites *et al*, 2018.

***Leishmania* species**	**Clinical form**	**Main clinical features**	**Natural progression**	**Risk groups**	**Main reservoir**	**Transmission**	**Relevant vectors**	**OW *vs.* NW**	**High-burden countries or regions**	**Estimated annual worldwide incidence**
Leishmania (Leishmania) donovani	VL and PKDL	Persistent fever, splenomegaly, weight loss, and anaemia in VL; multiple painless macular, papular, or nodular lesions in PKDL	VL is fatal within 2 years; PKDL lesions self-heal in up to 85% of cases in Africa but rarely in Asia	Predominantly adolescents and young adults for VL; young children in Sudan and no clearly established risk factors for PKDL	Humans	Epidemic anthroponotic	*Phlebotomus argentipes, Phlebotomus orientalis, Phlebotomus martini, Phlebotomus alexandri*	OW	India, Bangladesh, Ethiopia, Sudan, and South Sudan	50 000–90 000 VL cases; unknown number of PKDL cases
Leishmania (Leishmania) tropica	CL, LR, and rarely VL	Ulcerating dry lesions, painless, and frequently multiple	CL lesions often self-heal within 1 year	No well-defined risk groups	Humans, hyraxes	Urban anthroponotic	*Phlebotomus sergenti, Phlebotomus arabicus*	OW	Eastern Mediterranean, the Middle East, and northeastern and southern Africa	200 000–400 000 CL
Leishmania (Leishmania) aethiopica	CL, DCL, DsCL, and oronasal CL	Localized cutaneous nodular lesions; occasionally oronasal; rarely ulcerates	Self-healing, except for DCL, within 2–5 years	Limited evidence; adolescents	Hyraxes	Rural zoonotic	*Phlebotomus longipes, Phlebotomus pedifer, Phlebotomus sergenti*	OW	Ethiopia and Kenya	20 000–40 000 CL
Leishmania (Leishmania) major	CL	Rapid necrosis, multiple wet sores, and severe inflammation	Self-healing in >50% of cases within 2–8 months; multiple lesions slow to heal, and severe scarring	No well-defined risk groups	Rodents	Rural zoonotic	*Phlebotomus papatasi, Phlebotomus duboscqi, Phlebotomus salehi, Phlebotomus caucasicus*	OW	Iran, Saudi Arabia, north Africa, the Middle East, central Asia, and west Africa	230 000–430 000 CL

Legend: CL- cutaneous leishmaniasis; DCL - diffuse cutaneous leishmaniasis; DsCL - disseminated cutaneous leishmaniasis; LR - leishmaniasis recidivans; MCL - mucocutaneous leishmaniasis; NW - New World; OW - Old World; PKDL- post-kala-azar dermal leishmaniasis; VL- visceral leishmaniasis.

Table 1 (cont.). Leishmaniasis: etiological, clinical and epidemiological aspects. Adapted from Bates, 2007; Roque and Jansen, 2014; Akhoundi *et al*, 2016; Cotton, 2017; Burza *et al*, 2018 and Olivo Freites *et al*, 2018.

***Leishmania* species**	**Clinical form**	**Main clinical features**	**Natural progression**	**Risk groups**	**Main reservoir**	**Transmission**	**Relevant vectors**	**OW *vs.* NW**	**High-burden countries or regions**	**Estimated annual worldwide incidence**
Leishmania (Leishmania) infantum	VL and CL	Persistent fever and splenomegaly in VL; typically single nodules and minimal inflammation in CL	VL is fatal within 2 years; CL lesions self-heal within 1 year and confers individual immunity	Children under 5 years and immunocompromised adults for VL; older children and young adults for CL	Dogs, hares, foxes, and humans	Peridomestic zoonotic	*Phlebotomus Larroussius* subgenus (e.g. *P. ariasi, P. Perniciosus*), *Lutzomyia longipalpis, Lutzomyia cruzi*	OW and NW	China, southern Europe, Brazil, and South America for VL and CL; Central America for CL	6200–12 000 cases of Old World VL and 4500–6800 cases of New World VL; unknown number of CL cases
Leishmania (Leishmania) mexicana	CL, DCL, and DsCL	Ulcerating lesions, single or multiple	Often self-healing within 3–4 months	No well-defined risk groups	Rodents and marsupials	Sylvatic zoonotic	*Lutzomyia olmeca, Lutzomyia ayacuchensis*	NW	South America	Limited number of cases, included in the 187 200–300 000 total cases of New World CL
Leishmania (Leishmania) venezuelensis	CL	Ulcerating lesions	Not well described	No well-defined risk groups	Unknown	Zoonotic	*Lutzomyia olmeca* ? *Lutzomyia bicolor* ?	NW	Venezuela	Limited number of cases, included in the 187 200–300 000 total cases of New World CL
Leishmania (Leishmania) amazonensis	CL, DCL, and DsCL	Ulcerating lesions, single or multiple	Not well described	No well-defined risk groups	Possums and rodents	Sylvatic zoonotic	*Lutzomyia flaviscutellata*	NW	South America	Limited number of cases, included in the 187 200–300 000 total cases of New World CL

Legend: CL- cutaneous leishmaniasis; DCL - diffuse cutaneous leishmaniasis; DsCL - disseminated cutaneous leishmaniasis; LR - leishmaniasis recidivans; MCL - mucocutaneous leishmaniasis; NW - New World; OW - Old World; PKDL- post-kala-azar dermal leishmaniasis; VL- visceral leishmaniasis.

Table 1 (cont.). Leishmaniasis: etiological, clinical and epidemiological aspects. Adapted from Bates, 2007; Roque and Jansen, 2014; Akhoundi *et al*, 2016; Cotton, 2017; Burza *et al*, 2018 and Olivo Freites *et al*, 2018.

***Leishmania* species**	**Clinical form**	**Main clinical features**	**Natural progression**	**Risk groups**	**Main reservoir**	**Transmission**	**Relevant vectors**	**OW *vs.* NW**	**High-burden countries or regions**	**Estimated annual worldwide incidence**
Leishmania (Viannia) braziliensis	CL, MCL, DCL, and LR	Ulcerating lesions can progress to mucocutaneous form; local lymph nodes are palpable before and early on in the onset of the lesions	Might self-heal within 6 months; 2·5% of cases progress to MCL	No well-defined risk groups	Dogs, humans, rodents, and horses	Sylvatic zoonotic	*Lutzomyia wellcomei, Lutzomyia migonei, Lutzomyia neivai, Lutzomyia carrerai*	NW	South America	Majority of the 187 200–300 000 total cases of New World CL
Leishmania (Viannia) guyanensis	CL, DsCL, and MCL	Ulcerating lesions, single or multiple that can progress to mucocutaneous form; palpable lymph nodes	Might self-heal within 6 months	No well-defined risk groups	Possums, sloths, and anteaters	Sylvatic zoonotic	*Lutzomyia whitmani, Lutzomyia shawi, Lutzomyia anduzei, Lutzomyia ayacuchensis*	NW	South America	Limited number of cases, included in the 187 200–300 000 total cases of New World CL
Leishmania (Viannia) peruviana	CL	Ulcerating lesions, single or multiple	Not well described	No well-defined risk groups	Unknown, dogs?	Zoonotic	*Lutzomyia peruensis, Lutzomyia verrucarum*	NW	Peru, Bolivia	Limited number of cases, included in the 187 200–300 000 total cases of New World CL

Legend: CL- cutaneous leishmaniasis; DCL - diffuse cutaneous leishmaniasis; DsCL - disseminated cutaneous leishmaniasis; LR - leishmaniasis recidivans; MCL - mucocutaneous leishmaniasis; NW - New World; OW - Old World; PKDL- post-kala-azar dermal leishmaniasis; VL- visceral leishmaniasis.

Table 1 (cont.). Leishmaniasis: etiological, clinical and epidemiological aspects. Adapted from Bates, 2007; Roque and Jansen, 2014; Akhoundi *et al*, 2016; Cotton, 2017; Burza *et al*, 2018 and Olivo Freites *et al*, 2018.

***Leishmania* species**	**Clinical form**	**Main clinical features**	**Natural progression**	**Risk groups**	**Main reservoir**	**Transmission**	**Relevant vectors**	**OW *vs.* NW**	**High-burden countries or regions**	**Estimated annual worldwide incidence**
Leishmania (Viannia) panamensis	CL, MCL and DCL	Ulcerating lesions, single or multiple that can progress to mucocutaneous form	Not well described	No well-defined risk groups	Rodents, dogs?	Sylvatic zoonotic	*Lutzomyia gomezi, Lutzomyia hartmanni, Lutzomyia trapidoi, Lutzomyia yuilli*	NW	Central and South America	Limited number of cases, included in the 187 200–300 000 total cases of New World CL
Leishmania (Viannia) lainsoni	CL	Ulcerating lesions, single or multiple	Not well described	No well-defined risk groups	Rodents, porcupines	Sylvatic zoonotic	*Lutzomyia ubiquitalis*	NW	Brazil, Bolivia, Peru, Ecuador	Limited number of cases, included in the 187 200–300 000 total cases of New World CL
Leishmania (Viannia) lindenbergi	CL	Ulcerating lesions	Not well described	No well-defined risk groups	Unknown	Zoonotic	*Lutzomyia atunesi*?	NW	Brazil	Limited number of cases, included in the 187 200–300 000 total cases of New World CL

Legend: CL- cutaneous leishmaniasis; DCL - diffuse cutaneous leishmaniasis; DsCL - disseminated cutaneous leishmaniasis; LR - leishmaniasis recidivans; MCL - mucocutaneous leishmaniasis; NW - New World; OW - Old World; PKDL- post-kala-azar dermal leishmaniasis; VL- visceral leishmaniasis.

Table 1 (cont.). Leishmaniasis: etiological, clinical and epidemiological aspects. Adapted from Bates, 2007; Roque and Jansen, 2014; Akhoundi *et al*, 2016; Cotton, 2017; Burza *et al*, 2018 and Olivo Freites *et al*, 2018.

***Leishmania* species**	**Clinical form**	**Main clinical features**	**Natural progression**	**Risk groups**	**Main reservoir**	**Transmission**	**Relevant vectors**	**OW *vs.* NW**	**High-burden countries or regions**	**Estimated annual worldwide incidence**
Leishmania (Viannia) naiffi	CL	Ulcerating lesions, single and small	Not well described	No well-defined risk groups	Rodents, Anteaters	Sylvatic zoonotic	*Lutzomyia ayrozai, Lutzomyia squamiventris*	NW	Brazil, French Guyana	Limited number of cases, included in the 187 200–300 000 total cases of New World CL
Leishmania (Viannia) shawi	CL	Ulcerating lesions	Not well described	No well-defined risk groups	Rodents, sloths	Sylvatic zoonotic	*Lutzomyia whitmani*	NW	Brazil	Limited number of cases, included in the 187 200–300 000 total cases of New World CL
Leishmania colombiensis	CL and VL	Not well described	Not well described	No well-defined risk groups	Sloths	Sylvatic zoonotic	*Lutzomyia hartmanni*	NW	Colombia	Limited number of cases
Leishmania martiniquensis	CL and VL	Not well described	Not well described	No well-defined risk groups	Rodents and or marsupials?	Likely sylvatic zoonotic	Unknown	OW and NW	Martinique, Thailand	Limited number of cases
Leishmania siamensis	CL and VL	Not well described	Not well described	No well-defined risk groups	Rodents?	Likely sylvatic zoonotic	*Sergentomyia gemmea*?	OW and NW	Central Europe, Thailand, USA	Limited number of cases

Legend: CL- cutaneous leishmaniasis; DCL - diffuse cutaneous leishmaniasis; DsCL - disseminated cutaneous leishmaniasis; LR - leishmaniasis recidivans; MCL - mucocutaneous leishmaniasis; NW - New World; OW - Old World; PKDL- post-kala-azar dermal leishmaniasis; VL- visceral leishmaniasis.

2. The parasite, the host and their interactions in the fight for survival

All causative agents of vector borne diseases, must have the capacity to survive and thrive in two evolutionary distinct organisms, the vector and, the host. The *Leishmania* genus, which includes all of the abovementioned etiological agents of human leishmaniasis is an example of protozoan organisms that resisted simultaneously to 2 major evolutionary pressures and evolved no only to subsist, but to become dependent on both arthropods and vertebrates to prosper (Caljon *et al*, 2016). As a consequence of this co-evolutionary processes, *Leishmania* parasites show some peculiar characteristics (in comparison to other pathogenic unicellular microorganisms).

Leishmania parasites are flagellated eukaryotes (Phylum: Euglenozoa) possessing a single mitochondrion, with the concentration of the several copies of mitochondrial DNA in a single structure called kinetoplast (Class: Kinetoplastea). Because they have a single flagellum, they are grouped in the family Trypanosomatidae, together with other known vector-borne human pathogens (but not only) such as *Trypanosoma brucei* and *Trypanosoma cruzi*, causative agents of sleeping sickness and Chagas disease, respectively, also known NTDs (McGhee and Cosgrove, 1980; Stevens *et al*, 2001). The simplified taxonomy up to the species level (considering only the human pathogenic species) is represented in Figure 3.

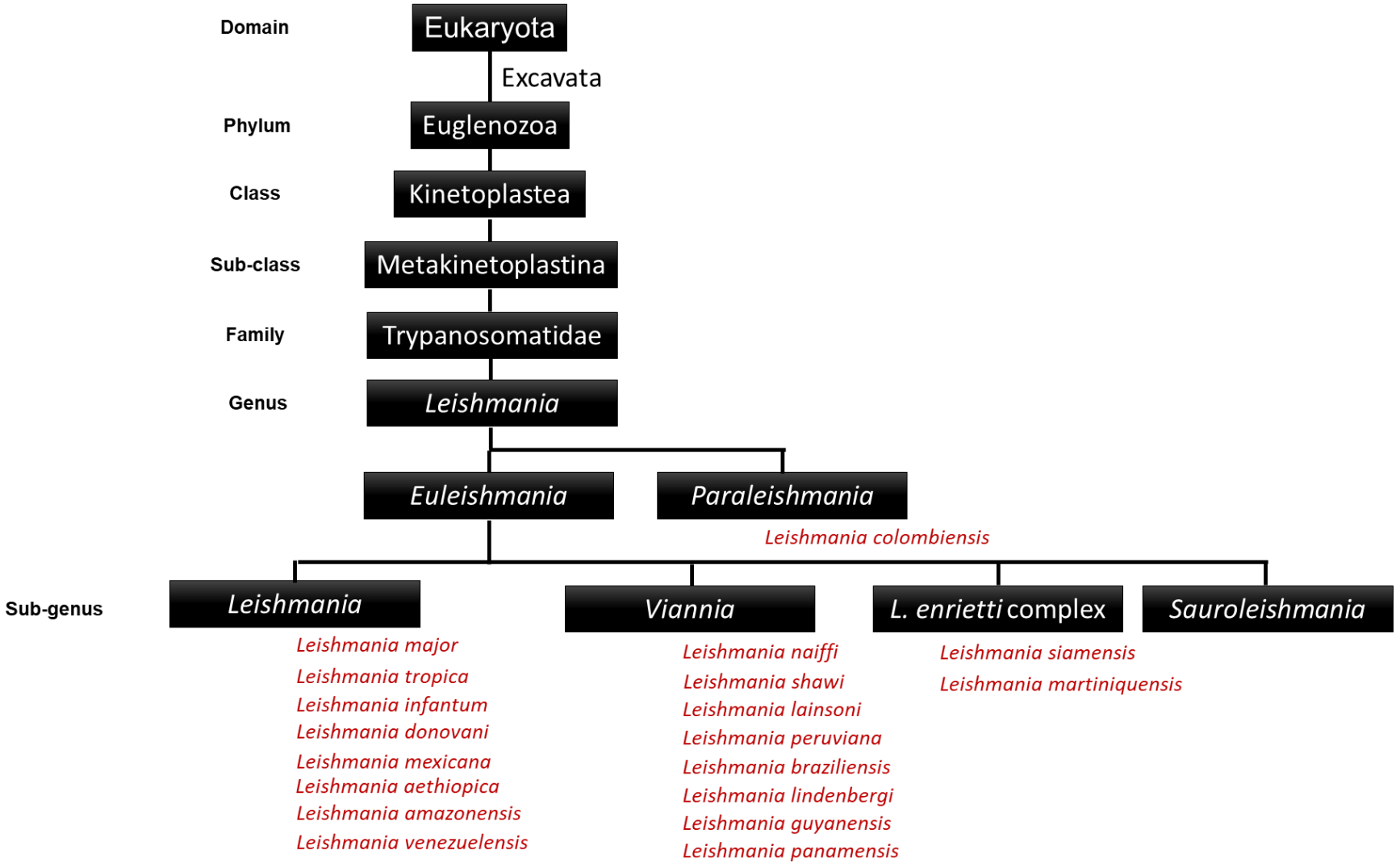


Figure 3. Taxonomy of *Leishmania* parasites with focus on human pathogenic species. Adapted from Akhoundi *et al*, 2016.

Furthermore, *Leishmania* parasites exhibit a variety of cell morphologies, essential to their survival in each step of their life cycle (digenetic or dixenous) - Figure 4 - that can be divided into two major cell types - promastigotes and amastigotes (Sunter and Gull, 2017) - which allow the parasites to prosper within the vector and the host, respectively. The two particular niches *Leishmania* parasites occupy in nature overall justify these two major morphologies. In the sand fly vector, *Leishmania* spp. reside extracellularly within their intestinal tract (Dostalova and Volf, 2012), while in the vertebrate hosts these parasites are almost strict intracellular organisms, subsisting and replicating within phagocytic cells' lysosomes (Moradin and Descoteaux, 2012). Consequently, motility is much more important comparing the first with the later contexts, which justifies the primary major difference observed comparing promastigotes and amastigotes, which relates to the flagellum. While promastigotes typically present long flagella, with at least the same length as the cell body, amastigotes have a residual flagellum with an intracellular location (Figure 5) (Gluenz *et al*, 2010). Also related to motility, the second microscopically visible difference relates to cell body length and shape. Again, while amastigotes are ovoid and smaller, *Leishmania spp.* promastigotes are larger and have an elongated cell shape (hydrodynamic), with a high degree of symmetry, essential for their directional motility (Figure 5) (Wheeler, 2017). On the other hand, the rest of the basic cell architecture (defined by cross-linked sub-pellicular corset microtubules) is conserved between these two *Leishmania* spp. main morphologies (Figure 5) (Sunter and Gull, 2017).

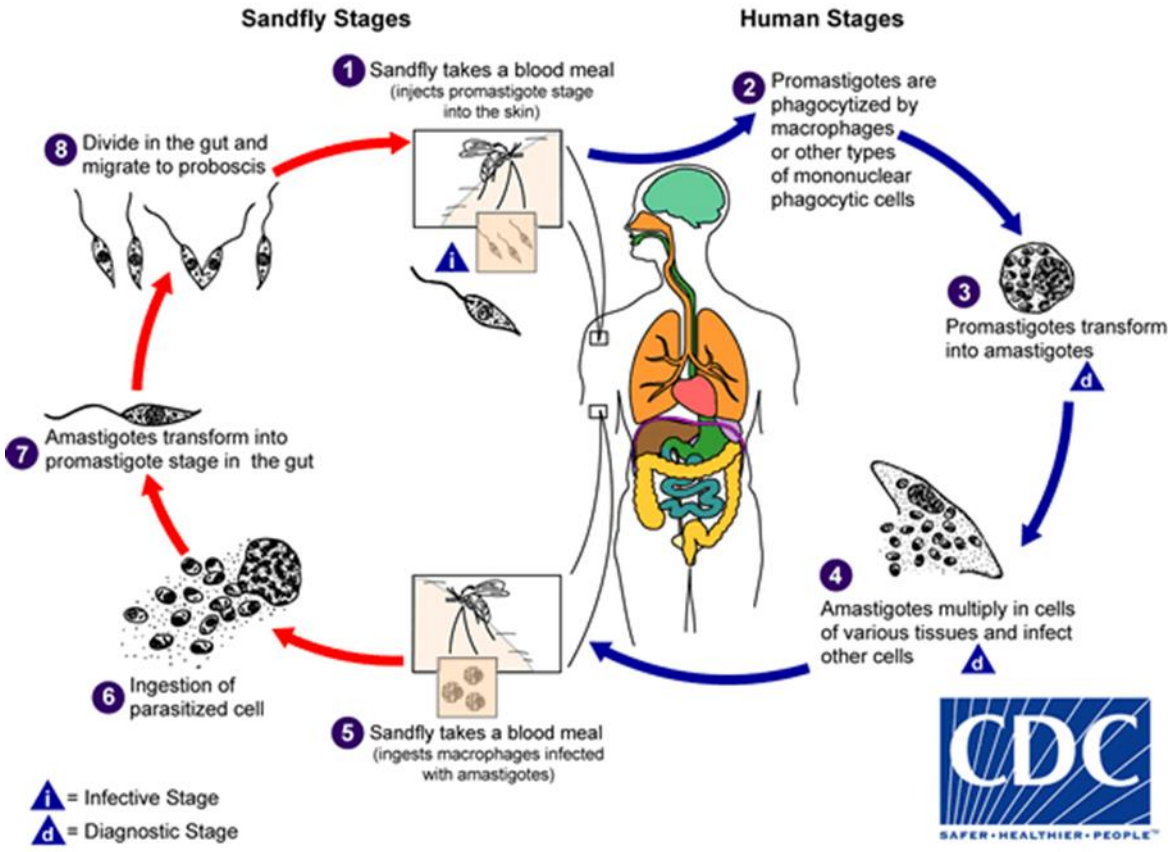


Figure 4. Simplistic representation of *Leishmania* parasites' life cycle. The cycle begins with the egestion of *Leishmania* spp. metacyclic promastigote parasites into the host skin by sand flies during the blood feeding process (1). Parasites are then phagocytized and ultimately reach their major niches, phagolysosome compartments of macrophages (2), where they differentiate to the amastigote form and multiply (3, 4). Parasites either remain in the skin, or reach the internal organs. Either way, infection progresses in the host with the multiplication of amastigotes and the infection of new cells (4). When parasites reach a given threshold (not strictly, but many times associated with overt disease) they can be ingested (usually within infected cells) by a non-infected sand fly during the blood feeding process (5, 6). Inside the sand fly, parasites undergo a series of morphological alterations (starting by the differentiation to the promastigote form - 7), replicate and ultimately give rise to the metacyclic promastigote infective forms (closing the cycle) that locate in the sand fly anterior midgut (8). Reproduced from CDC, 2017.

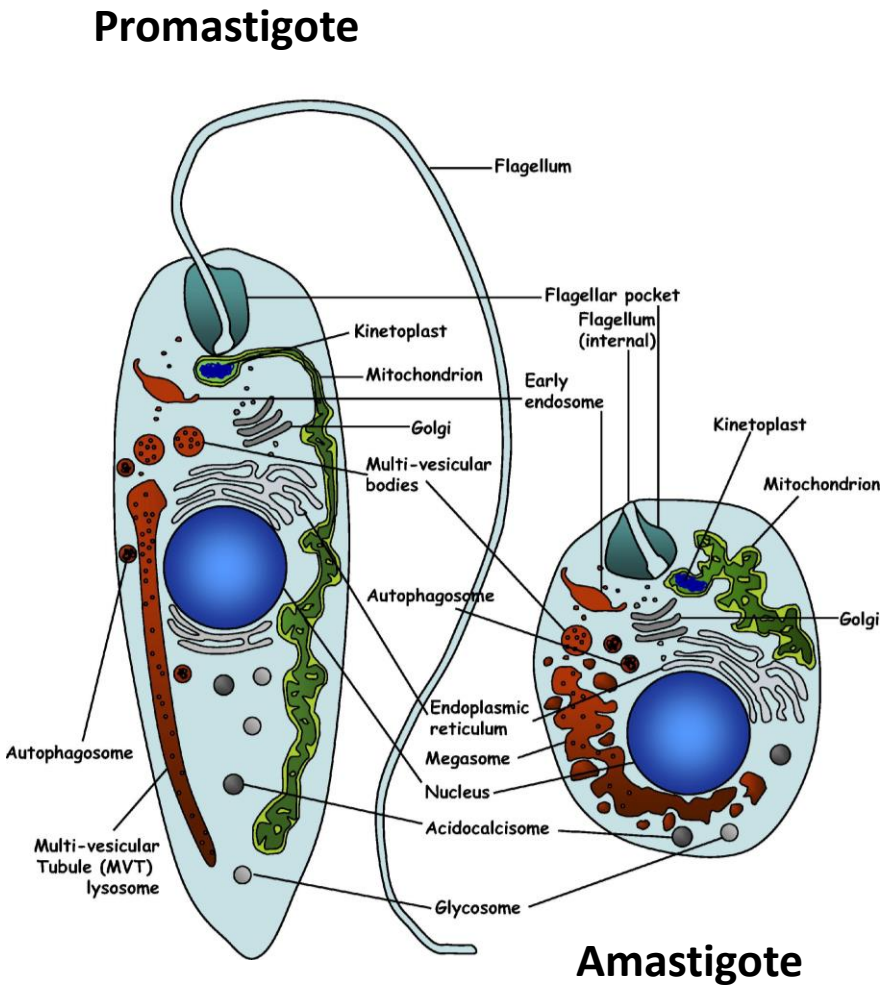


Figure 5. Comparing *Leishmania spp.* promastigotes and amastigotes. Adapted from Besteiro *et al*, 2007.

Apart from the morphology, all *Leishmania* spp. developmental forms are distinguished by their nutritional requirements, their growth rate, and ability to divide, and the regulated expression of their surface molecules, once again to cope, survive and thrive within distinct niches, with different “selective pressures” (Besteiro *et al*, 2007; Sunter and Gull, 2017). Remarkably, these adaptations occur quite fast, translated into differentiation processes that can be triggered by several factors, such as variations in the availability and type of nutrients, pH, temperature, and availability of oxygen (Besteiro *et al*, 2007; Sunter and Gull, 2017).

The understanding of each adaptation process experienced by *Leishmania spp.* parasites is ultimately essential for the comprehension of the pathogenic processes, pre-requisite for the development of any effective control measures. Regarding the host-parasite interface, to know the host immune determinants essential for parasite elimination is as vital as to know the several strategies *Leishmania* spp. parasites developed and use to overcome the host immune pressure, discussed in the published review “Deception and manipulation: the arms of *Leishmania*, a successful parasite” (Cecilio *et al*, 2014).

frontiers in IMMUNOLOGY

REVIEW ARTICLE
published: 20 October 2014
doi: 10.3389/fimmu.2014.00480

Deception and manipulation: the arms of *Leishmania*, a successful parasite

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Leishmania spp. are intracellular parasitic protozoa responsible for a group of neglected tropical diseases, endemic in 98 countries around the world, called leishmaniasis. These parasites have a complex digenetic life cycle requiring a susceptible vertebrate host and a permissive insect vector, which allow their transmission. The clinical manifestations associated with leishmaniasis depend on complex interactions between the parasite and the host immune system. Consequently, leishmaniasis can be manifested as a self-healing cutaneous affliction or a visceral pathology, being the last one fatal in 85–90% of untreated cases. As a result of a long host–parasite co-evolutionary process, *Leishmania* spp. developed different immunomodulatory strategies that are essential for the establishment of infection. Only through deception and manipulation of the immune system, *Leishmania* spp. can complete its life cycle and survive. The understanding of the mechanisms associated with immune evasion and disease progression is essential for the development of novel therapies and vaccine approaches. Here, we revise how the parasite manipulates cell death and immune responses to survive and thrive in the shadow of the immune system.

Keywords: *Leishmania*, immunomodulation, apoptosis, innate immunity, acquired immunity

INTRODUCTION

Parasitism is defined as a "non-mutual symbiotic relationship between species, where one species, the parasite, benefits at the expense of the other, the host," Such relationship occurs during leishmaniasis, where the protozoan *Leishmania* spp. takes advantage of its mammalian host in order to survive and thrive.

Leishmania is a genus of trypanosomatid protozoa that combines over 30 species, of which 11 have significant medical and veterinary importance (1). These parasites have a complex digenetic life cycle, with some particularities, requiring a vertebrate host and an insect vector. The alimentary tract of female *Phlebotomus* spp. and *Lutzomyia* spp. sandflies is colonized by the extracellular form of the parasite, the flagellated, and motile promastigote. Within the insect midgut, *Leishmania* undergoes several developmental changes that culminate in the infectious developmental form of the parasite: the metacyclic promastigote. During the insect blood feeding, the parasite infectious forms are released into the mammal host dermis and quickly uptaken by mono and polymorphonuclear (PMN) cells. Ultimately, in the phagolysosome of macrophages, promastigotes will differentiate into the non-motile amastigote form and multiply. The cycle is completed when the sandfly takes another blood meal, recovering free amastigotes or infected cells (1–3).

Leishmaniasis is endemic in 98 countries, 72 of which are developing nations and 13 correspond to the least developed ones, being considered by the World Health Organization as a Neglected Tropical Disease (4, 5). Over 350 million people reside in areas with active parasite transmission (6). Annually, an estimated 1.5–2 million develop symptomatic disease, and approximately 50,000 die, mostly children (4, 7). Climate changes and population mobility can contribute to the increase of the vector activity and, consequently of the disease incidence (8, 9). The infection caused by *Leishmania* spp. can lead to different clinical manifestations depending on complex interactions between the parasite and the host immune response. The disease is normally divided into three main categories: cutaneous, mucocutaneous, and visceral. Cutaneous leishmaniasis is the most extensively studied form of the disease, usually appearing as a self-healing skin ulcer or dermal granuloma that may need several months or years to heal (10). In some cases, these ulcers can become chronic (11). While most *Leishmania* species cause lesions confined to small areas of the skin, a few, such as *L. braziliensis,* cause diffuse lesions that may even spread to mucosal tissues leading to the mucocutaneous form of the disease (12). Finally, visceral leishmaniasis, the most severe leishmaniasis form, is caused by *Leishmania donovani* and *Leishmania infantum*. It is characterized by fever, cachexia, hepatosplenomegaly and hypergamaglobulinemia and, when untreated, can be fatal (13). In endemic countries, *Leishmania* has gained prominence as an opportunistic pathogen in HIV positive and other immunocompromised patients (8, 14). Leishmaniasis is also a major veterinary concern, as dogs are the main reservoir for the parasite in South America and southwestern Europe (15).

There is no human vaccine available at the moment. Nonetheless, prevention of infection through vaccination seems to be a viable option, since in endemic areas the majority of infected persons do not develop clinical symptoms and previous infection leads to robust immunity against the parasite (16). In the absence

of vaccines, control of the disease relies on prophylaxis and treatment, reviewed elsewhere (17, 18). Treatment options are limited, present significant toxicity and require, with the exception of oral miltefosine, administration in ambulatory conditions (18). Drug resistance is also a growing limitation of some anti-leishmanial therapies (19). Therefore, it is essential to develop novel treatment options and vaccine strategies. Such goal has its cornerstone on the solid knowledge of the details of parasite infection. For this, different strategies that *Leishmania* uses to manipulate the immune system to establish infection will be revised here.

PLAYING WITH DEATH TOWARD THE ESTABLISHMENT AND MAINTENANCE OF INFECTION

Apoptosis, or programed cell death, is a physiological and essential process for the maintenance of general cellular homeostasis. In immunology, this mechanism is indispensable for elimination of autoreactive immune cells (20, 21) and control of the proliferative response (22, 23). Programed cell death also plays a key role in the resolution of infections produced by intracellular pathogens (24). However, and as a result of the continuous host-microbe co-evolutionary process, *Leishmania* developed strategies for using apoptosis to its own benefit.

DEAD PARASITES ARE ESSENTIAL FOR THE SURVIVAL OF FREE PROMASTIGOTES

Parasite cell death, reviewed elsewhere (25–27), seems to be very relevant for the deception of the initial immune response. Some authors described that the presence of apoptotic parasites is essential for successful infection of mice susceptible to cutaneous leishmaniasis. Indeed BALB/c mice did not develop disease after intradermal infection with purified virulent non-apoptotic parasites (28, 29). The need for dead parasites in the infective inoculum is related with the exposure of phosphatidylserine (PS) in the outer leaflet of the parasite cytoplasmic membrane. The exposure of this phospholipid enables a silent invasion, inducing the production of anti-inflammatory cytokines such as TGF-β (30, 31). In fact, a recent study shows that the administration of a PS-targeting antibody after C57Bl/6 mice intradermal infection with *L. amazonensis* promastigotes renders the animals more resistant to the infection (32). Thereby, and as represented in **Figure 1**, the inoculation of equal proportions of dead and live parasites in the mammalian host may allow the silent entry of *Leishmania* into the first cells recruited to the inoculation site (28, 33).

MODULATING APOPTOSIS OF NEUTROPHILS AT THE INOCULATION SITE

It is accepted that macrophages are the cells predominantly infected in leishmaniasis. However, they are neither the first nor the only to be recruited to the site of inoculation. Several evidences support the early recruitment of neutrophils to the inoculation site. Two hours after natural infection of C57Bl/6 mice with *L. major*, neutrophils are predominantly found (34). Such granulocyte infiltration was also seen upon intradermal infection of either BALB/c or C57Bl/6 mice with *L. infantum* and *L. major*, respectively (35, 36), as well as after subcutaneous infection with *L. amazonensis* or *L. major* promastigotes (37–39). Furthermore in a murine air pouch model, *L. major*, and to a lower extent *L. donovani*, predominantly induced the recruitment of neutrophils 6 h after infection (40, 41). Interestingly, the air pouch system revealed that *L. major* derived extracellular vesicles induced the same type of cellular recruitment as parasites (40). These studies preceded the description of Wilson et al. who saw neutrophils infiltration 1 h after intradermal inoculation of *L. donovani* promastigotes in hamsters (42). Although the role of neutrophils during infection is not consensual, several evidences support the capacity of *Leishmania* to modulate their life span. Traditionally, neutrophils show a relatively short life span (43), but *Leishmania* can successfully delay their programed cell death for up than 24 h, potentially benefiting from the protection of a safe intracellular niche (44). However, other studies show induction of neutrophil apoptosis after parasite intake (35). These contradictions may be due to differences in the genetic background of the animal model used (BALB/c versus C57Bl/6), as well as in the parasite inoculation route (45). The delay in the natural apoptotic process of infected neutrophils was related to an inhibition of the pro-caspase-3 processing (44), and the consequent diminishment of caspase 3, a well-known apoptosis executer in neutrophils (46). Moreover, a recent publication clarified the mechanisms by which *L. major* contributes for the neutrophil apoptosis inhibition, showing that the key event is the activation of the extracellular signal-regulated kinases (ERK1/2) survival pathway (47). Sarkar and colleagues showed that the parasite upregulates ERK1/2 phosphorylation, leading to the delay of neutrophil apoptosis (48). Also, this work unveiled additional players of the apoptotic machinery responsible for neutrophil life span enhancement. Among these the anti-apoptotic proteins, Bfl-1 and Bcl-2 were upregulated, preventing the release of cytochrome *c*

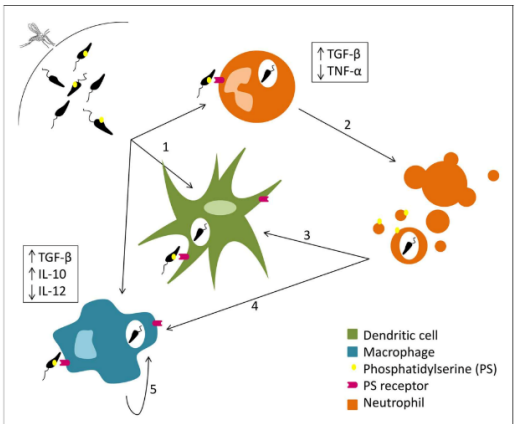


FIGURE 1 | Silent entry of *Leishmania* into the host cells. Live and dead parasites are engulfed by phagocytes. The recognition of the externalized phosphatidylserine present on the cellular membrane of dead parasites induces TGF-β secretion and TNF-α downregulation (1). Neutrophil apoptosis is delayed by *Leishmania* (2). Both dendritic cells (3) and macrophages (4) remove neutrophil apoptotic bodies carrying *Leishmania* promastigotes and secrete TGF-β and IL-10. Macrophages (5) can also phagocyte parasites extruded within other macrophage membrane blebs, which in turn promotes the secretion of IL-10.

from the mitochondria and the downstream activation of caspases. Additionally, processing of the pro-apoptotic Bid was inhibited and the Fas expression reduced, preventing apoptosis triggering (48). This delay of neutrophil death may be essential for the arrival of a sufficient number of antigen-presenting cells (APCs), namely macrophages, and dendritic cells (DCs), to the inoculation site.

"TROJAN HORSE" STRATEGY

After being infected, dying neutrophils secrete different chemotactic factors for macrophages (49, 50); cells that then remove apoptotic neutrophils by phagocytosis and secrete the anti-inflammatory cytokine TGF-β (50). High amounts of IL-10 and low amounts of interleukin (IL)-12 may also contribute for the silent entry of *L. major* into macrophages (51) as shown in **Figure 1**. The parasite can, therefore, arrive to its primary host cell unnoticed and proceed with the infection process, using the so-called "Trojan horse" strategy (52). TGF-β seems to be essential for the establishment of infection not only by *L. major* but also by *L. amazonensis*, although conclusions about the exploitation of the "Trojan horse" strategy in this case cannot be withdrawn (50, 53). DCs have also been related with this tactic. Ribeiro-Gomes et al. recently described in a mouse model of intradermal infection with *L. major* that skin resident DCs uptake apoptotic infected neutrophils and, as a consequence, the activation of *Leishmania*-specific $CD4^+$ T cells is prevented somehow (35). Other authors suggested that free parasites silently enter into host cells taking advantage of nearby neutrophil apoptotic bodies with exposed PS (54).

BUYING TIME BY PROLONGING THE LIFE OF MACROPHAGES

When promastigotes reach macrophages, its definitive cellular host, a new step of the infective process begins with their differentiation into amastigotes. Therefore, inhibition of apoptosis may be once more essential for *Leishmania* to protect its niche, enabling the differentiation into the amastigote form that is fully adapted to the phagolysosome. Extensive data exists concerning the capacity of the parasite to increase the life span of infected macrophages. The first description was made by Moore and Matlashewski, who reported that *L. donovani* infection of murine bone marrow-derived macrophages (BMM) represses macrophage apoptosis through a mechanism dependent on the secretion of TNF-α (55). Since then, numerous studies addressed this issue, unveiling some intracellular mechanisms that could explain the death delay. External ATP is known to trigger death in macrophages when injured or stressed, by its binding to purinergic receptors of the P2X family (56, 57). Interestingly, Kolli et al. showed that *L. amazonensis* releases nucleoside diphosphate kinase (NdK), preventing ATP-induced cytolysis of J774 macrophages (58). Further studies are, however, required to access the relevance of NdK in the context of infection. The ERK1/2 pathway also plays a role in the prevention of macrophage apoptosis. Kamir and colleagues described a protein produced by *L. major* that shows structural homology with the human macrophage inhibiting factor (MIF) and exerts similar effects. Indeed this MIF ortholog induced ERK1/2 kinases activation in a CD74-dependent manner, subsequently resulting in the inhibition of macrophage apoptosis *in vitro* (59). The mitochondrial apoptotic pathway is also modulated by *Leishmania*. BMM infected with *L. major* showed enhanced survival that was related with the prevention of cytochrome *c* release by mitochondria (60), observation possibly explained by the involvement of an anti-apoptotic signaling pathway (61). Ruhland and colleagues showed that *L. major* block macrophage apoptosis through the phosphatidylinositol 3′-kinase (PI3K)/protein kinase B (Akt) signaling pathway. Briefly, Akt phosphorylates the pro-apoptotic Bad, deactivating it, and preventing the release of mitochondrial cytochrome *c* (62), which avoids downstream activation of the effector caspase-3 (60). Similar results were obtained with DCs (63, 64). More recently, it was also shown that apoptosis triggered by oxidative burst is prevented by *L. donovani*. Although infected macrophages were capable of ROS production, a complete abrogation of the downstream caspase cascade was observed due to thioredoxin mediated selective induction of suppressors of cytokine signaling (SOCS) proteins (65). A direct responsibility of a parasitic protein was not addressed in these studies, but we cannot exclude the role of phosphoglycans since there are studies that relate them with apoptosis delay in *L. infantum*, *L. major*, and *L. donovani*-infected macrophages (66, 67). Notwithstanding, the parasites capacity to delay macrophage apoptosis is yet to be shown *in vivo*.

Although parasites delay macrophage death, they cannot prevent it. However, when an infected macrophage dies, *Leishmania* is able to escape. A recent study showed that *L. amazonensis* amastigotes are transferred from cell to cell when the donor host macrophage delivers warning signals of imminent apoptosis (**Figure 1**). Interestingly, that transfer happens without full exposure of the parasite to the extracellular milieu: the parasites are extruded from the host macrophages within membrane blebs rich in phagolysosomal membrane components, which are in turn phagocytized by nearby macrophages that will then secrete the infection promoting cytokine IL-10 (68).

REMOVAL OF EFFECTOR T CELLS BY APOPTOSIS

Modulation of cell death is also used by parasites as a way to directly alter the acquired immune response by elimination of effector cells. Felix de Lima et al. showed that apoptosis levels in both peripheral blood and spleen T lymphocytes from *L. infantum* naturally infected dogs are higher in comparison to control animals. The authors concluded that immunosuppression associated with chronic infection is due to accelerated rates of T cell apoptosis, which in turn contributes to white pulp disorganization in the spleen and diminished T cell levels in peripheral blood (69, 70). Furthermore, active human cutaneous leishmaniasis caused by *L. braziliensis* was associated with increased apoptosis of $CD8^+$ and $CD4^+$ T cells (71). Interestingly, all of these studies linked T cells apoptosis with active disease. However, the mechanisms are yet to be unveiled. The death receptors apoptotic pathway may be involved, as Fas and FasL expression in human splenic lymphocytes is increased in acute disease (72). Furthermore, the correlation between T cell apoptosis and pathophysiological states was further accessed using mouse infection models. In fact infection of susceptible, but not resistant mice with *L. donovani* induced apoptosis of splenic $CD4^+$ T cells after *in vitro* stimulation (73). In this case, the mechanisms involved in apoptosis induction, start to be disclosed. Reckling et al. showed that the pro-apoptotic Bcl-2 family member

Bim possibly has a role in T cell apoptosis in a mouse model of infection with *L. major* (74). Moreover, in another mouse model infected with *L. donovani*, authors concluded that T cell apoptosis could be related with downregulation of PKC and ERK1/2 activities. Ser/Thr phosphatase seems to have a major role in the initiation of this process by dephosphorylation of key molecules of different T-lymphocyte signaling pathways (75).

Table 1 resumes the topics described above, overviewing the modulation of apoptosis by *Leishmania* in different cell types.

OVERCOMING THE IMMUNE LEISHMANICIDAL MACHINERY

Leishmania is one of the few intracellular pathogens that can live and replicate inside the harsh environment of a mature phagolysosome. Apart from this parasite, only *Coxiella brunetti* resides during its entire replicative cycle inside that cellular compartment, as reviewed by Voth and Heinzen (76), while other intracellular pathogens that preferentially infect macrophages escape the phagocytic pathway (77). *Leishmania* must, therefore, cope with different effector molecules from the innate immune response in order to survive.

AVOIDING CELL LYSIS AND TAKING ADVANTAGE OF OPSONIZATION

The first challenge *Leishmania* encounters in the mammalian host is the complement system (78). Traditionally, promastigote complement resistance is associated with two *Leishmania* glycocalyx components (79): lipophosphoglycan (LPG) and the metalloprotease leishmanolisin (GP63). *Leishmania major* parasites deficient for both these molecules demonstrated high complement sensitivity (80–82). LPG avoids the ultimate step of the complement cascade through prevention of the attachment of the C5b-C9-complex to the parasite surface (83, 84). On the other hand, GP63 inactivates C3b preventing the formation of the C5 convertase complex (85, 86). Albeit, Dominguez et al. showed that under physiological conditions 85–100% of *L. donovani*, *L. infantum*, *L. major*, and *L. amazonensis* promastigotes are killed by complement after 2.5 min in human blood (87). Yet, it was also published that as soon as 1 min after *L. amazonensis* and *L. donovani* contact with human blood, infected granulocytes were easily found (88). Therefore, it is essential for the parasite to escape the complement onslaught by quickly entering a phagocytic cell.

Once again *Leishmania* glycocalyx components are used to subvert the innate immune system enhancing the phagocytosis of the parasites. Both GP63 and LPG can directly interact with the host cell surface through binding to the fibronectin receptor and the mannose/fucose receptor, respectively (89–92). Moreover, iC3b, the cleavage product of C3 by GP63, can function as an opsonin (85), and LPG interacts with the early inflammatory C-reactive protein, which triggers phagocytosis (93, 94). Interestingly, iC3b is a ligand of the complement receptor 3 (CR3) (95), and this interaction is directly related with the downregulation of IL-12 production by macrophages (96). The mechanism by which this downregulation happens is not known; however, we may not exclude a toll like receptor (TLR) inhibition since C5a, another complement component, has a negative impact on the TLR-4 induced IL-12 synthesis (97). This may ultimately contribute for the silent entry of the parasites into the host cells.

Table 1 | Apoptosis modulation during *Leishmania* infection.

Cell type	Alteration of apoptosis related molecules	Outcome	Key player	References
Neutrophils	Phosphorylation of ERK1/2 Upregulation of BX-1 and Bcl-2 Inhibition of Bid and pro-caspase 3 processing Prevention of mitochondrial cytochrome *c* release Downregulation of Fas expression	Apoptosis inhibition	Unk	(48)
Macrophages	Decrease of extracellular ATP Phosphorylation of ERK1/2 Activation of PI3K/Akt signaling pathway Deactivation of Bad Induction of SOCS proteins Inhibition of pro-caspases 3 and 7 processing	Apoptosis inhibition	NdK Lm1740MIF Unk Thioredoxin	(58) (59) (62) (65)
Dendritic cells	Upregulation of BX-1 and Bcl-2? Prevention of mitochondrial cytochrome *c* release? Inhibition of pro-caspases 3 and 7 processing	Apoptosis inhibition	Unk	(63, 64)
T cells	Upregulation of Bim? Deactivation of ERK1/2 Downregulation of Bcl-2 Increase of mitochondrial cytochrome *c* release Upregulation of pro-caspase 3 processing	Induction of apoptosis	Unk Ser/Thre phosphatase	(74) (75)

Akt, protein kinase B; ERK, extracellular signal-regulated kinases; MIF, macrophage inhibiting factor; NdK, nucleoside diphosphate kinase; SOCS, suppressors of cytokine signaling; Ser/Thr, serine/threonine; Unk, unknown.

TOWARD A SUCCESSFUL DIFFERENTIATION: ALTERATIONS DURING THE PHAGOLYSOSOME MATURATION PROCESS

After promastigote entry into the host cell, *Leishmania* needs to differentiate to the amastigote form. Since promastigotes cannot survive in the harsh environment of the phagolysosome (low pH, hydrolases), a delay of phagolysosomal fusion was considered essential for the parasite differentiation process (98). Such delay has been described for *L. major*, *L. infantum*, and *L. donovani* via mechanisms that may or may not involve LPG (98, 99). However, with *L. mexicana* and *L. amazonensis*, this was not proved (100–103). For these parasites, the large parasitophorous vacuoles found in macrophages dilute the hydrolytic enzymes upon lysosome fusion to a level below their effectiveness, allowing promastigotes to differentiate without any requirements of fusion delay (100). In the case of *L. donovani*, it was shown that LPG impairs the association of synaptotagmin V to phagosome membranes, inhibiting the recruitment of the vesicular proton-ATPase and preventing their acidification, allowing promastigote to amastigote differentiation (104). *Leishmania donovani* LPG was also associated with retention of the small GTPase Cdc42 at the phagosome membrane, leading to F-actin accumulation around the phagosome and presumably interfering with vesicle trafficking and phagosome maturation (105, 106).

ROLE OF GP63 IN THE DEFENSE AGAINST ANTIMICROBIAL PEPTIDES

Inside a phagolysosome, fully differentiated or not, *Leishmania* has to deal with other components of the innate immune system: the antimicrobial peptides (AMPs). AMPs are structurally diverse cationic proteins with intrinsic antimicrobial activity, playing normally by disruption of cell surface membranes resulting in osmotic lysis of the pathogen. They can be found both intra and extracellularly, and most of them are constitutively produced and secreted (when applicable) (107, 108). Some human AMPs present activity against *Leishmania*. For example, Kulkarni et al. showed that cathelicidin, an intracellular AMP present in macrophage lysosomes, can kill up to 50% of *L. major* and *L. amazonensis* parasites (109). The same group showed in a different study that α-defensins, produced by neutrophils, also kill *L. major* parasites (110). GP63 play a key role in the defense against these peptides, as it was shown that gp63 KO promastigotes were efficiently killed in a dose dependent manner by AMPs (109).

COPING WITH REACTIVE OXYGEN AND NITROGEN SPECIES (ROS AND RNS)

Once inside the host cell, ROS and RNS are the cellular major arms against *Leishmania*. $NO^{\bullet}$ is synthesized by nitric oxide synthase (NOS) during the conversion of L-arginine to L-citrulline, while $O_2^{\bullet}-$ and other reactive oxygen species (ROS) are generated by the membrane-bound NADPH-dependent oxidases (NOX). These reactive species contribute for the generation of others as $ONOO^-$, $NO_2^{\bullet}$, and nitrogen trioxide (111). Although NO is considered the most relevant microbicidal molecule, ROS are also associated with disease susceptibility since NOX deficient mice are more susceptible to *L. donovani* and *L. major* infection (112, 113). However, unlike what happens with inducible NOS (iNOS) KO mice, NOX deficient mice eventually control the infection (112–114). Therefore, the parasite needs to somehow neutralize these reactive species and/or prevent their production to avoid a certain death by oxidative stress. The inflammatory cytokine TGF-β produced by infected phagocytes shifts the L-arginine metabolism toward the production of L-ornithine through the activation of arginase (115, 116). This metabolic shift leads to a decrease in NO secretion favoring intracellular *Leishmania* growth (117). Glycocalyx components can also play a role in the protection of *Leishmania* parasites from ROS. A genetic rescue of a *L. amazonensis* GP63 deficient strain increased its intramacrophage survival potential, which was probably related with inhibition of ROS generation (118, 119). In turn, LPG not only prevents ROS generation through inhibition of NOX recruitment to the phagosome membrane, but also directly scavenges these reactive species (81, 120). Glycosylinositolphospholipid (GILP), another component of the glycocalyx, may also be important during the amastigote form, suppressing macrophage iNOS expression and, consequently, NO production (121). Finally, we cannot disregard the intrinsic antioxidant machinery of *Leishmania*, whose most important components are trypanothione synthase and trypanothione reductase. The last one is essential for the fight against ROS and NOS, once disruption of the trypanothione reductase gene renders the parasites susceptible to intracellular killing by macrophages (122). A recent publication shows that *L. donovani* activates multiple own enzymatic mechanisms for the detoxification of ROS and NOS (123). Some of these enzymes have already been associated with protection against reactive species, including the *L. infantum* peroxiredoxins LicTXNPx and LimTXNPx, *L. major* pteridin reductase, and *L. donovani* superoxide dismutase (124–126).

Table 2 discusses the different ways by which components of the *Leishmania* glycocalyx prevents parasite killing by innate immune response.

MODULATING THE IMMUNE RESPONSE THROUGH ALTERATION OF CYTOKINE AND CHEMOKINE SIGNALING AND PRODUCTION

Cytokines are cell signaling mediators, which affect cell function in an autocrine, paracrine, or endocrine manner. Interference with the normal cytokine production is a powerful weapon that the parasite can use for the modulation of immune function. It is generally accepted that production of IL-12 by macrophages and DCs is associated with resistance against *Leishmania*. This cytokine induces naive T cells maturation toward an IFN-γ producing Th1 phenotype (resistant to infection), which in turn induce macrophage M1 activation and elimination of parasites (127, 128). Th2 cytokines, namely IL-4 regarding cutaneous leishmaniasis and IL-10 and TGF-β in the case of visceral disease, have been related with disease susceptibility and progression by induction of an M2 macrophage phenotype (129–131). Therefore, parasites seem to modulate the immune response toward a Th2 phenotype. However, this Th1/Th2 straight polarization seems only to be observed in some murine models, and cannot be fully applicable to human diseases (132). The Th1/Th2 paradigm (reviewed elsewhere) (133, 134) states that Th1 and Th2 cells counter-regulate each other. That would imply that *Leishmania*-induced polarization of the immune response toward a Th2 phenotype would suppress a Th1 immune response.

Table 2 | Glycocalyx components: overcoming innate immune leishmanicidal machinery.

Glycocalyx component	Species	Protective role	Mechanism	References
LPG	*L. major*	Inhibition of complement-mediated lysis	Prevention of attachment of the C5b-C9-complex	(83)
	L. donovani	Promotion of phagocytosis to escape the	Interaction with C-reactive protein and direct binding	(91, 93, 94)
	L. mexicana	extracellular milieu	to phagocytes receptors	
	L. donovani	Delay of phagolysosome maturation process	Inhibition of the recruitment of vesicular proton-ATPase	(104)
	L. donovani	Reduction of leishmanicidal reactive species	Inhibition of ROS generation	(81, 120)
	L. major		ROS scavenging	
GP63	*L. major*	Inhibition of complement-mediated lysis	Inactivation of C3b	(85, 86)
	L. infantum			
	L. major	Promotion of phagocytosis to escape the	The C3b inactivation product functions as an opsonin	(85, 89, 92)
	L. infantum	extracellular milieu	Direct binding to phagocytes receptors	
	L. donovani			
	L. major	Prevention of antimicrobial peptide mediated lysis	Proteolytic degradation of the antimicrobial peptides	(109)
	L. amazonensis	Reduction of leishmanicidal reactive species	Inhibition of ROS generation	(119)
GILP	*L. major*	Reduction of leishmanicidal reactive species	Suppression of iNOS expression and NO production	(121)

GILP, glycosylinositolphospholipid; iNOS, inducible nitric oxide synthase; LPG, lipophosphoglycan; NO, nitric oxide; ROS, reactive oxygen species.

However, what is observed in human disease is a peculiar mixed cytokine response, variable, depending on the infective species (132, 133, 135).

LEISHMANIA MODULATES TLR SIGNALING

Toll like receptors recognize a variety of pathogen-associated molecular patterns (PAMPs), from proteins to nucleic acids. Upon engagement, TLRs mediate the activation of different transcription factors, such as nuclear factor-κB (NF-κB) and interferon-regulatory factors (IRFs), leading to the production of inflammatory cytokines (136, 137). Induction of cell mediated immunity (138–140) and promotion of NO production (141) are other two known TLR triggered responses against *Leishmania* infection. Nevertheless, the parasite developed strategies that interfere with TLR associated signaling cascades subverting the traditional pro-inflammatory responses. *Ex vivo* experiments suggest that TLR-2 performs a minor role in initiating the synthesis of pro-inflammatory cytokines, namely IL-12, during mice infection with *L. infantum* (142). Chandra et al. showed that *L. donovani* can shift TLR-2 responses toward a Th2 immune response, with downregulation of IL-12 production in macrophages, through MAP kinase inactivation (143). The crosstalk between TLR-2 and CCR-5 (which expression is dependent on the expression of the first one) was also described as relevant in *L. donovani* infection, promoting parasite internalization and inducing a Th2 immune response (144). Moreover, the interaction between TLR2 and LPG was shown do decrease TLR-9 expression leading to a lesser inflammatory profile (145). Nevertheless, the interplay between *Leishmania* and TLRs is highly complex and needs further clarification, once there are several reports showing that LPG-TLR interactions can also result in increase of anti-leishmanial responses by effector cells (146).

The capacity of *Leishmania* to interact with regulatory proteins of the host may also be relevant for TLR signaling modulation. As an example, *L. donovani* exploits a host negative TLR regulator, the deubiquitinating enzyme A20, to inhibit the TLR-2-mediated pro-inflammatory gene expression, consequently suppressing IL-12 and TNF-α production (147). It was also described that *L. donovani*, along with *L. mexicana* and *L. major*, uses the macrophage tyrosine phosphatase SHP-1 to inactivate kinases involved in TLR signaling (148). As happens with TLR-2, *Leishmania* exploits host TLR regulators to deal with TLR-4 activation. Gupta et al. showed that *L. donovani* parasites alter the ubiquitination pattern of TRAF3, preventing its degradation, which is required for the effective cytosolic translocation of the TLR-4-anchored multiprotein complex. As a consequence, NF-κB is silenced leading to a downregulation of IL-12 and TNF-α production (149). Furthermore, *L. amazonensis* amastigotes can suppress TLR-4 activation on DCs via rapid degradation of intracellular signaling proteins (JAK/STAT, NFκB, and IRF) leading to a decrease in IL-12 production (150). The deubiquitinating enzyme A20 also has a role in the inhibition of the TLR-4-mediated pro-inflammatory response. However, in this case, the regulation is an indirect consequence of active disease promoted by the high levels of TGF-β that infected cells produce (151). Another "macrophage imbalance" mediated by TLR-4 signaling manipulation was described by Shweash et al. These authors reported that *L. mexicana* promastigotes are able to prolong and enhance PGE_2, NO, and arginase production through TLR-4, and consequently achieve the reduction of macrophage released IL-12 (152). Finally, *Leishmania* can impair TLR signaling through prevention of receptor ligand interaction. Here, the player is ectoin-like serine peptidase inhibitor, produced by *L. major*, which inhibits neutrophil elastase and consequently prevents TLR-4 activation (153, 154). Ultimately, TLR-4 signaling inhibition in macrophages induces an

Table 3 | Strategies of TLR signaling modulation by *Leishmania*: an overview.

TLR	Species	Key player	Mechanism of modulation	Reference
TLR 2	*L. donovani*	Unk	Shift to Th2 immune response	(143)
	L. donovani	Deubiquitinating enzyme A20	Inhibition of TLR-mediated pro-inflammatory gene expression	(147)
	L. donovani			
	L. mexicana	SHP-1	Inhibition of TLR-mediated pro-inflammatory gene expression	(148)
	L. major			
	L. major	LPG	Downregulation of TLR-9 expression	(145)
TLR-4	*L. amazonensis*	Unk	Degradation of intracellular signaling proteins	(150)
	L. donovani	Deubiquitinating enzyme A20/SHP-1	Inhibition of TLR-mediated pro-inflammatory gene expression	(151)
	L. major	Ecotin-like serine peptidase inhibitor	Shift to Th2 immune response	(154)
	L. mexicana	Unk	Enhancement of PGE_2, NO, and arginase production	(152)

LPG, lipophosphoglycan; NO, nitric oxide; PGE_2, prostaglandin E_2 SHP, sarcoma homology 2 domain phosphatase-1; Th, T helper; TLR, toll like receptor; Unk, unknown.

M2b phenotype that correlates with higher IL-10 levels and a Th2-type immune response (154). **Table 3** collects the data discussed above.

INFLUENCING CHEMOKINE PRODUCTION

As an intracellular pathogen, *Leishmania* depends on the initial recruitment of host cells for successful establishment and perpetuation of infection. Chemokines are small proteins that induce and regulate the migration of immune cells, and their expression is known to be modulated by *Leishmania* spp. (41, 155). Several studies reported the upregulation of numerous chemokines (RANTES/CCL5, MIP-1α/CCL3, IP-10/CXCL10, MCP-1/CCL2, MIP-1β/CCL4, MIP-2/CXCL1, and IL-8/CXCL8) after *L. major*, *L. donovani*, *L. tropica*, *L. infantum*, and *L. panamensis* inoculation (156–161). Interestingly, few of these chemokines attract neutrophils, which can be another *Leishmania* mediated immune modulation strategy. Although neutrophils may be a possible vehicle for *Leishmania*, facilitating infection, it was described that exacerbated neutrophil recruitment is associated with parasite killing (162). On the other way, it was also shown that skin lesions of *L. major* infected mice mainly contained Th2 cell-attracting chemokines, such as CCL7 (163, 164). The absence of Th1 cell-attracting chemokines in these lesions may reflect the downregulation of the expression of genes linked with Th1 trafficking, such as the ones coding for CXCR3 chemokines (165). Last but not least, it was described that *Leishmania* may also profit from malnutrition to impair chemokine secretion and to establish infection (158, 166). Interestingly, differential expression of chemokines induced by distinct parasite strains leads to various infection and disease outcomes. As an example, human infection with *L. mexicana* may lead either to a self-healing cutaneous form or to a non-healing cutaneous disease, associated with the increased expression of CCL2 and CCL3, respectively (167). This differential chemokine expression was also seen in human infection with *L. panamensis* (168), and may be related with parasite virulence, once in a mouse model infected with two strains of *L. braziliensis* (highly virulent versus less virulent) a differential chemokine expression profile was observed (169). Elaboration of these studies would be of great interest, particularly regarding the parasite virulence factors responsible for the induction of the chemokine profiles seen in non-healing/severe pathologies, which will unveil new parasite immunomodulatory players.

INTERFERING WITH CYTOKINE PRODUCTION

Although cytokines are important throughout the whole *Leishmania* infectious process, they are fundamental during the acquired immunity phase. IL-12 is mainly produced by APCs, particularly by DCs (170), and is related with important cytokines that mediate very different outcomes of *Leishmania* infection, such as IFN-γ, IL-10, and IL-4. Therefore, the interference with IL-12 is a recurrent phenomenon in *Leishmania* infection. *Leishmania major* was found to deplete cholesterol, inhibiting the assembly of an IL-12-inducing CD40 signalosome and modifying the cell effector functions (171). Others have reported that *L. major* infection directly down-regulates IL-12 production through a CD40 signaling-regulation (172). Furthermore, *L. mexicana* and *L. donovani* were also found to impair LPS-induced IL-12 production by BMM through cysteine proteinase mediated NF-κB degradation (173, 174). Others have correlated IL-12 downregulation with *Leishmania* evasion mechanisms, probably through PI3K/Akt signaling pathway modulation (175–179). In a recent study, *Batf3*$^{-/-}$ mice, that lack the major IL-12 producing and cross-presenting subsets CD8α^{+} and CD103^{+} DCs, showed enhanced susceptibility to *L. major* infection partially due to reduced IFN-γ and increased IL-4 and IL-10 secretion (180). IFN-γ is released by Th1 cells triggering the leishmanicidal activity of macrophages via expression of the inducible NO synthase which, in turn, leads to the killing of intracellular *Leishmania* (181). Thus, several reports on prevention of IFN-γ secretion and/or action by the parasite exist. Ray et al. showed that infection of macrophages with *L. donovani* causes a decrease in the phosphorylation of the IFN-γR-α subunit, which consequently affects the receptor expression (182). Furthermore, GP63 was related with reduction of IFN-γ producing cells in BALB/c mice infected with *L. amazonensis* (183). Finally, our group reported that the non-secreted *Leishmania* protein *Lm*S3arp is also associated with downregulation of IFN-γ production by splenocytes (184). It was described that regulatory T cells (Tregs) may have a role in the downregulation of IFN-γ, in a murine model infected with *L. amazonensis* (185). However, it

is yet to be unveiled whether and how parasites are able to control these cells. Furthermore, the role of Tregs in infection progression and pathology diverges, depending on the infecting *Leishmania* species. While Tregs are associated with disease exacerbation and parasite persistence, in the infection context with *L. donovani* and *L. major*, respectively, *in vivo* experiments with *L. amazonensis* shown that Tregs aid in disease resolution (185–188). Additionally, Ehrlich et al. demonstrated *in vivo* that both the transfer of Tregs to chronically infected animals with *L. panamensis*, and their treatment with rIL-2/anti-IL-2 Ab complex for Treg expansion contributed for disease amelioration, showing the protective role of Tregs in *L. panamensis* infection and a possible immunotherapeutical role of these cells (189). The immunosuppressive IL-10 has long been associated to visceral disease pathogenesis (190), being not only important in the establishment of infection but also during parasite persistence through the direct inhibition of Th1 cell development, preventing the resolution of the infection (191). In fact, IL-10 receptor blockade or IL-10 KO mice renders animals resistant to *L. donovani* infection (192, 193). The major source of IL-10 in both cutaneous and visceral leishmaniasis is controversial. Some works proposed T regs and Th2 lymphocytes as the main IL-10 producers (190, 194–197), while others claim that Th1 lymphocytes are the main IL-10 source (190, 194, 198–200). Notwithstanding, the parasite can also promote IL-10 production by other cells. For instance, *L. braziliensis* amastigotes and promastigotes induce the secretion of this cytokine by PBMCs (201). This IL-10 secretion was shown to be mediated by phagocytosis of opsonized parasites in an *in vivo* model of low dose infection with *L. major* (202) and also with *L. amazonensis* and *L. mexicana* (203, 204). The *Leishmania* secreted protein *Li*TXN1 is also involved in the promotion of IL-10 production by spenocytes (205). Apart from IL-10, IL-4 also induces Th2 responses (206) and is particularly involved in the promotion of cutaneous leishmaniasis. Tabatabaee et al. suggested that *L. major* secrete immunosuppressive factors that promote IL-4 production by lymphocytes (207). This cytokine was shown to interfere with the synergy of IFN-γ/FasL that contributes to macrophage activation and killing of intracellular *L. major* (208). There is, however, some contradictory studies showing that IL-4 promotes IL-12 production by bone marrow-derived DCs (BMDC) and resistance to the disease (209, 210). Hurdayal et al. clearly showed that DC specific IL-4 receptor alpha (IL-4Rα)-deficient BALB/c mice became hypersusceptible to *L. major* infection, due to a decrease in IL-12 and an increase in IL-10 production by DCs (211). These contradictory observations with IL-4 might be possibly explained by the fact that a low infection dose with *L. major* induces a Th2 response in C57BL/6 mice, whereas high doses induce a Th1 response, both dependent on IL-4 production by lymphocytes (212). Considering the fact that, in average, sandflies transmit not more than 1000 parasites per bite, an induction of Th2 response might be expected in a real situation (213).

Other cytokines have been studied in the context of *Leishmania* infection. IL-17, for instance, has been involved in the outcome of cutaneous leishmaniasis (214–216). Although there are not many studies showing *Leishmania* modulation of this cytokine, some clues exist about how this can happen. Castellano et al. showed that *L. amazonensis* antigens possibly induce a decrease in the percentage of $CD3^+CD4^+IL\text{-}17^+$ human cells, at least in cases of HIV/*Leishmania* co-infection (217). Interestingly, patients with signs of active disease present lower levels of Th17 cytokines (218, 219). Yet, more studies are needed to discover whether *Leishmania* can directly modulate IL-17 production or if it acts on other interlinked cytokines such as IL-6 and IL-23 (201, 216). IL-1β was also shown to influence the clinical course of leishmaniasis, and is strictly related with inflammasome activation, a general but powerful antimicrobial strategy in innate immunity (220). A recent study showed that *Leishmania* can prevent caspase-1-dependent IL-1β activation through a C-type lectin (SIGNR3) mediated signaling process, which consequently favors parasite persistence (221). The parasite key player responsible for this signaling modulation is, however, yet unknown. Finally, IL-13, IL-21, and IL-27 may also have a role in leishmaniasis, either preventing or inducing pathology (222–225).

IMPAIRING CELLULAR FUNCTION

Leishmania is able to control the acquired immunity through the impairment of effector cells function. Antigen processing and presentation by APCs is necessary for the efficient priming of effector T cells which, in turn, will generate a directed and specific immune response (226). Through phagocytosis of parasite debris or intracellular parasite degradation, APCs process and present *Leishmania* antigens (227). Both major histocompatibility complex (MHC) I and MHC II antigen presentation are related with *Leishmania* elimination, although only the second one is essential for complete parasite clearance (212, 228). *Leishmania* can interfere with antigen processing and presentation, consequently modulating once again the immune function.

LEISHMANIA INTERFERES WITH ANTIGEN PRESENTATION BY PROFESSIONAL CELLS

In 1987, Reiner et al. described that *L. donovani* decreases macrophage expression of both MHC I and MHC II molecules (229). Others have also reported a *L. major* related downregulation of MHC molecules in DCs (230), which can be mediated by direct parasite internalization of these molecules (231–233). Interestingly, *L. donovani* extracellular vesicles were shown to inhibit MHC-II expression in human monocyte-derived DCs (234). Furthermore, both *L. pifanoi* and *L. amazonensis* amastigotes interfere with the macrophage antigen processing process by sequestration of antigens from the MHC II pathway, through a mechanism involving targeted vacuolar fusion (235, 236). However, prevention of surface-expressed MHC class II-peptide complexes is not the only way by which the parasite impairs antigen presentation (**Figure 2**). *L. donovani* was shown to interfere with BMM antigen presentation by modulating the capacity of surface MHC class II-peptide complexes to engage the T cell receptor (TCR) (237). An increase in the infected cell membrane fluidity by cholesterol depletion and ceramide generation may justify this inefficient engagement (238, 239). Adhesion molecules are also important in the process of antigen presentation. They help during the initiation of contact between APCs and T cells, required for the subsequent formation of the immunological synapse. Bimal et al. reported that particularly $CD4^+$, but also $CD8^+$ T cells, from patients with active visceral leishmaniasis caused by *L. donovani* express

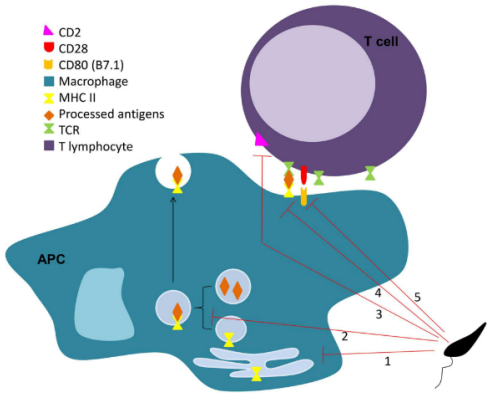


FIGURE 2 | *Leishmania* interferes on MHC II antigen presentation process. *Leishmania* impairs the antigen presentation process through several mechanisms. The parasite is responsible for the downregulation of MHC II in APC (1), sequestration of antigens from the MHC II pathway (2), limitation of the MHC II-peptide-TCR engagement (3), and down-regulation of co-stimulatory (4), and adhesion molecules (5) on APCs and lymphocytes, respectively.

less CD2 than the ones from healthy subjects (240). *In vitro* and *in vivo* studies must, however, be performed to confirm that this downregulation of CD2 in $CD4^+$ T cells is caused directly by the parasite. Co-stimulatory molecules are necessary for the full activation of T cells by APCs, which expression can be downregulated by *Leishmania*. For instance, Kaye et al. showed that BMM infected with *L. donovani* expressed lower levels of co-stimulatory molecules B7.1 and heat stable antigen than the non-infected controls (241). Mbow et al. also reported that Langerhan cells of BALB/c mice infected with *L. major* showed a down-regulation of B7.1 expression (242).

LEISHMANIA-INDUCED CELLULAR ANERGY AND EXHAUSTION

The lack of co-stimulatory molecules on APCs, particularly in DCs, can be a consequence of another immune modulation strategy used by *Leishmania*, the inhibition of cell maturation/activation. The induction of cellular unresponsiveness or anergy is the ultimate weapon that *Leishmania* uses in the fight against the immune system. Impairment of APC function was reported by our group. Briefly, BMDC infection with *L. infantum* promastigotes counteracts LPS-triggered activation. Parasites avoided the upregulation of transcription and surface expression of CD40 and CD86 co-stimulatory molecules on BMDC, through activation of the PI3k/Akt pathway and the impairment of NF-κB transcription factor (243). This DCs activation/maturation arrest was also described for *L. amazonensis* infection on mice and human cells (150, 217, 231, 244). *Leishmania* has also been associated with T cell exhaustion (245). Gautam et al. described that IFN-γ production by $CD8^+$ effector cells was absent in active human visceral leishmaniasis. These cells expressed elevated levels of Cytotoxic T Lymphocytes Antigen 4 (CTLA-4) and programed death protein 1 (PD1) (246), negative regulators of T cell activation associated with T cell anergy and exhaustion (247). Similar results were also reported by Esch and colleagues, regarding not only CD8, but also CD4 T cells (248). This topic was recently reviewed by our group regarding *Leishmania* and other parasitic infections (249).

CONCLUSION

Remarkable progresses were made in the past years in the knowledge of immunomodulation by *Leishmania*. As a result of a long parasite-host co-evolutionary process, this organism can escape or fight the immune system using diverse and complex strategies. However, the knowledge produced is sometimes dispersed and contradictory, reflecting several variables such as infecting species and different infection models. Notwithstanding, it is now clear that the parasite can modulate cell death, alter the maturation process of the phagolysosome, modulate cytokine, and chemokine production by host cells, and impair cell function, in order to silently enter in host cells and successfully differentiate and infect. Furthermore, *Leishmania* released material seems to have by itself some immunomodulatory potential. Therefore, the study of the parasite exoproteome may contribute for the discovery and characterization of the yet unknown arms that the parasite uses to achieve victory against the immune system. The unraveling of the agents responsible for this modulation will help us to define the requirements for infection and disease. This will ultimately become the cornerstone that will contribute to develop novel strategies to fight the disease. Although not discussed in this review, but not less important, the pressure that the parasite exerts in the host cells metabolism is now an area of growing interest. The nascent field of immunometabolism will also contribute significantly for the full understanding of the infectious process.

ACKNOWLEDGMENTS

This work was funded by FEDER funds through the Operational Competitiveness Programme – COMPETE and by National Funds through FCT – Fundação para a Ciência e a Tecnologia under the project FCOMP-01-0124-FEDER-019648 (PTDC/BIA-MIC/118644/2010). Pedro Cecílio, Begoña Pérez-Cabezas, and Nuno Santarem are supported by fellowships from the European Community's Seventh Framework Programme under grant agreements No. 603181 (Project MuLeVaClin), No. 603240-2 (Project NMTryPI), and No. 602773 (Project KINDRED), respectively. Joana Maciel is supported by a post-doctoral fellowship from the FCT project grant No. PTDC/BIA-MIC/118644/2010. Vasco Rodrigues is supported by a doctoral fellowship from FCT code SFRH/BD/64064/2009. The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript. The authors would like to acknowledge COST Action BM1202: European Network on Microvesicles and Exosomes in Health and Disease (Me-HaD).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 July 2014; accepted: 19 September 2014; published online: 20 October 2014.

Citation: Cecílio P, Pérez-Cabezas B, Santarém N, Maciel J, Rodrigues V and Cordeiro da Silva A (2014) Deception and manipulation: the arms of Leishmania, a successful parasite. Front. Immunol. 5:480. doi: 10.3389/fimmu.2014.00480

This article was submitted to Microbial Immunology, a section of the journal Frontiers in Immunology.

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A few recent studies posterior to the publication of the review above, still on the topic of parasite immunomodulation, deserve to be highlighted. The first one shows how the parasites can take advantage of the host cell redox machinery. *Leishmania donovani* parasites were recently demonstrated to use a host-cell detoxifying enzyme (Heme Oxygenase-1) to inhibit reactive oxygen species generation and downregulate host cytokine responses, and thus contribute to parasite survival (Saha *et al*, 2019). The remaining ones relate with the most studied subject on *Leishmania* immunomodulation, the impact on chemokines/cytokines. Interestingly, *L. major* parasites were demonstrated to directly cleave CXCL1 (*via* parasite proteases, independently of host-factors), inactivating its biological activity and therefore, to potentially escape or tune-down host innate immune responses (Yorek *et al*, 2019). Additionally, interleukin (IL)-27 was implicated in disease susceptibility in mouse models of VL (Perez-Cabezas *et al*, 2016; Quirino *et al*, 2016). The *Leishmania infantum* infection-induced IL-27, a result of the endosomal Toll-Like Receptors (TLR) induced type I interferon (IFN) response, was shown to act as a major regulator of the IL-17A-neutrophil axis, consequently promoting susceptibility to infection (Quirino *et al*, 2016). Although this response seems to be mouse-strain specific (infection-induced induction in BALB/c mice *versus* no change in C57BL/6 mice), the fact that active VL patients' present high serum levels of IL-27 makes these observations relevant to human disease (Perez-Cabezas *et al*, 2016). On the other hand, another cytokine, IL1-β, seems to have dual roles in disease outcome, depending on the parasite species studied (which is not difficult to imagine, recognizing the complete distinct physiopathologic contexts of the different leishmaniasis forms). Comparing two *Leishmania major* strains causing healing and non-healing disease, respectively, in a murine model of CL, Charmoy *et al.* showed that the disease susceptibility was mediated by IL-1β secretion (Nlrp3 inflammasome dependent) associated with localized neutrophil recruitment (Charmoy *et al*, 2016). The treatment of murine cutaneous lesions with IL-1β seems to support these findings (Patil *et al*, 2018), as also does the association of IL-1β with the severity of cutaneous and visceral disease in humans (Fernandez-Figueroa *et al*, 2012; Moravej *et al*, 2012; Costa *et al*, 2013). Still, other studies have shown that for *L. amazonensis, L. braziliensis* and *L. infantum* infections, inflammasome dependent IL-1β signaling mediate resistance to infection (Lima-Junior *et al*, 2013; Chaves *et al*, 2019).

3. The vector: the forgotten variable of the equation

The main vector of *Leishmania* spp. parasites are phlebotomine sand flies. Taxonomically, they are arthropods and insects included in the Diptera Order (two winged flies), Nematocera suborder, Psychodidae Family and Phlebotominae subfamily (Akhoundi *et al*, 2016). More than 900 sand fly species or subspecies are recognized and grouped within six genera: *Phlebotomus* (13 subgenera) *Sergentomyia* (10 subgenera), and *Chinius* (4 species) from the Old World, and *Lutzomyia* (26 subgenera and groups), *Brumptomyia* (24 species), and *Warileya* (6 species) from the New World (Akhoundi *et al*, 2016). Female sand flies (with the exception of a few autogenous species), apart from sugar, need to take a blood meal in order to develop and lay eggs. Importantly, the genera *Lutzomyia* and *Phlebotomus* are the ones that contain the anthropophagous species (although some *Sergentomyia* may also feed in humans) that are therefore relevant for human disease transmission (Lane, 1993).

Contrarily to mosquitoes, sand flies do not depend on water sources to complete their life cycle (ECDC, 2014). Still, humidity is an important factor that together with temperature are detrimental for sand fly development (Kasap and Alten, 2005; Erguler *et al*, 2019). This justifies the fact that sand fly distribution is restricted to areas that have temperatures above 15.6 ºC for at least three months of the year (ECDC, 2014), which still corresponds to the greatest portion of the world – from latitude 50ºN to latitude 40ºS (although they are absent from New Zealand and the Pacific islands) (Figure 6) (Killick-Kendrick, 1999).

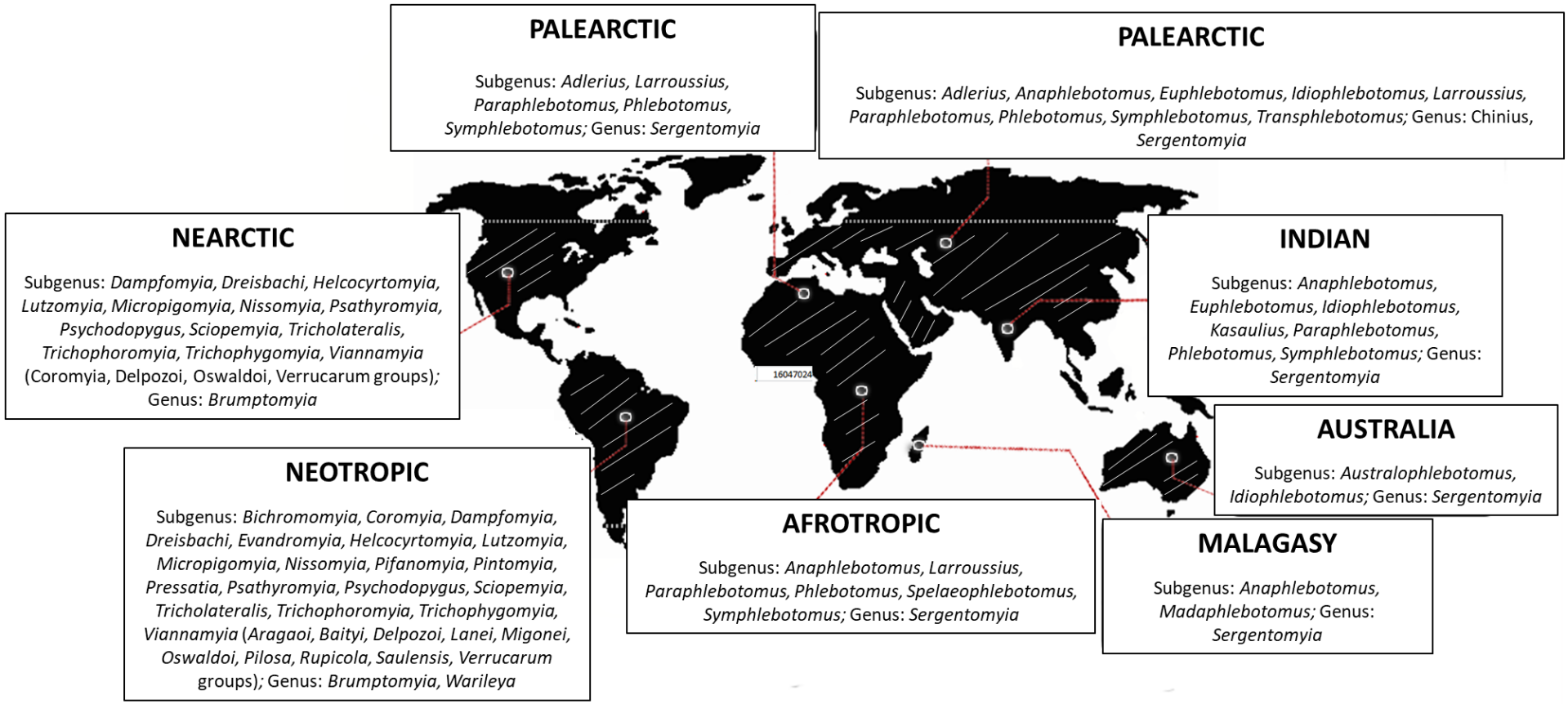


Figure 6. Sand fly distribution map by genus/subspecies. Global distribution between latitude 50ºN and latitude 40ºS (demarked by white horizontal lines), excluding New Zealand and the Pacific islands. Adapted from Akhoundi *et al*, 2016.

Sand fly life cycle contemplates 4 major stages: eggs, larvae, pupae, and adults (Figure 7). On average, a female sand fly deposits 30 to 70 eggs in places chosen based on humidity and presence of organic matter (e.g. cracks and holes in the ground, animal burrows/dens, thermite mounds, leaf litter…) (Killick-Kendrick, 2001; Service, 2012). Frequently, 4 to 20 days after oviposition, eggs hatch, although this timing is likely delayed in cooler weather (Service, 2012). There are four larval instars, and larval development is usually completed after 20 to 30 days, depending on the sand fly species, as well as temperature and availability of food. This period can however prolong to several months in sand fly species that diapause to cope with winter (only full grown larvae diapause - instar 4) (Killick-Kendrick, 2001; Service, 2012). Pupation usually takes from 6 to 13 days with the resultant emergence of adults during the hours of darkness, often just before dawn (Service, 2012). Males usually emerge before females. Adult life-expectancy in the wild has hardly been determined for sand flies, particularly considering females. Still, it is known that female sand flies normally undergo more than one gonotrophic cycle (Rutledge and Gupta, 2002). The timing of egg development is 5 to 8 days after blood-feeding, although some species are known to feed multiple times before properly developing the eggs (Rutledge and Gupta, 2002). Most sand fly species are exophagic (feed outside), although some are known to be endophagic and endophilic (feed and rest in houses), being therefore qualified as domestic or peridomestic species. A more detailed description of sand fly biology, behavior, and morphology (used to distinguish sand fly species) can be found on (Lane, 1993; Killick-Kendrick, 2001; Rutledge and Gupta, 2002; Service, 2012). Still, much remains less than clear regarding these subjects.

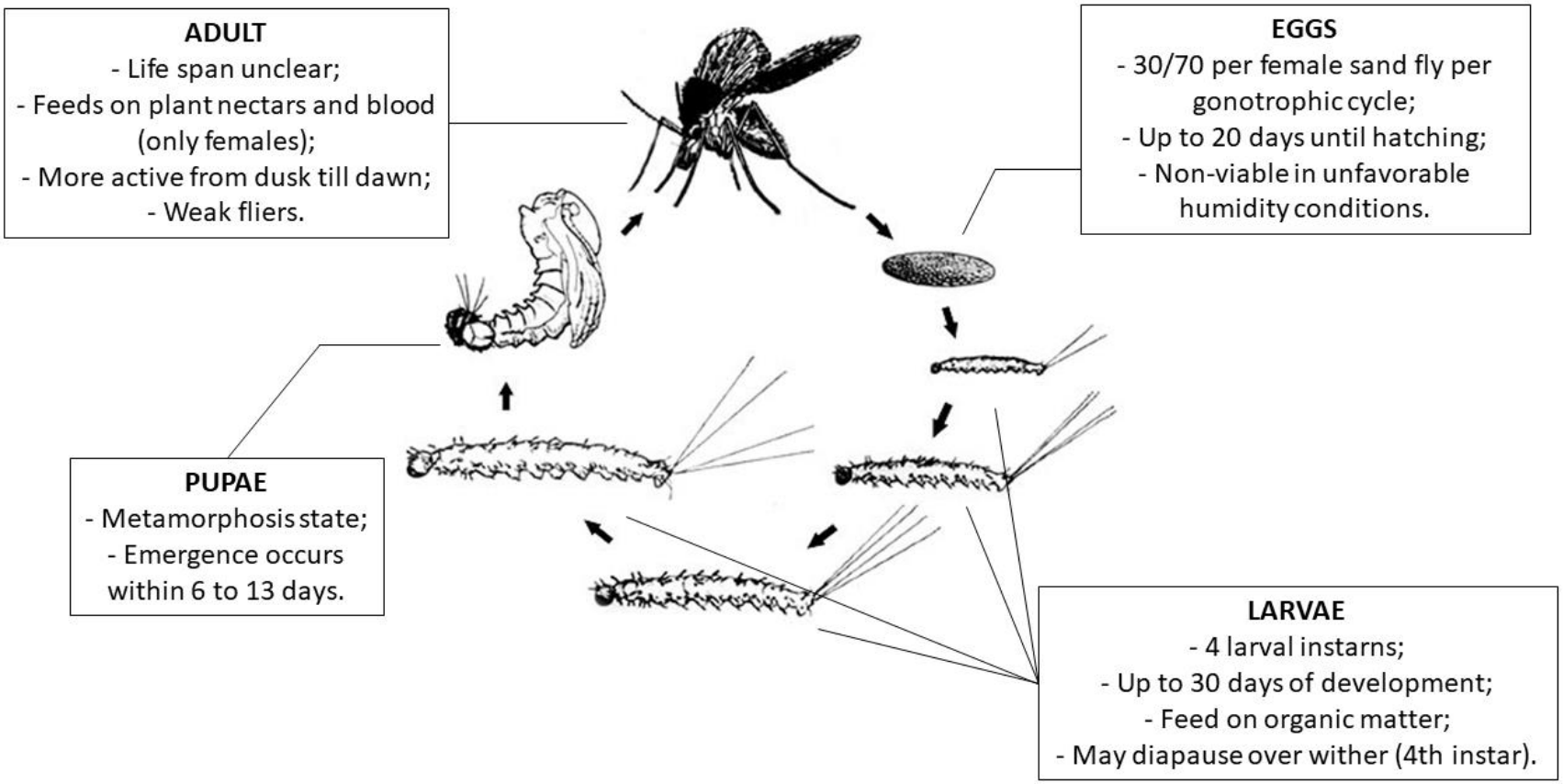


Figure 7. Schematic representation of sand flies life cycle. Adapted from Dillon 2008 and Akhoundi *et al*, 2016.

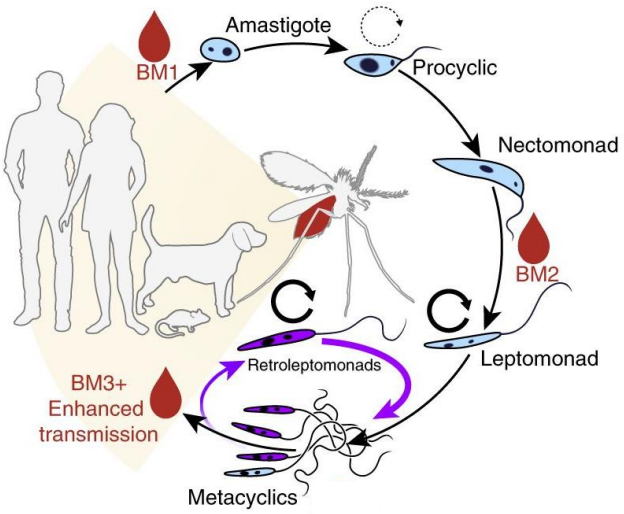
The need for blood to give rise to a new sand fly generation (and the perpetuation of the species), associated with the notion that sand flies are weak fliers (reports state that adults usually disperse 100 meters or less from their larval habitats), makes feeding-preferences relevant only in the context of high (and diverse) host-abundance (Service, 2012). In other words, many times feeding depends on host availability; sand flies will take blood from the closest permissive available source (although the engorgement outcome may vary) (ECDC, 2014; Gebresilassie *et al*, 2015). Contrarily to mosquitoes which are usually vessel feeders, sand flies are blood-pool feeders. They use their toothed mandibles in a scissors-like manner to lacerate the host's skin, disrupting cells and causing an extravascular pool of blood, from which they ingest the blood meal (Lane, 1993; USA_Department_of_Defense, 2015). Importantly, during this process, through salivation, sand flies introduce a number of pharmacologically active molecules into the skin, to facilitate feeding. The sand fly salivary proteome, although limited in number (usually less than 40 secreted proteins according to transcriptomic studies) is quite diverse considering function (Abdeladhim *et al*, 2014). While a vasodilator molecule promotes the increase of local blood circulation, a molecule with apyrase function inhibits platelet aggregation through destruction of the agonist adenosine diphosphate (APD), and together with molecules that inhibit the blood coagulation cascade and the classical pathway of the complement system, counteract an efficient hemostatic response (Abdeladhim *et al*, 2014). Additionally, other proteins have relevant immunomodulatory properties, that are however not important for the feeding process (Abdeladhim *et al*, 2014).

Although sand flies are mostly recognized as leishmaniasis vectors, they transmit other pathogens, such as bacteria and viruses. Carrion's disease is a sand fly transmitted biphasic illness caused by *Bartonella bacilliformis* bacteria in Central/South America (Pons *et al*, 2016). It is characterized by either intermittent febrile states (called Oroya fever) also with hepatic involvement that can lead to death in the absence or delay of treatment (when infected individuals are naïve); or by cutaneous lesions called Peruvian warts (when infected individuals were previously exposed to the bacteria) (Pons *et al*, 2016). Arboviruses are also human pathogens transmitted by sand flies, particularly the ones belonging to the *Phlebovirus* genus (order Bunyavirales, family Phenuiviridae), enveloped single-stranded RNA(-) viruses (Moriconi *et al*, 2017). At least 9 virus "species" are recognized, that contain 70 antigenically distinct viruses and exclude 33 other viruses, yet to be classified (Elliott and Brennan, 2014). Most of the sand fly transmitted viruses cause uncomplicated to moderate fever states known (when identified) as "sand fly fever" or "pappataci fever". However, one

particular agent, Toscana virus has a marked tropism for central and peripheral neurological systems, potentially causing neuro-invasive infections such as meningitis or encephalitis (Peyrefitte *et al*, 2005; Alkan *et al*, 2013; Howell *et al*, 2015; Moriconi *et al*, 2017).

Leishmania spp. - sand fly interactions studied in laboratory conditions led to the separation of sand fly vectors in two major groups: permissive and restrictive vectors (Volf and Myskova, 2007). As the names indicate, while the first group allows the development of distinct *Leishmania* species (e.g. *L. longipalpis* and *P. arabicus*), the second displays a remarkable specificity for the *Leishmania* species they transmit in nature (e.g. *P. papatasi* and *P. sergenti*) (Volf and Myskova, 2007). These differences are thought to be related to parasite attachment to the sand fly midgut, defined as an essential mechanism for infections to proceed within the sand fly (Kamhawi, 2006; Dostalova and Volf, 2012). However, while we understand these interactions in restrictive vectors (mediated by very specific ligand-receptor interactions), a lot is yet unknown regarding permissive ones (Dostalova and Volf, 2012). What we know in this regard considering restrictive (or specific) vectors comes from the study of *L. major* development within *P. papatasi* sand flies. In this context, parasites' attachment to the midgut is mediated by the binding of *L. major* Lipophosphoglycan (LPG) molecules to a specific sand fly midgut receptor, a galectin (β-galactoside binding family of lectins) (Kamhawi *et al*, 2004). Importantly, although LPG molecules are very abundant surface proteins, transversal to all *Leishmania* species, they are also polymorphic (particularly the 10–30 phosphoglycan repeating units) and different, not only considering parasite species but also parasite strain and even parasite stage (Kamhawi *et al*, 2004; Forestier *et al*, 2014). And these differences explain both vector restrictiveness, and the *L. major* parasites attachment-detachment processes to *P. papatasi* midguts, dependent obviously on the specificity of ligand-receptor interactions (Kamhawi *et al*, 2004). On the other way, vector permissiveness suggests broader or even non-specific binding processes. Although these processes are yet to be fully understood, the involvement of sand fly midgut "sticky proteins" is a considered hypothesis (e.g. O-linked glycoproteins with mucine-like properties) (Myskova *et al*, 2016).

The development of *Leishmania* parasites within the sand fly midgut is quite complex (Figure 8), with distinct differentiation processes that are required for the establishment of a successful infection.



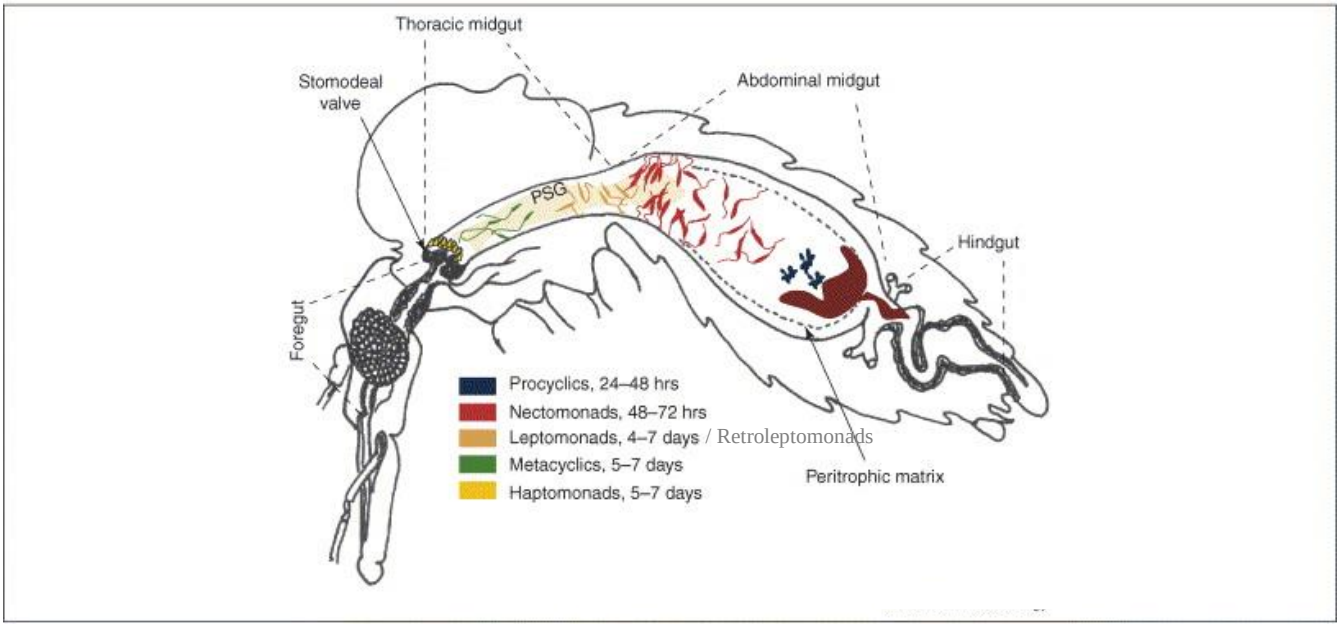


Figure 8. *Leishmania* development within the sand fly midgut: "life cycle" and representation of different parasite stages and their locations. Adapted from Kamhawi, 2006 and Serafim *et al*, 2018.

Although most of *Leishmania* species are suprapylarian (development restricted to the midgut), the ones from the subgenera *Viannia* enter the hindgut before migrating forward to the midgut (peripylarian parasites) (Nieves and Pimenta, 2000). The first differentiation step occurs not long after the ingestion of the infected blood meal by sand flies. Due to changes in conditions (such as the decrease of temperature and the increase in the pH), the amastigotes differentiate into procyclic promastigotes, weakly motile forms (Dostalova and Volf, 2012). This first differentiation step is extremely important, once the parasites within the blood meal need to resist the digestive proteases, which pose the first and one of the most significant barriers to parasite survival (Rogers, 2012). Importantly, procyclic promastigotes were demonstrated to be more resistant to proteolytic attack than the parasites in the transitional state (amastigote to promastigote forms) (Ramalho-Ortigao *et al*, 2010). This is probably due to the known dynamic changes of the parasites' glycocalyx components: e.g. comparing amastigotes with promastigotes, the latter have a higher content of LPG in their membrane (Rogers, 2012; Forestier *et al*, 2014). Procyclic promastigotes are also the first replicative forms found within the sand fly vector, increasing parasite numbers, important to the next step of the vector infection cycle (Dostalova and Volf, 2012). The blood meal is separated from the sand fly midgut by a type I peritrophic matrix. This structure found in blood fed insects has mainly a semi-barrier protective role: i) against the possible damage caused to midgut microvilli by the concentrated digestive environment; ii) against the potentially devastating effects of one of the blood digestion by-products, heme; and iii) against potential pathogens (Secundino *et al*, 2005). *Leishmania* parasites need to escape this structure in order to establish an infection. Around 48 hours after the blood meal ingestion, procyclic promastigotes begin to slow their replication and differentiate into strongly motile and long forms called nectomonad promastigotes. These developmental forms are the ones that escape from the peritrophic matrix into the midgut lumen (Dostalova and Volf, 2012). Furthermore, these parasite forms are also responsible for the attachment to the sand fly midgut, another crucial step for the completion of *Leishmania* cycle within the vector, since it prevents the parasites to be eliminated together with the blood meal remnants when they are defecated (Wilson *et al*, 2010; Dostalova and Volf, 2012; Sadlova *et al*, 2018). The life cycle then continues with the migration of nectomonad promastigotes towards the anterior midgut and their differentiation into leptomonad promastigotes. These shorter parasite forms are another replicative insect stage and are responsible for the population of the sand fly anterior midgut as well as the secretion of promastigotes secretory gel (PSG), important for the transmission process

(Rogers *et al*, 2002; Gossage *et al*, 2003). Eventually, and because the increase of parasite numbers has as a consequence the limitation of nutrients availability, the leptomonads undergo another differentiation step called metacyclogenesis giving rise to the parasite stage infective to vertebrate hosts, the metacyclic promastigote (Ramalho-Ortigao *et al*, 2010; Dostalova and Volf, 2012). Although the metacyclogenesis determinants are yet to be fully understood, the nutritional deprivation hypothesis (and probably a resultant quorum sensing mechanism) makes sense and is supported by at least a study that shows that the absence of purines promotes parasite differentiation to metacyclic forms (Serafim *et al*, 2012). Metacyclic parasites have a smaller body, and a long flagellum, responsible for their extremely fast motility (Ramalho-Ortigao *et al*, 2010). Additionally to metacyclics, leptomonad promastigotes are thought to attach to the sand fly stomodeal valve and give rise to the haptomonad promastigote forms, the less studied vector-derived parasite form, whose function is yet to be disclosed (Sunter and Gull, 2017). Importantly, more than morphologically, all these parasite stages were demonstrated to be transcriptionally distinct (in different extents) (Coutinho-Abreu *et al*, 2019).

Up to two years ago metacyclic promastigotes were thought to be terminally differentiated parasite forms. In nature, sand flies are expected to take a blood meal every 5 to 6 days, to complete as many gonotrophic cycles as possible (Ready, 2013). Consequently, the nutrient deprivation environment occurring within the sand fly midgut (in the obvious context of *Leishmania* infection) is expected to be transient. This notion consequently changes the traditionally envisioned *Leishmania* cycle within the vector (linear) that needs to be adapted to the ecological reality (with an associated dynamicity). Serafim *et al.* showed the consequences of a second blood meal (non-infected) in experimental sand fly infections. The most important one led to the breaking of the dogma that metacyclics are the last stage, considering *Leishmania*-vector parasite forms. The revisited vector life cycle includes a new parasite stage, called retroleptomonads, originated by the de-differentiation of metacyclic parasites in the context of newly available nutrients, a consequence of blood intake by infected sand flies (Serafim *et al*, 2018). As the name indicates, this newly described parasite form is morphologically closer to leptomonad promastigotes, as it appears to be functionally: contrarily to metacyclics and similarly to leptomonad promastigotes, retroleptomonads are replicative forms (Serafim *et al*, 2018). Eventually, when the stress conditions are re-established in the midgut (with the defecation of the second blood meal remnants), retroleptomonad parasites “differentiate back” to metacyclic promastigotes (Serafim *et al*, 2018). Importantly, retroleptomonad replication has as a consequence better vector

infections, both quantitatively (increased parasite numbers per midguts) and qualitatively (more homogeneous populations of metacyclic promastigotes) (Serafim *et al*, 2018). These findings, aligned with the ecological context, may suggest that the development of a successful infection in wild sand flies is a gradual process, dependent on parasite amplification boosted by the intake of multiple blood meals by the sand fly vector (Serafim *et al*, 2018). Even if initially the parasite numbers are residual, the intake of a second blood meal five days after the ingestion of *Leishmania* amastigotes will boost leptomonads replication, increasing parasite numbers up to a point that favors differentiation to metacyclics (a third blood meal may be necessary, depending on the initial infectious inoculum). Still, this may not be enough to originate a productive infection, known to be dependent on the infectious inoculum (at least experimentally) (Loeuillet *et al*, 2016; Serafim *et al*, 2018). Nevertheless, the de-differentiation of metacyclic promastigotes into replicative retroleptomonads upon the intake of subsequent blood meals by infected sand flies will potentiate the development of "better" infections and increase vector competence up to a point that transmission is the most likely scenario (assuming the sand fly survives long enough).

Considering *Leishmania* spp. transmission to vertebrate hosts, the infectious inoculum is not exclusively constituted by metacyclic parasites. The deposition of metacyclics into the host skin is dependent on their regurgitation by the sand fly vector. Importantly, this process is probably the result of a clever adaptation of *Leishmania* spp. parasites. The filamentous proteophosphoglycans secreted by leptomonad promastigotes form a gel-like plug called PSG, as mentioned above, that difficult the sand fly feeding process. Because the infected sand fly digestive tracts are clogged, in an extent dependent on parasite burdens [the more parasites, the bigger the plug (Serafim *et al*, 2018)], to facilitate blood intake (trying to unclog the anterior midgut), sand flies regurgitate (Bates, 2007). This way, mostly metacyclic promastigotes are egested into the host skin (in the context of a mature sand fly infection), but not alone. We know today that the infectious inoculum is composed of many relevant factors, both parasite and vector-derived. The *Leishmania*-derived proteophosphoglycans, part of the PSG plug, that are regurgitated together with parasites, were demonstrated to contribute to disease exacerbation (in the context of both CL and VL) (Rogers, 2012). A possible mechanism proposed was the modulation of early innate pathways involved in response to a wound. PSG was shown to potentially accelerate wound healing in the skin (Giraud *et al*, 2018), which in turn is known to be by itself a potential infection-enhancer stimulus (Baldwin *et al*, 2007; Abdoli *et al*, 2017). Also parasite-derived exosomes were

shown to be part of the infectious inoculum, and to potentiate disease, in both CL and VL animal models (Atayde *et al*, 2015; Perez-Cabezas *et al*, 2019). Alteration of cell recruitment patterns, with modulation of cell behavior, were mechanisms shown to be involved in exosome-mediated potentiation of infection (Atayde *et al*, 2015; Perez-Cabezas *et al*, 2019). In respect to the vector-derived infection enhancers, both sand fly gut microbiota and sand fly saliva were demonstrated to have a role. Equally to every known gastrointestinal tract in nature, sand fly midguts harbor a diverse microbiological community. Importantly, the colonization of the vector intestinal tract was shown to be extremely important for *Leishmania* parasites development within the sand fly (Kelly *et al*, 2017). Additionally, these sand fly bacterial midgut colonizers were shown to be co-egested together with *Leishmania* parasites and the abovementioned parasite-derived infection enhancers at the moment of transmission, additively contributing to disease establishment (Dey *et al*, 2018). The egested microbes were shown to trigger the inflammasome, leading to a rapid production of IL-1β and a sustained neutrophil infiltration at the site of the vector bite (Dey *et al*, 2018). Neutrophils' essential role in *Leishmania* infection-establishment was shown for *L. major* parasites more than a decade ago (Peters *et al*, 2008), although some studies point to an infection-protective role (Carlsen *et al*, 2015). All of these midgut-derived immunomodulatory factors (and *Leishmania* parasites) will join the sand fly saliva in the host skin. And more than to impact host hemostasis, sand fly saliva was shown to modulate host immunity. Sand fly salivary proteins were shown to favor (in most contexts) anti-inflammatory local immune responses, benefic for parasites' establishment, and consequently, to potentiate infection and disease (Gomes and Oliveira, 2012).

4. Disease control: towards the ultimate goal of transforming neglected into negligible

The global reach and burden of human leishmaniasis justify the need for disease control. However, due to the epidemiological complexity of this spectrum of diseases, that is not an easy task to accomplish. If we think on a "common anthroponotic human infectious disease" (e.g. airborne human-to-human transmission), the focus required is limited to the causative agent, the host, and their interactions. Therefore, only two populations must be considered, the afflicted individuals, and the non-afflicted, but at risk of developing infection/disease, being the ultimate disease control goal the decrease of the first and the increase of the second populations (thinking simplistically without considering immune status). The early identification of active cases (through appropriate diagnosis) and their management (quarantine; treatment…), if properly performed (having obviously in consideration transmission patterns and timings), many times are enough to contain an epidemic (Syme, 2007; Lima *et al*, 2015; WHO, 2018b). Decrease of the populations at risk, on the other way (e.g. through vaccination), is also an effective measure that should be applied in parallel with the previous one to promote the essential decrease in disease incidence, and consequently, achieve disease control (Goldstein *et al*, 2017). The facts that leishmaniases are both vector-borne diseases and mainly zoonosis, obviously contribute to the increase of the variables to consider when we think of disease control.

Although proper diagnosis [revised by (Sundar and Rai, 2002; Burza *et al*, 2018; Ibarra-Meneses *et al*, 2020)] and treatment [revised by (de Menezes *et al*, 2015; Goncalves *et al*, 2019; Souto *et al*, 2019)] in leishmaniasis (even with all the recognized limitations that deserve to be overcome) are as important as for any infectious disease, since they are the main means to tackle disease burden, considering the already infected individuals (decrease mortality rates, prevent or diminish morbidity), their effect in terms of disease incidence are limited (Biswas *et al*, 2017; Kamhawi, 2017), since parasite circulation is perpetuated by the interaction of sand flies with the many animal reservoirs of *Leishmania* parasites. There is obviously one great exception, VL in the Indian subcontinent, where *Leishmania donovani* transmission is anthroponotic, as far as we are concerned (Singh *et al*, 2016). This is one of the main reasons why WHO and partners chose this region for the application of VL elimination campaigns (WHO, 2010; Uniting_to_Combat_NTDs, 2012) - with the final goal

to reduce annual disease incidence to <1 per 10.000 cases per epidemic block by 2020, values expected to be met according to some mathematical models (Le Rutte *et al*, 2017). Still, we have to keep in mind that these campaigns combined surveillance and control not only of disease, but also of the vector, being the focus on the vector the major contributor for long-term control outcomes, at least according to some predictions (Stauch *et al*, 2011; Le Rutte *et al*, 2018).

If even in the case of anthroponotic disease, vector control is important, considering zoonotic leishmaniasis, disease control is only potentially achievable through combined vector and reservoir control. Typical sand fly control approaches rely on the use of insecticides/repellents. Frequently these approaches are used in houses and animal shelters (insecticide residual spraying) to reduce the incidence of sand fly exposures indoors, and are better effective in the urban *versus* non-urban contexts (Alexander and Maroli, 2003). In sylvatic areas, the same spraying approach can be used in sand fly resting areas (intrinsically more difficult thinking on coverage constraints). Additionally, the spraying can be combined with a barrier method, such as the use of bed-nets that can even be insecticide-impregnated (Alexander and Maroli, 2003). Importantly, systematic reviews point to an association between these methods and decrease at least in CL incidence (Gonzalez *et al*, 2015). Additionally, less conventional approaches can be used (most of them still with at least minimal applicability), such as bait and trap tactics (e.g. based on the pheromone-induced attraction of sand flies) (Alexander and Maroli, 2003; Andrade *et al*, 2008), paratransgenesis (Hurwitz *et al*, 2011a; Hurwitz *et al*, 2011b), or even the introduction of genetically modified sand flies in the field, either sterile, or more resistant (or even refractory) to infection, strategy mostly studied for mosquitoes (Carvalho *et al*, 2015). Pertaining to reservoir control, the main efforts have turned to dogs, responsible for the perpetuation of *L. infantum* parasites both in South America and in the Mediterranean basin (Dantas-Torres, 2007). The most common measure applied (and recommended) is the use of pyrethroid impregnated collars that prevent the potential transmission of disease, but not necessarily infection of the animal (Leite *et al*, 2018). Interestingly, studies show that this measure may be sufficient to reduce disease incidence in humans (Courtenay *et al*, 2019). In parallel, a more controversial measure is still applied in several countries, the euthanasia of seropositive dogs, although more and more pieces of evidence support the failure of dog culling as an effective control strategy (Dantas-Torres *et al*, 2019). One of the main reasons is the existence of potential alternative reservoirs that may contribute to the perpetuation of *Leishmania* parasites. This aligns with the main limitation of both reservoir and vector

control, particularly (but not only) in non-urban areas. Most of the *Leishmania* reservoirs are wild animals, meaning that *Leishmania* life cycle is maintained in the wild, a “natural barrier” to the adaptation of any control measure. Consequently, there will always be a probability of human exposure to sand flies, and potentially to *Leishmania* parasites.

Therefore, it is recognized by the scientific community that the most logical way (and maybe the only one) to achieve disease control is by decreasing the population at risk. In other words to convert susceptible into resistant individuals, which can be achieved through vaccination, discussed in the published book chapter “Vaccines for human leishmaniasis: where do we stand and what is still missing?” (Cecilio *et al*, 2018).

Chapter 5

Vaccines for Human Leishmaniasis: Where Do We Stand and What Is Still Missing?

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75000

Abstract

Responsible for up to 30,000 deaths annually, leishmaniasis is a complex spectrum of diseases endemic in 97 countries around the globe. Disease control relies heavily on the early diagnosis and treatment of the active cases (relevant for anthroponotic disease), although it is widely accepted that a prophylactic vaccine for human leishmaniasis is the way to achieve the successful elimination of human disease (taking in consideration the vast list of non-human reservoirs that enable the perpetuation of parasites all around the globe). The notion that infection leads to strong and long-lasting immunity against leishmaniasis supports vaccination as an achievable goal. However, and in spite of the different candidates tested along the years, till date, we still do not have an approved vaccine for humans. In this chapter, we will explore the last advances made in the field of vaccines against *Leishmania* without forgetting the historical perspective, essential to the understanding of the road already undergone. We will then discuss the correlates of disease and protection, still neither consensual nor definitive, as well as the issue of pre-clinical to clinical translation. The complete understanding of these issues will be essential for the approval of a successful vaccine for human leishmaniasis.

Keywords: leishmaniasis, human vaccines, correlates of protection, cellular immunity, cross-protection

1. Introduction

Vaccination is undoubtedly one of the greatest achievements of modern medicine, responsible, together with the use of antimicrobials and access to clean water and sanitation, for the global human demographics transformation in the past two centuries [1–3]. The apparently

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insignificant proportion of the world population, whose lives are spared annually, thanks to vaccines (0.04 or 0.1%, if we include deaths avoided by smallpox eradication), is equivalent to up to 3 (or 8) million lives spared per year and a cumulative of more than half a billion deaths avoided just in the twentieth century [4, 5]. Nevertheless, and notwithstanding the significant and successful global efforts toward the goal of universal health protection/promotion, the picture could be much better. On the one hand, just by improving global vaccination coverage, an additional 1.5 million deaths could be avoided yearly [5]. On the other hand, there are still many deadly infectious diseases, whose prevention through vaccination is theoretically possible but for which there are no vaccines approved [6–8]. There are different compatible explanations/hypothesis that together justify it. The first one has to do with legal and ethical reasons: to test/approve/administer a pharmaceutical product nowadays is harder than it was 100 years ago [9]. Also in a chronologic point of view, it is not surprising that there are still no vaccines available for emerging diseases (e.g., Zika or MERS-CoV) [10, 11]. Other reasons have to do directly with the convergence of the nature of the pathogens with the evolution of vaccine technologies [12]: (i) almost all vaccines available till date are humoral based, which is not the best option against intracellular pathogens (e.g., *Leishmania* spp., *Trypanosoma cruzi*) [13, 14] and (ii) there are pathogens with immune-evasion strategies dependent on high antigenic variability that poses a challenge in vaccine development [6, 15]. Lastly, but not least important, there are diseases more relevant from an economic standpoint than others: many diseases for which there are still no vaccines available affect almost exclusively the poorest of the poor (neglected tropical diseases—NTDs) [8, 16, 17].

Fortunately, with the arrival of the new millennium, WHO/UN initiatives such as the Millennium Development Goals (Goal 6, Target 3) and more recently the Sustainable Development Goals (Goal 3, Target 3.3) contributed to an increase in the awareness on the NTDs and consequently the investment on strategies to control them [18, 19]. The best example of concrete measures undertaken to "end the neglect" is given by the London Declaration on NTDs, signed in 2012 by 20 parties (including governmental organizations, non-profits and pharmaceutical companies) and endorsed thereafter by many others, that proposes to meet the goals set by the WHO Roadmap to overcome the global burden of NTDs (2012–2020), that include the elimination of five diseases and the control of five others. One of the potential short-term controllable NTDs is the fatal form of leishmaniasis [20].

Endemic in 97 countries around the globe, leishmaniasis is a complex spectrum of diseases [21, 22]. The first layer of complexity is given by its vector-borne nature, which introduces an extra variable (the phlebotomine vector) to the binomium host pathogen. The second one is given by the 20 *Leishmania* species known to cause human diseases (usually in a species/disease-manifestation-specific fashion), which is mostly but not exclusively of zoonotic origin (there are no animal reservoirs recognized for *L. donovani*) [23]. The third one relates to the infectious process, which frequently does not lead to an overt disease but instead to a chronic and "benign" asymptomatic state [24]. These are some of the main challenges to consider within the topic of disease control, which relies heavily on the early diagnosis and treatment of the active cases (whose influence in the diminishment of disease incidence should be relevant in anthroponotic *versus* zoonotic leishmaniasis) [25, 26]. Although till date there is no vaccine available against human leishmaniasis, not only is it widely accepted that the development of an effective vaccine is possible but also it is recognized that vaccination is the only viable option to achieve zoonotic disease elimination [25].

http://dx.doi.org/10.5772/intechopen.75000

With this chapter, we propose to explore the broad anti-*Leishmania* vaccines field, with humans as the focus population. Starting from a historical perspective, we will clarify where we stand today by discussing the different candidates and approaches followed along the years, situating them in the vaccine development pipeline. Additionally, we will debate what is missing (focusing mainly, but not only, on the correlates of protection and the disease models) as a way to substantiate why currently there are no vaccines against leishmaniasis approved for humans.

2. Vaccines for human leishmaniasis: where do we stand?

2.1. Leishmanization as the proof of principle of vaccines against leishmaniasis

The close relation of the human host and *Leishmania* parasites is quite ancient: there is evidence of parasite genetic material (identified retrospectively) in mummies from the year 2000 B.C. [23, 27]. However, the major breakthroughs in the leishmaniasis field were only achieved starting from the beginning of the twentieth century, with the identification of the causative agent(s), the incrimination of the vector(s), and consequently the understanding of parasite(s) life cycle and the distinct physiopathologic mechanisms that characterize each of the leishmaniasis forms [23, 28]. The definitive allocation of leishmaniasis within the infectious (or communicable) diseases, in convergence with the "success of variolation" and the birth of vaccination [29], boosted the investigation of the anti-*Leishmania* immune response envisioning the development of an effective prophylactic approach. The first reports date from early 1900 and are based on either contemporary common "medical practices" from Old World Cutaneous Leishmaniasis (CL) endemic countries or directly on evidence produced in human clinical trial-like studies [30, 31]. The general conclusions of these pioneer "vaccine studies" that used as inoculum either material from CL patient's ulcers or live parasites collected from *in vitro* cultures (*L. tropica*) were (i) only the individuals that developed a lesion and then self-healed were resistant to reinfection and (ii) reinoculation of immune individuals led to what is nowadays known as Type I delayed type hypersensitivity (DTH) reaction [30, 31]. Such studies established the dogma accepted today by the scientific community—"previous infection leads to robust immunity against *Leishmania*"—and were the proof of principle of the only prophylactic approach clinically used against leishmaniasis known as leishmanization.

Leishmanization was no more than the controlled induction of the cutaneous disease to prevent the consequences of natural infection, such as the scarification of exposed body parts (particularly the face) and the consequent life-long psychosocial impact and simultaneously to decrease the disease incidence in hyperendemic areas [32, 33]. In the 1970s and 1980s, several trials were performed using live virulent *L. major* parasites with promising results (up to 80% efficacy, **Table 1**) [32, 34–36]. This vaccine approach was accepted in countries such as the former Soviet Union, Iran, Israel, and Uzbekistan [32, 36, 37]. However, it was generally abandoned (with the exception of Uzbekistan, where it is still a licensed approach according to the most recent reviews on the field [34, 36, 38]) due to a number of concerns such as: (i) some individuals (1–2/10,000 inoculations) developed non-healing lesions, hard to resolve with chemotherapy [32, 39]; (ii) live vaccines (even the attenuated) are contraindicated to immuno-suppressed individuals [40] (whose worldwide prevalence has increased in the modern days, due not only to the HIV pandemic but also, for instance, to the increase of organ transplantation

procedures [41]); (iii) batch-to-batch variability issues of such complex immunogens raise reproducibility concerns [36, 42]; and (iv) complex logistics are usually associated with live vaccines [42].

2.2. An overview of the vaccine candidates against human leishmaniasis explored since leishmanization until the present day

The knowledge produced by leishmanization trials and campaigns conducted at the end of the last century is the most important evidence that the development of a vaccine against leishmaniasis is quite far from being impossible. The quest for such an essential pharmaceutical, indispensable for the achievement of global disease control, has been continuous (in a scale proportional to the funding for NTD research) and fruitful if we consider the number of candidates and different approaches tested. Here we will separate them into five major groups: live vaccines ("leishmanization like"), first-, second-, and third-generation vaccines, and vector-derived vaccines. **Table 1** compiles the information to be discussed in the next subheadings, presenting not only the different candidates/approaches tested along the years but also the disease form they were destined to prevent, their placement in the vaccine development pipeline, and the main findings reported.

2.2.1. Live vaccine candidates

The success of leishmanization is still used to support the investigation of vaccine approaches based on live parasites (called by some as leishmanization revisitation [38]), that according to the authors have the advantage of at least partially reproducing the normal infectious process (and consequently induce a "close-to-natural" anti-*Leishmania* memory) [43]. This includes for some candidates the long-term parasite persistence in the site of inoculation that will continuously boost the immune system and prevent the loss of immunity to reinfection [44–46]. The first two approaches explored, relying on parasite persistence as the key to effectiveness, are readaptations of leishmanization directed toward the prevention of visceral disease and proposed the controlled infection with either virulent *L. major* parasites or with a virulent but dermotropic *L. donovani* strain to promote heterologous or homologous protection against visceral disease caused by viscerotropic *L. infantum* or *L. donovani* strains, respectively [38, 47–49]. Still, both approaches, although shown effective in the pre-clinical context, will unlikely proceed in the vaccine development pipeline, mainly due to the safety concerns always raised by the use of virulent pathogens. As a way to partially overcome this barrier, different live vaccine approaches proposed the use of attenuated parasites, that would still mimic the natural infection (although in a sub-clinical form) and induce anti-*Leishmania* memory but in most of the cases would then be completely eliminated. In the pre-genomic era (but not only) chemically and physically attenuated parasites were shown to be effective, in pre-clinical trials, against CL, muco-cutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL) [50–52]. These attenuation approaches that did not assure a homogeneous parasite population (with an unpredictable potential of reversion to the virulent form) were almost completely replaced by the genetically modified parasites in the post-genomic era. Two main groups of genetically modified *Leishmania* parasites were used in the pre-clinical context: loss-of-function mutants

Type	Approach/vaccine candidate(s)	Disease form	Vaccine development pipeline	Efficacy/outcome	Reference
	Inoculation of live, virulent *L. major* parasites: Leishmanization	CL	Effective clinical use in the former Soviet Union, Israel and Middle East* (discontinued)	About 80% efficacy	[31, 34, 36]
	Heterologous protection mediated by inoculation of live, virulent *L. major*	VL	Pre-clinical studies in mice	No effect in BALB/c mice; protection in C57Bl/6 mice	[46–48]
Live vaccines	Inoculation of a dermotropic *L. donovani*	VL	Pre-clinical studies in mice	Protection against challenge with viscerotropic *L. donovani* in BALB/c mice	[37]
	Physically attenuated parasites	CL/ MCL/ VL	Pre-clinical studies in mice and hamsters	Homologous protection for *L. major*, *L. tropica*, *L. amazonensis*, *L. donovani* and *L. braziliensis*; no effect for *L. infantum*	[49–51]
Non-defined composition; live, attenuated and/ or drug-sensitive parasites (through culture, chemical, radiation or genetic manipulation)	Chemically attenuated parasites (N-nitrosamines/ antibiotic pressure)	CL/ MCL/ VL	Pre-clinical studies in mice and dogs	Homologous protection for *L. major* and *L. mexicana* in BALB/c mice; promising results for *L. infantum* in dogs	[51]
	Genetically attenuated parasites (**Lmaj**dhfr-ts, **Lmex**CystProt, **Lmaj**LPG2, **Lmaj**PPM, **Ld**Cen1, **Li**HSP70-II, **Ld**p27, **Ld**ALO and **Ld**BT1 null mutants; **Li**SIR2 sKO)	CL/ MCL/ VL	Pre-clinical studies in mice/hamsters/dogs/ macaques	Homologous protection for *L. major* in mice, but not monkeys; *L. mexicana* in mice and hamsters; *L. infantum* in mice and *L. donovani* in mice, hamsters and dogs; heterologous protection for *L. major* in mice, *L. braziliensis* in mice and hamsters and *L. infantum* in dogs (mediated by *L. donovani* KO parasites)	[51–55]
	Genetically modified parasites (gain of function)—suicide mutants: *L. major* tk-cd+/+ (susceptible to Ganciclovir and 5-flurocytosine), *L. amazonensis* alad-pbgd+/+ (used in the context of photodynamic vaccination)	CL/VL	Pre-clinical studies in mice and hamsters	Homologous long-term protection (lesion free) in mice for *L. major*; heterologous protection against *L. donovani* mediated by *L. amazonensis*: 99% reduction in parasite loads and suppression of disease	[50, 56, 57]

Type	Approach/vaccine candidate(s)	Disease form	Vaccine development pipeline	Efficacy/outcome	Reference
	Immunization with non-pathogenic *L. tarentolae* (wild type or genetically modified strains producing LPG3, **Ld**A2 or **Ld**A2/CPA/CPB)	VL	Pre-clinical studies in mice and dogs	Promising results in mice and dogs	[50, 60, 61]
First generation vaccines	ALM adjuvanted with BCG	CL/VL	Pre-clinical and human clinical studies	Protection in macaques against *L. donovani* challenge; poor efficacy in humans. Protection in mice against *L. major* infection; clinical studies with disappointing results	[54, 65, 67, 68]
	Alum-ALM adjuvanted with BCG	CL/VL	Pre-clinical and human clinical studies	Immunogenic and safe in humans; protective (single dose) in macaques challenged with *L. donovani*; moderate efficacy against canine visceral leishmaniasis; protection in BALB/c mice against challenge with *L. major*	[54, 56, 69]
	Autoclaved *L. donovani*	VL	Pre-clinical studies in mice	Significant levels of homologous protection	[70]
	Phenol or Heat inactivated *L. guyanensis, L. braziliensis* and *L. amazonensis* adjuvanted with BCG	CL/ MCL	Human clinical studies	52% Efficacy in endemic area (phenol inactivation); no protection against *L. amazonensis* infection (heat inactivation)	[65, 67]
	Merthiolate-killed *L. amazonensis* (with/without BCG)	CL	Pre-clinical and Human clinical studies	Protection in mice not reproduced in humans	[65, 73, 74]
Non-defined composition; whole killed parasites or parasite fractions	Sonicated *L. donovani* (whole cell or soluble antigens) adjuvanted (MPL-A, BCG, liposomes)	VL	Pre-clinical studies in mice hamsters and monkeys	Good homologous protection in all species; liposomal formulation elicits the best protection in mice	[36, 54, 71, 72]
	Liposomal *L. major* soluble antigen adjuvanted with CpG	CL	Pre-clinical studies in mice	Significant levels of homologous protection	[36]

Type	Approach/vaccine candidate(s)	Disease form	Vaccine development pipeline	Efficacy/outcome	Reference
	Fucose-Manose ligand adjuvanted with saponin	VL	Pre-clinical studies in mice and hamsters; "clinical" studies in dogs	Protection in mice and hamsters challenged with *L. donovani* (homologous); effective heterologous protection (against *L. Infantum*) in dogs; transmission blocking potential; commercialized as a canine anti-*Leishmania* vaccine with the name Leishmune in Brazil (commercialization license suspended in 2014)	[66, 75]
	L. infantum or *L. amazonensis* excreted-secreted antigens adjuvanted with saponin	VL	Pre-clinical/"Clinical" studies in dogs	Significant, long-lasting protection against canine VL in a field trial in an endemic area (**Li**); promising results in terms of heterologous protection against *Leishmania infantum* (**La**); commercialized as a canine anti-*Leishmania* vaccine with the name CaniLeish in Europe (**Li**)	[54, 66, 76, 77]
Second generation vaccines	Membrane proteins: native **Ld**Dp-72, gp63 and PSA-2 and recombinant **Li**LCR1, **Ld**HASPB1, KMP-11 and gp63; adjuvanted (BCG, CpG-ODN, MPL-SE, IL-12, saponin, cationic nanoparticles, liposomes)	CL/VL	Pre-clinical studies in mice, dogs and macaques	Promising results regarding homologous protection in mice; dubious protection in monkeys against *L. major* challenge (gp63)	[50, 54, 71, 83–85]
	"Soluble proteins": recombinant **Ld**A2, **Li**PHB, **Ld**F14, **Ld**p27, **Ldp**SP **Ld**P45, **Ld**PDI, **Ld**TPI, **Ld**TPR, **Li**P0, **Lmaj**STM1, **Li**TDR-1, **Lb**Hyp, EiF5a, eIF-2, NH, CPA and CPB, SMT, PEPCK, Histone H1, Heat shock proteins (HSP), **Li**Ribosomal proteins, **Li**Hypothetical amastigote-specific protein, cysteine proteinases, LACK; adjuvanted (BCG, ALD, *P. acnes*, CpG-ODN, MPL-SE, IL-12, saponin, cationic nanoparticles)	CL/ MCL/ VL	Pre-clinical studies in mice, hamsters and dogs; *ex vivo* human studies	Promising results in mice and hamsters; a major limitation is that most of the antigens were not tested in superior models; positive response in *ex vivo* human studies for **Ld**eIF-2; partial homologous and heterologous (*L. infantum*) protection in dogs and heterologous protection in mice challenged with *L. infantum* and *L. amazonensis* (**Ld**A2: licensed veterinary product in Brazil—LeishTec)	[36, 50, 54, 67, 84, 85, 87–90]

Type	Approach/vaccine candidate(s)	Disease form	Vaccine development pipeline	Efficacy/outcome	Reference
Defined antigens: (native) or produced through DNA recombinant technology (more frequent)	Peptides: CPA, GP63, LmSTI1, LiKMP-11, PEPCK; often associated DC-based vaccination or nano-sized vaccine-delivery systems; adjuvanted (MPLA, CpG-ODN)	CL/VL	Pre-clinical studies in mice	Partial protection for *L. infantum*; differential protection for *L. major*	[71, 89, 93, 94]
	Fusion protein/polyprotein: Q protein, Leish-F1 (Leish 110-f), Leish-F2 (Leish 110-f), Leish-F3, Leish-F3+, KSAC, 8E + p21 + SMT, KMP-11 + LJL-143 + Leish-F3 + (in virosomes), rLiHyp1 + rLiHyp6 + rLiHyV+rHRF multiepitope; adjuvanted (BCG, Saponin, CpG-ODN, GLA-SE, MPLA, ALD and MPL-SE)	CL/VL	Pre-clinical studies in mice, hamsters, dogs and macaques; human clinical studies	Promising results in mice (CL and VL) and hamsters; protection conferred to dogs against challenge with *L. infantum* (Q protein, Leish 110-f, Leish-F1, KSAC); protection of macaques challenge by *L. major* (Leish-F1); vaccines safe and immunogenic in humans (Leish-F1, Leish-F2 and Leish-F3); licensed veterinary product in Europe (Q protein—Letifend)	[54, 84, 85, 95–105]
Third generation vaccines	DNA plasmidic vaccines (usually self adjuvanted): LdPDI, tuzin, HbR, A2, Histones+p36, LACK, TSA + LmSTI1, gp63, KMP-11, CPB, ORFF, NH36, TRYP, PSA-2, γGCS, PEPCK, LeIF, GP63 + HSP70, LeIF+/orTSA; MIDGE-Th1 vectors encoding conserved T-cell epitopes from KMP11, TSA, CPA, CPB, and P74	CL/VL	Pre-clinical studies in mice, hamsters, dogs and macaques; *ex vivo* human studies	Generally good protective responses in mice and hamsters correlated with the induction of Th1 immunity; partial (Histones+p36) and good (LACK, cysteine proteinase) protection in dogs; protection in macaques (TSA + LmSTI1); effective in mice in immuno-chemotherapeutic approaches (MIDGE Th1); strong possibility of human immunogenicity (MIDGE Th1)	[36, 50, 54, 84, 85, 89, 110–117]
DNA vaccination and/or modified expression systems	Recombinant viral vectors: recombinant/modified vaccinia virus expressing TRYP, LACK, KMP-11; recombinant Influenza virus expressing LACK; (non-replicative) recombinant adenovirus expressing A2, Leish-F3 or KMP-11-HASPB; recombinant lentivirus expressing KMP11-HASPB	CL/VL/ PKDL	Pre-clinical studies in mice, dogs and macaques; human clinical studies	Promising results obtained in all animal models; vaccine safe and immunogenic in humans (replication defective adenovirus coding for KMP-11-HASPB)	[50, 83, 119–123]

Type	Approach/vaccine candidate(s)	Disease form	Vaccine development pipeline	Efficacy/outcome	Reference
	Live recombinant bacterial vectors: *Lactococcus lactis* expressing A2 and LACK+IL-12; recombinant *S. typhimurium* vaccine strains expressing gp63, Linj08.1190 and Linj23.0410; recombinant *L. monocytogenes* (attenuated) expressing LACK	CL/VL	Pre-clinical studies in mice	Different results obtained, varying from disease exacerbation (A2 *L. lactis*), to limitation of pathology (LACK *L. monocytogenes*) or protection	[51, 124]
Vector-derived vaccines	Th1 immunity inducing sand fly salivary proteins: recombinant or DNA encoding LJM-19 (SALO), PdSP-15, PpSP15 (also *L. tarentolae* based), PpSP-44, LJM-143, LJM-17, LJM-11 (also *L. monocytogenes* based); alone, or in combination with common anti-*Leishmania* vaccine	CL/ MCL/ VL	Pre-clinical studies in mice, hamsters, dogs and macaques	Evidences or described effect in protection from (natural) infection in all animal models, except with PpSP-44 which leads to exacerbation of cutaneous disease	[128–135]
Recombinant or DNA coding for sand fly derived proteins (including heterologous expression systems)	Insect-based transmission blocking vaccine: anti *P. papatasi* galectin (sand fly midgut protein) antibody	CL	*In vitro* and *in vivo* insect studies (artificial feeding)	86% reduction of sand fly-midgut *L. major* infection; impairment of metacyclogenesis	[136–137]

A2, amastigote specific protein 2; ALM, autoclaved *L. major*; ALO, arabino-1,4-lactone oxidase; Cen, centrine; BCG, Bacillus Calmette–Guérin; CL, cutaneous Leishmaniasis; CPA/B, cysteine peptidase A/B; CystProt, cysteine proteinase; dhfr-ts, dihydrofolate reductase-thymidylate synthase; eIF, elongation factor; GCS, glutamylcysteine synthetase; GLA, glucopyranosyl lipid A; gp, glycoprotein; HASP, hydrophilic acylated surface protein; HbR, hemoglobin receptor; HSP, heat shock protein; IL, interleukin; KMP, kinetoplastid membrane protein; LACK, *Leishmania* homolog of receptors for activated c-kinase; Ld, *L. donovani*; Li, *L. infantum*; LJL, *Lutzomyia longipalpis* Jacobina large; LJM, *Lutzomyia longipalpis* Jacobina medium; Lmaj, *L. major*; Lmex, *L. mexicana*; LPG, lipophosphoglycan; MIDGE, minimalistic immunogenically defined gene expression; MCL, mucocutaneous leishmaniasis; MPL, monophosphoryl lipid A; NH, nucleoside hydrolase; ODN, oligodeoxynucleotides; ORFF, open reading frame fragment; P0, acidic ribosomal protein P0; PDI, protein disulphide-isomerase; PdSP, *Phlebotomus duboscqi* salivary protein; PEPCK, phosphoenolpyruvate carboxykinase; PHB, prohibitin; PKDL, post kala-azar dermal Leishmaniasis; PPM, phosphomannomutase; PpSP, *Phlebotomus papatasi* salivary protein; PSA, promastigote surface antigen; SE, stable emulsion; SIR, silent information regulator; SMT, sterol 24-c-methyltransferase; TDR, thiol-dependent reductase; TPI, triose phosphate isomerase; TPR, trypanothione reductase; TRYP, tryparedoxin peroxidase; TSA, thiol-specific antioxidant; VL, visceral leishmaniasis. 'Supposedly still used in some extent in Uzbekistan [56].

Table 1. Different anti-*Leishmania* vaccine candidates explored in the last century.

(knock-out) and gain-of-function mutants (knock-in). In respect of the first group, nine null mutants [*L. major* dihydrofolate reductase-thymidylate synthase (dhfr-ts$^{-/-}$), *L. mexicana* Cysteine proteases (CPA/CPB$^{-/-}$), *L. major* lipophosphoglycan 2 (LPG2$^{-/-}$), *L. major* phosphomannomutase (PPM$^{-/-}$), *L. donovani* Centrin (Cen$^{-/-}$), *L. infantum* heat shock protein 70 type II (HSP70-II$^{-/-}$), *L. donovani* amastigote specific protein p27 (p27$^{-/-}$), *L. donovani* arabino-1,4-lactone oxidase (ALO$^{-/-}$) and *L. donovani* biopterin transporter 1 (BT1$^{-/-}$)], and one single knock-out [*L. infantum* silent information regulatory protein 2 (SIR2$^{+/-}$)] were proven, in most cases, as effective vaccine candidates (CL, MCL, and VL) [52–56]. Concerning the second group, two gain-of-function mutants were shown effective as vaccines for CL and VL. Both trials relied on the generation of "suicidal mutants" that would be completely eliminated from the immunized host either by the action of chemotherapeutics [L. major thymidine kinase (herpes simplex virus), cytosine deaminase (Saccharomyces cerevisiae) knock-in: tk-cd$^{+/+}$], or by photodynamic therapy (*L. amazonensis* δ-aminolevulinate dehydratase, porphobilinogen deaminase knock-in: alad-pbgd$^{+/+}$) [51, 57, 58]. Yet, although safer in theory than both live virulent and pre-genomic attenuated vaccine candidates, post-genomic live attenuated vaccines still raise safety concerns, both due to the potential for reversion to virulence (higher for gain-of-function parasites but not negligible for knock-out parasites as was reported [59]) and due to the potential risk to the immunosuppressed (that was not explored in most of the trials). The last tested live vaccine approaches we will discuss here propose the use of closely related non-pathogenic parasites as a way to overcome all the live vaccine safety-related red flags. *Leishmania tarentolae* parasites infect reptiles but are unable to generate a sustained infection in humans (although able to enter into human phagocytic cells, there is no evidence of efficient intracellular replication) [60]. Importantly, they share >90% of the gene content with the other *Leishmania* species [60] which makes these parasite species an innocuous source of native *Leishmania* antigens (although some of the important virulence factors of pathogenic parasites that may be essential to the induction of a protective prophylactic response are missing). Using this premise, both wild-type and genetically modified (LPG3, amastigote-specific protein (A2), or A2/CPA/CPB knock-in) non-pathogenic parasites were reported, in the pre-clinical context, as promising vaccine candidates for VL [52, 61, 62].

2.2.2. First-generation vaccine candidates

Together with live attenuated vaccines, killed whole pathogens or fractions of them (inactivated and fraction vaccines) comprise a large proportion of the approved vaccines for humans today [63]. In line with what happened chronologically in modern vaccinology, killed/fractionated vaccines against leishmaniasis were developed both contemporarily and posteriorly to the "leishmanization era," to answer to the safety concerns associated with live virulent/attenuated vaccines. The main advantage of first-generation vaccines in relation to the live vaccine counterparts is consequently their innocuity: the pool of antigens in its native form will still be "delivered" and elicit a specific memory response (diversity in antigenic *repertoire* given by live parasites will be at least partially maintained), while no pathology is expected, even in immunocompromised individuals (no infection = no disease) [64]. This, however, may as well be a disadvantage: while regarding live vaccines, the antigen delivery will be sustained; that will not be true for killed vaccines that may require more than one administration (prime homologous boosts immunization schemes) and/or the co-administration of an immune response enhancer or adjuvant (usually not required in live vaccine approaches) [65], which may or may not be enough to generate long-lasting protection. Additionally, all of the manufacturing and logistics

issues discussed earlier for leishmanization (and live vaccines in general) are also applicable to killed/fractionated vaccines. Notwithstanding, first generation vaccines for leishmaniasis are the better studied ones in the clinical context (the only leishmaniasis vaccine candidates which have undergone phase 3 clinical trials) [66], by itself very relevant for the anti-Leishmania vaccine development field, and are available today in the market as approved vaccines for canine VL (Leishmune® in Brazil and CaniLeish® in Europe) [67]. The better recognized vaccine candidate within this sub-topic is the autoclaved *L. major* (ALM) adjuvanted with BCG, tested in the pre-clinical and clinical contexts, with promising results in the first that were not confirmed in the second [55, 66, 68, 69]. This candidate was then optimized by adsorption of the antigenic fraction to alum (alum-ALM + BCG) and retested once again in both pre-clinical and clinical contexts (CL and VL), with reported different degrees of efficacy in animal models and good immunogenic and safety profiles in humans [55, 66, 70]. A similar parasite-killing approach was used with *L. donovani* parasites, tested in a vaccine pre-clinical trial for VL (mice) that revealed significant homologous protective potential [71]. In parallel, a different inactivation strategy (sonication) was used also with *L. donovani*, and the obtained total or soluble antigens were used together with MPL-A, BCG, or liposomes as vaccine candidates for VL in pre-clinical trials with promising results in all models tested (mice hamsters and monkeys) [37, 55, 72, 73]. Only two other candidates were tested in the clinical context, this time in the New World as CL and/or MCL vaccines. One of them was a trivalent formulation of phenol or heat-inactivated *L. guyanensis*, *L. braziliensis*, and *L. amazonensis* adjuvanted with BCG [66, 68], while the other consisted of merthiolate-killed *L. amazonensis* (with/without BCG) [66, 74, 75]. Curiously, in line with what was verified in the Old World with ALM-BCG, although effective in the pre-clinical context, both candidates generally failed as human vaccines [66, 75]. Apart from crude extracts, parasite fractions have been tested. Liposomal *L. major* soluble antigens adjuvanted with CpG were tested as a vaccine candidate for CL with significant levels of homologous protection observed in mice [37]. A glycoproteic fraction of *L. donovani* parasites (fucose-mannose ligand—FML) adjuvanted with saponin [67, 76] and *L. infantum* (or *L. amazonensis*) excreted-secreted proteins (ESP) also adjuvanted with saponin [55, 67, 77, 78] were tested as vaccine candidates for VL in canines, whose determined efficacy, and safety profiles, was sufficient to warrant their registration as veterinary vaccines (*L. donovani* FML as Leishmune®—out of the market nowadays—and *L. infantum* ESP as CaniLeish®). Nevertheless, they were never tested in the human clinical context, which may be due to different reasons, all connected to the notion that the requirements needed for the approval of a human pharmaceutical are much more strict than the ones required in the veterinary context: (i) the heterogeneous antigen formulation, harder to standardize, may have been considered an obstacle or (ii) the data obtained in the pre-clinical context may not have been sufficient (vaccines conferred only partial protection [67]).

Although it is a topic we do not explore in this chapter, it is important to stress that killed vaccines, different from what was observed in the prophylactic context, have shown great promise in a therapeutic context (revised in [79]).

2.2.3. Second-generation vaccine candidates

The birth and evolution of the molecular biology field contributed immensely to the rhythm of science in general. Today, the production of a single antigen is usually easily achievable,

as it is the possibility of scaling-up the process to an industrial level. Second-generation vaccines are a consequence of this scientific evolution (although some are native proteins, most of them are recombinant antigens) and consist of defined antigens, generally together with an immune response enhancer. They are usually accepted by the scientific community, as well as by the regulatory entities that so far have approved three vaccines for human use (including the hepatitis B recombinant vaccine that replaced the traditional plasma-derived one [80]). The main advantage of these vaccines in relation to the ones earlier discussed is the defined composition that allows an easier standardization. Another advantage we can think of is the elimination of immuno-dominance events that invariably occur if a complex antigen mixture is used as a vaccine and may hinder the potential of good vaccine candidates [81]. As disadvantages, the following should be considered: (i) the limited duration of antigen availability might impact the memory pool and limit the "protection window" [82] (more complex immunization schemes have to be used) and (ii) recombinant proteins, usually expressed in heterologous systems, may be slightly different from native proteins (particularly concerning post-translational modifications [83]) which might impact their immunogenic potential (more relevant for humoral responses, considering conformational epitopes).

Second-generation vaccines against leishmaniasis are the group with higher representativeness. Here, for the sake of clarity, we separate them into four different groups: membrane and soluble proteins (full single recombinants), peptides, and polyproteins (multivalent), whose main candidates are enumerated in **Table 1**. The studies from fractionated parasites postulated that parasite membrane proteins had a good vaccine potential. Because of that, and also due to their relative abundance, relevant in terms of antigen presentation, many membrane proteins were explored as vaccine candidates in the pre-clinical context for both CL and VL with promising results [51, 55, 72, 84–86]. Among these is the well-known, and extensively studied in the context of anti-*Leishmania* vaccination, kinetoplastid membrane protein-11 (KMP-11) [87]. Importantly, most of these proteins were identified by classical immuno-proteomic approaches considering always the amastigote parasite form as the most relevant in the human infectious process and are known virulence factors. This fact is also true for most of the non-membrane proteins (we name here "soluble proteins") also tested in the last decades as vaccines against leishmaniasis, although most of them only in rodent models of CL, MCL, and VL (translatability to humans is not assured) [37, 51, 55, 68, 85, 86, 88–91]. Ribosomal proteins (e.g., P0), metabolic enzymes (e.g., TPI), stress-related proteins (e.g., HSP), antioxidant-machinery components (e.g., TPR), and even hypothetical proteins (**Table 1**) are found among them. One of these candidates, *L. donovani* A2, is today a licensed veterinary vaccine against leishmaniasis in Brazil—LeishTec® (that needs however to be optimized, according to a recent efficacy field trial performed in an endemic area with high transmission rates [92]). In the past few years, the development of vaccine candidates against leishmaniasis became more refined and rationale based, following the trends of twenty-first-century vaccinology [93]. New studies are now usually based in an initial *in-silico* prediction of immunogenicity, validated later ideally through *ex vivo* studies using samples from exposed human individuals, all performed before the design of any clinical trial. Furthermore, the antigens/antigen portions should be "broad spectrum"—conserved in all the pathogenic *Leishmania* spp.—and very different from human "self-antigens." From the application of such approaches and selection criteria, promising new candidates were proposed. Among them, peptide vaccines, chosen from immunogenic portions of known vaccine candidates such as KMP-11, were tested pre-clinically, often associated with DC-based vaccination

strategies or nanosized vaccine-delivery systems [72, 94, 95]. Interestingly, a recently published work proposes a peptide vaccine candidate (from *Leishmania* phosphoenolpyruvate carboxykinase—PEPCK) that may be effective for both VL and CL should the results obtained in the pre-clinical context translate into the clinical one [90]. However some argue that to use a single antigen, or a peptide as vaccine, may be less than optimal, considering that there will be a limitation in terms of epitope diversity. To answer to this, some propose the use of defined polyantigen vaccines (fusion proteins or mixed recombinants), also rationale based, as a way to generate "first-generation-like" second-generation vaccines, increasing epitope diversity and consequently in theory enhancing recognition by human T cells (**Table 1**) [55, 85, 86, 96–101]. Some of these candidates are among the second-generation vaccines that went further in the vaccine pipeline. Q protein (a fusion protein containing portions of *L. infantum* p2a, p2b, and P0 ribosomal proteins and histone H2A) that was demonstrated effective in a pre-clinical trial in dogs infected with *L. infantum* is today the newest approved vaccine for veterinary use—Letifend® [102, 103]. Leish-F1 [fusion protein containing epitopes from *Leishmania* elongation initiation factor (LeIF), thiol-specific antioxidant (TSA), and *Leishmania major* stress-inducible protein 1 (LmSTI1)], Leish-F2 (same immunogenic portions as Leish-F1 but his tagged), and Leish-F3 (fusion protein containing portions of *Leishmania* nucleoside hydrolase and sterol 24-c-methyltransferase), which revealed promising and safe candidates in the pre-clinical context (for both CL and VL), were tested in phase I/II clinical trials that confirmed the translatability of results obtained with animal models to humans [97, 104–106]. Nevertheless, the researchers involved in these clinical trials think that there is still space for improvements and recently presented an improved version of Leish-F3 (with cysteine protease B as an extra fused antigen—Leish-F3^{+}) that is going through the pre-clinical phase of the vaccine development pipeline, with promising results, either alone [99] or in combination with KMP-11 and the vector-derived antigen LJL-143, within a virosomal formulation [96].

We cannot end this sub-section without stressing that generally these second-generation vaccine candidates require the co-administration of adjuvants to warrant their efficacies as vaccines for leishmaniasis. **Table 2** resumes the relevant information on the topic, extensively covered by two recently published reviews [107, 108].

2.2.4. Third-generation vaccine candidates

The notion that intradermal or intramuscular injection of a plasmid into an animal model would be enough to generate antigen-specific immune responses was responsible for the creation of a new arm in the vaccine research field. Although Initially DNA vaccines were not as well accepted as first- and second-generation vaccines, not only due to potential ethical implications (injection of foreign genetic material into humans that could, for instance, integrate within the human genome) but also due to safety concerns such as the possible generation of autoimmune pathologies initiated by the generation of anti-DNA immune responses [109]. However, these potential issues of DNA vaccines were, with time, shown to be irrelevant, both through extensive pre-clinical research and through several clinical trials performed that confirmed DNA vaccines as safe and immunogenic in humans (although for some candidates, the immunogenicity data was not as promising as expected) [109, 110]. Yet, contrary to the other vaccine approaches discussed earlier, we still have no data from phase IV studies of DNA vaccination, since till date there is no third-generation vaccine approved for human use (although there are already four

Adjuvant	Class	Mechanism of action	Type of immune response	Licensed for use in human vaccines
Aluminum mineral salts	Particulate formulation; antigen depot	NALP3, ITAM, antigen delivery, IL-1 secretion, necrosis, inflammasome	Antibody, Th2	✓ (adjuvant of different commercially available vaccines)
Simple or emulsified Lipid A analogues (e.g., GLA, MPL)		TLR-4 agonists	Antibody, Th1	✓ (in combination with Alum in HBV and HPV vaccines)
Imidazoquinolines (e.g., Imiquimod, R848)		TLR-7, TLR-8 agonists	Antibody, Th1	X (clinically tested in cancer immuno-therapy)
CpG-ODN	Immuno-modulatory molecule	TLR-9 agonists	Antibody, Th1, Th2, CD8+ T cells	X (clinically tested in HBV, malaria, influenza and anthrax vaccines and in cancer immuno-therapy)
Saponins (e.g., QuilA, QS21)		Unknown	/	X (clinically tested in combination with cholesterol in HCV, influenza and HPV vaccines and in cancer immuno-therapy)
Nanoparticles (e.g., Virosomes*, Liposomes)	Particulate formulation	Antigen delivery; cross-presentation enhancer*	Antibody, Th1, Th2	✓ (HAV and *Influenza* vaccines)

Adapted from [106, 107].

* The asterisk means that only virosomes are cross presentation enhancers (asterisk in both)

Table 2. Main adjuvants used in anti-*Leishmania* vaccines development.

approaches approved for veterinary use) [109, 110]. However, considering that third-generation vaccines are the most recent approaches (studies started in the 1990s), it is likely a matter of time until the approval of the first DNA vaccines considering some advantages attributed to them: (i) they are easy to design, produce, and scale up (potentially more cost-effective); (ii) they are quite stable, which minimize distribution and logistics-related complications; and (iii) they can induce both humoral and cellular immune responses (including $CD8^+$-mediated cytotoxicity) [110]. Here, we categorize third-generation vaccines in three clusters: DNA vaccines, viral heterologous expression systems, and live bacterial expression systems. DNA vaccines are the more expressive in respect of the number of candidates explored, containing the simplest vaccine candidates: consist of usually non-adjuvanted plasmids (the "real DNA vaccines"). Similar to what was described for second-generation vaccines, both membrane (e.g., KMP-11 and gp63) and non-membrane antigens (e.g., NH, CPB, HSP70, and A2) were explored in the context of plasmid vaccine candidates (**Table 1**), pre-clinically, using animal models for both CL and VL [37, 51, 55, 85, 86, 90, 111–118]. Interestingly, many of the candidates tested as second-generation vaccines (and particularly those that have shown some degree of promise) were retested as DNA vaccines, either individually or in "multi-antigen" approaches (e.g., KMP-11, A2, LACK, and TSA+LmSTI1), showing the adoption of a rationale-based vaccine development [114]. The general reproduction of the results obtained with second-generation vaccines, after immunization with their DNA counterparts (CL and VL models, including mice, hamsters, dogs, and macaques), validated these approaches as potentially effective agents in

the context of anti-*Leishmania* vaccine prophylaxis [51, 55, 85, 86, 90, 114]. In this sub-group, we would like to highlight the LEISHDNAVAX approach, recently proposed for VL. Completely based in a modern vaccine development approach (rationale based), this vaccine candidate, shown to protect mice from an intravenous challenge with *L. donovani*, is composed of five individual plasmid (MIDGE-Th1 vectors) coding for five *Leishmania* antigenic determinants, chosen based on inter-species conservation, "pan-immunogenic" potential (in different human populations), and content of T-cell-restricted epitopes (KMP11, TSA, CPA, CPB, and P74) [112]. This approach, which, according to the authors, is a candidate for clinical trials, has as the main advantage the modular nature: the vaccine is multivalent, but the antigens are not fused together, allowing the rapid modification and adaptation of the vaccine (exchange, addition, or elimination of antigens) [112]. Still within third-generation vaccines, more complex candidates were explored as well, in the form of heterologous expression systems. Among them are viral vectors, referred to as an improvement of classical DNA vaccines, once in one way allow *in situ* antigen expression, and also have an intrinsic adjuvant activity (mediated by pathogen-associated molecular patterns (PAMPs) immune recognition) [119]. One important prerequisite of such vectors is their relative innocuity, being in most cases either human-approved vaccine strains (which have the same counter-indications for immuno-compromised individuals) or replicative-deficient strains. Till date, more than 5 viral-recombinant vaccines (using as viral platforms modified vaccinia virus, influenza virus, non-replicative adenovirus and lentivirus) coding for *de facto* effective antigens such as KMP-11, LACK, Leish-F3 and HASPB, were tested in the pre-clinical context for both CL and VL, with promising results obtained in all animal models used (mice, dogs and macaques) [51, 84, 120–123]. Remarkably, one of them was the first third-generation anti-*Leishmania* vaccine candidate to undergo human clinical trials. The adenoviral-based vaccine (non-replicative strain) expressing a self-cleaving polyprotein (L. donovani KMP-11+HASPB) was shown safe and immunogenic in humans, inducing particularly specific $CD8^+$ T cell responses, and importantly is being proposed as, more than a prophylactic vaccine, a therapeutic vaccine destined to aid in the control of post-kala-azar dermal leishmaniasis—PKDL ("the neglected form of leishmaniasis" in respect of the anti-*Leishmania* vaccine studies) [124]. Last but not the least, some bacterial-based heterologous systems were proposed as anti-*Leishmania* vaccines although with disappointing results in some cases [52]. Also, to these ones, because they are live organisms, the disadvantages discussed earlier for live attenuated vaccines apply (such as counter-indication to immuno-suppressed) with the exception of the use of non-pathogenic organisms, such as *Lactococcus lactis*. From these candidates, we highlight the recombinant *Salmonella typhimurium* vaccine strains and the attenuated *Listeria monocytogenes* expressing different *Leishmania* antigens (e.g., gp63 and LACK), the ones that have shown the most promising results, although only in rodent models of CL/VL [52, 125].

2.2.5. Vector-derived vaccine candidates

It has become clear that to consider the sand fly vector only from the perspective of vector-control strategies would be not only reductive but also contribute to a major delay in the achievement of the disease elimination objective. The anti-*Leishmania* vaccine field became more complex from the moment Kamhawi, Belkaid, and colleagues showed that a previous exposure to uninfected sand fly bites (or to sand fly saliva) would be enough to confer protection against CL [126, 127]. Curiously, the anti-saliva-generated DTH responses were shown to be sufficient to negatively impact *Leishmania* parasites (indirectly). And importantly, such responses are apparently not

influenced by constant saliva exposure that could induce tolerization [128]. Such pieces of evidence supported the exploitation of defined sand fly salivary proteins as anti-*Leishmania* vaccine candidates (either as single recombinant proteins or DNA vaccines—both plasmids and heterologous systems—alone or in multivalent approaches together with *Leishmania*-derived antigens) [129–136]. Several antigens, derived from different sand fly species from both New [129–131, 136] and Old [133–135] Worlds, were explored in the pre-clinical context in models of CL, MCL, and VL, most of them with promising results. The most relevant candidate is PdSP15, which was shown to be protective against cutaneous disease in different models, including in non-human primates (DNA protein prime-boost approach) [133–135]. Another candidate that deserves to be highlighted is LJM-19 (or SALO), which was demonstrated simultaneously as a good candidate against visceral ("homologous protection") and mucocutaneous ("heterologous protection") disease [131, 136]. Still within vector-based anti-*Leishmania* vaccine approaches, and although it is an option which is exploited very little, we believe that transmission blocking vaccines deserve to be mentioned. Such vaccines will act by impacting parasites' development within the vector, impeding, therefore, their transmission to a new host [137]. For their engineering, however, the insect midgut proteins that allow parasite attachment during development (assuming such a process is dependent on specific interactions) have to be identified, which was described only for *Phlebotomus papatasi* (galectin—PpGalec) [138]. Interestingly, this study that shows that flies pre-fed with PpGalec murine pre-immune serum and posteriorly infected with *L. major* parasites were reproducibly less infected than the controls (an 86% decrease in the number of parasites retained in the midgut after blood meal excretion which led to at least a 5-fold reduction in the frequencies of mature infection development) is a proof of principle of *Leishmania* transmission blocking vaccines that may be used, for instance (but not only), in animal reservoirs and still impact human disease incidence [137, 138].

2.3. Questions that deserve to be answered

As a connecting point between the current and subsequent sections, we raise some questions for which we still do not have a clear answer today. The first one is if the development of a pan-*Leishmania* vaccine *sensum latum* (both prophylactic and therapeutic; for endemic and non-endemic individuals; against all disease forms) is something over-ambitious. And such a question makes sense, not only because of the time and investment that are expected to be involved—for the case of leishmaniasis, the non-existence of prophylactic agents implies the "faster is better" motto. For instance, in our recent work, we show that the pre-administration of a salivary antigen, followed by a boost with the same salivary antigen together with two other parasite-derived proteins, has a direct impact in the immunogenicity of the latest [96], which may suggest that vaccines for endemic individuals may not work equally in non-endemic ones and *vice versa*. This point is particularly relevant if we use vector- and parasite-derived components in the vaccines against leishmaniasis, which is related with the second question we pose: should vaccines for leishmaniasis always contemplate both parasite- and vector-derived components? Studies that show the improvement of parasite-derived vaccine candidates when co-administered with vector-derived antigens support this hypothesis [54, 64]. However, there are still some issues that have to be addressed, such as the possibility of tolerance induction, that is known to be dependent on the amount of antigen [139] (expected to be higher in a defined antigen-based vaccination approach, compared with a sand fly bite). Furthermore, another question relates to clinical research. How can we test the effectiveness of safe and immunogenic vaccine candidates? The last phase III

clinical trials were performed more than half a century ago and against the cutaneous disease. But, contrary to other deathly parasitic diseases, such as malaria [140], to perform controlled infections with *L. infantum*, *L. donovani* or even *L. braziliensis* or *L. guyanensis* would be unethical, to say the least. Therefore, such trials would have either to evaluate cross-protection to cutaneous disease (controlled infection with *L. major* that still raises ethical issues) and extrapolate results to the mucocutaneous/visceral forms or be designed and conducted directly as phase IV clinical trials (although to use a placebo in this context would probably also not be admissible).

3. Vaccines for human leishmaniasis: what is still missing?

So far, and consciously, we described the different vaccine candidates explored till the present days as vaccines against leishmaniasis, highlighting only their effectiveness in a qualitative way (effective/non-effective, promising or not) and not discussing the immune mechanisms linked to those results: first, because **Table 1** contemplates vaccine candidates developed for the different leishmaniasis forms, whose pathogenic mechanisms are distinct (and not completely understood) [141] and additionally, because the correlates of protection (that may also be distinct, depending on the disease form) are still far from being well established (they are neither consensual nor definitive). Such facts may have different justifications, as (i) we are still missing key insights concerning vector-parasite-host interactions (both in disease and in health states); (ii) the translation value of the animal models used is limited; or (iii) the models used are not adequate.

3.1. From "mice to man": the issue of animal models, correlates of protection, and translation

Being *Leishmania* parasites obligatory intracellular pathogens (in the mammalian host), it is not surprising that humoral-based responses will be less important than cellular-based ones. Indeed, in animal models of VL, the absence of B-cells contributed to decreased susceptibility to infection [142, 143]. Additionally, it has been shown that antibody-opsonized parasites are more efficiently taken by phagocytes that will become "permissive hosts" due to the high IL-10 and low IL-12 secretion phenotype induced by antibody Fc-receptor (FcγR) interactions [144–147]. Importantly, one of the hallmarks of human disease, is hypergammaglobulinemia (that correlates with disease severity), resultant from a polyclonal B-cell activation, being consequently most of the circulating antibodies non-parasite specific [148–150]. Still, and because the development and role of humoral responses in leishmaniasis is controversial and not completely understood, they may be important [151, 152]. For instance, the type and functionality of the antibodies may be relevant from the standpoint of a vaccine approach, considering lytic functions [e.g., antibody-dependent cell-mediated cytotoxicity (ADCC)] or even "Th1-inducing" FcγR ligation [153]. Yet, even for the proper mounting of effective antigen-specific humoral responses, cell-mediated immunity is of paramount importance [154].

What is known today regarding cellular immune responses to *Leishmania* infection was built on top of the Th1/Th2 paradigm defined on the basis of susceptibility *versus* resistance to *L. major* infection (one of the known CL etiologic agents) [155]. Indeed, the IL-12-mediated IFN-γ production by *Leishmania*-specific $CD4^+$ T cells is essential to promote the switch on of the oxidative cell-parasiticidal machinery, important for infection control both in animal models and in human

disease [156–158] (although in mucocutaneous forms, inflammation is also the cause of pathology [159]). However, while in cutaneous disease a general correlation between Th1 *versus* Th2 responses and immunity *versus* susceptibility is observed, in VL, where the major source of the regulatory cytokine IL-10 is *Leishmania*-specific Th1 cells (Tr1), that is not observed. This mechanism of self-regulation (to prevent inflammation-mediated tissue damage) contributes to parasite persistence [160]. Yet, most of the anti-*Leishmania* vaccine studies rely on the quantification of the levels of IL-10 and IFN-γ-secreted *ex vivo* in response to either the vaccine antigen or to parasite total proteins and use the Th1/Th2 paradigm as a justification for the candidate potential. Others use multi-parametric flow cytometry (or ELISPOT) that allows the characterization of individual cell populations and the disclosure of which cytokines they are producing (most of the times after an *ex vivo* stimulation step): often IFN-γ *versus* IL-10 (individually) or more recently multi-functional T cells [161]. Still, one may claim that results based on such approaches may have a limited validity due to the artificiality of the system: (i) the type and amount of antigen used in the recall and (ii) exclusion of parasite immuno-modulatory potential. To measure directly cytokine expression in the target organ (in an efficacy pre-clinical trial), as is sometimes done in CL models, is a way to bypass the potential limitations of *ex vivo* stimulation approaches. Importantly, the correlates of protection proposed and used should always correlate well with parasite burdens. Another issue that deserves to be emphasized is the cell type(s) we need to look at. Although $CD4^+$ T cells are important in anti-*Leishmania* immunity, so are $CD8^+$ T cells (important from both therapeutic and prophylactic standpoints) that are however many times almost not accounted for in vaccine studies [162]. These cells are nowadays known to be important for resistance to *Leishmania* infection and cure, either by production of IFN-γ (that will activate the microbicidal machinery) or by secretion of cytotoxic mediators that will directly kill infected cells [70, 163, 164]. And because of this, usually, the secretion/expression of IFN-γ or granzyme-B by *ex vivo*-recalled $CD8^+$ cells is used to qualify their responsiveness and considered as potential correlates of protection. Having in consideration what was referred above for $CD4^+$ T cells, an additional problem of translation must be considered. It is known that human $CD8^+$ T cells (and other cytotoxic subsets) produce an antimicrobial peptide (granulysin) with direct parasite-cytotoxic effect, while murine cells do not. Curiously, the infection of a humanized mouse model (granulysin knock-in) with *T. gondi* and *T. cruzi* was less severe than in WT animals, as probably will be reproduced with *Leishmania* spp. [165]. Another important factor to be considered in vaccine effectiveness evaluation is the relevance of the local *versus* systemic responses. Although in CL models, most of the times "specific-systemic responses" are investigated (e.g., recall experiments using splenocytes), it was recently shown that *Leishmania*-specific skin-resident $CD4^+$ T cells are able to confer protection to cutaneous diseases, independently of the central/effector memory pool [166]. However, the immune response in the skin is often not accounted for. Although natural infection (independently of the disease form) always begins by the deposition of parasites in the host dermis (excluding vertical transmission and accidental "human-made" infections resulting from, e.g., blood transfusions), most of the animal models used today in vaccine studies, particularly if we consider VL, completely bypass the skin (controlled infections are performed most of the times either intravenously or intraperitoneally). Therefore, in one way, we may be losing information on the contribution of skin immunity to the protective potential of a given vaccine candidate but on the other hand we may be "overloading the system" and induce responses quite different than the normal ones (too many parasites = excessive inflammation or immuno-modulation)

[167]. Additionally, most of the times in experimental infections, and in this case not only in VL models, the vector is completely disregarded. Importantly, vector saliva was shown to exacerbate infection in different disease models [126, 168, 169]. Also, we have to consider the vector microbiome as a potential infection modulator, as it has been hypothesized [170]. Additionally, parasite-excreted-secreted virulence factors (e.g., promastigote secretory gel and extracellular vesicles/exosomes), or death parasites, all expected to be part of the natural infectious inoculum, were also shown to promote infection [171–173]. Probably, one or the combination of all of these components was the factor responsible for the data published by Peters et al. [174] that have shown the loss of efficacy of the ALM vaccine candidate when tested in the sand fly *versus* needle challenge contexts ("reproducing" the results obtained in the clinical trials of a similar vaccine candidate—ALM + BCG). All of the above discussed point to the use of pre-clinical models to test vaccines that should be as close to what is observed in nature (bearing in mind that even a laboratory-based sand fly transmission model will not be indistinguishable from the natural one, considering the expected differences in the microbiomes [175] and the heterogeneity in vectorial capacities [176]. To improve the chances of translatability (even if the correlates of protection were concrete, the use of an inadequate model would "invalidate" the results), the minimum requirements of vaccine development pre-clinical infection model should be the co-inoculation of parasites together with vector saliva (particularly if the vaccine candidate consists [partially] of vector-derived antigens) in the host dermis, naturally or artificially, by needle injection. No model is perfect and pre-clinical investigation shall ever replace clinical research. However, the system simplification, which is generally used in scientific research as a way to eliminate noise, can also be the reason of loss of translatability. Most of the models used in vaccine development studies have a defined and identical genetic background—they are inbred [177]. Interestingly, vaccine candidates show contradictory results concerning efficacy, depending on the inbred murine model used [178]. We need to have in mind that humans, the target population of the vaccines, are quite a heterogeneous population, with more than 7000 HLA alleles identified so far to which we have to add heterozygosity favored by natural selection [179]. To address this issue, we can start by the vaccine engineering phase that should be more and more rational (using reverse vaccinology approaches [180]) and predict the immunogenicity in different human populations, as a proof of principle that is expected to be validated first pre-clinically and then clinically.

In respect of these three subjects (correlates of protection, animal models, and translatability of pre-clinical studies to humans) that have major overlaps and cannot be separated, there are still too many shades of gray to account for. As a way to eliminate the fogginess, it will be important to identify the divergent and common points of many anti-*Leishmania* vaccine pre-clinical and/or translational studies performed so far. The field would gain a lot from the elaboration and publication of bibliographic statistic studies such as meta-analysis or systematic reviews. Additionally, as suggested by Gannavaram and colleagues, the leishmaniasis research field needs to turn to more complex approaches, such as systems vaccinology, to be able to answer the questions that the community posed a while ago but still remain *quasi*-unanswered [181].

3.2. From "man to mice": the insufficiency of prospective studies

Leishmaniasis animal models have been undoubtedly an extremely useful tool to understand better the host–parasite interactions that influence either resistance to infection or disease

development [157, 182]. This is true for both cutaneous and visceral diseases, although much more relevant in the latest. It would be both unethical and dangerous to biopsy diseased individuals spleen, liver, or bone marrow (target organs of the viscerotropic *L. infantum* and *L. donovani*) just to better understand the infectious process. However, an animal model, even when it combines both conceptual and facial validities, is still just a model; in other words, translation to human health and disease may not always be achievable. In other diseases, prospective studies in human populations have produced valuable information not only from epidemiological and pathological standpoints but also applicable to the vaccine development field [183, 184]. On the other way, till the present days, most of the prospective studies performed in leishmaniasis had an epidemiological character (as invaluable in what respects the common goal of the community, which is disease control) [24, 44]. The development of such studies, focusing on systemic immune responses (particularly cellular based), would be of paramount importance to better understand both disease and resistance in leishmaniasis. For that, there are two target populations that deserve to be studied longitudinally: cured individuals and asymptomatics. While the following of the first population would help to answer the questions related to long-lasting immunity, the following of the second would help to define the potential host factors that determine susceptibility *versus* resistance. Yet, we have to consider as a possible limitation of studies with asymptomatics the less-than-clear and consensual definition of these individuals [24]. Nevertheless, the information generated by such studies would be then possibly "translated back" to animal models, used to better define the correlates of protection to improve vaccine design.

4. Conclusion

Today we still do not have a vaccine approved for human leishmaniasis (regardless of the disease form). Many candidates were tested in the last century, and up to nowadays only vector-derived vaccines were not tested in the clinical context; for all the other parasite-derived candidates, regardless of the vaccine generation they are part of, we have proof of principle of at least immunogenicity and safety (in human healthy individuals) and therefore a precedent is open. Yet, the efficacy clinical trials performed so far (the last more than 50 years ago), excluding leishmanization, were overall disappointing. Such information is however as valuable as any positive result and should be used from a perspective of "learning from our mistakes." There are still many questions to be answered in the anti-*Leishmania* vaccine development field, such as which parameters should be used as correlates of protection and how we should test our vaccine candidates in a way that warrants translation to the clinical context. Additionally, to define the vaccine effectiveness in the clinical context in a controlled way is essential. To address all of these issues, the vaccine development should be more and more rationale based, taking advantage of the modern and of the ancient. Observational studies of target human populations associated with systems biology may for instance help once and for all to disclose the health *versus* disease determinants and contribute to the final establishment of flawless correlates of protection. Additionally, immuno-informatic tools may help to design or refine (through a reverse vaccinology approach) the future vaccine(s) for human leishmaniasis.

Acknowledgements

This work was funded by the project NORTE-01-0145-FEDER-000012, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). Additionally, this work was funded in part by the Intramural Research Program of the NIH, National Institute of Allergy and Infectious Diseases. PC was supported by Foundation for Science and Technology (FCT), Portugal, through the individual grant SFRH/BD/121252/2016.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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II. OBJECTIVES & RESULTS

1. Scope of the thesis

The fact that today there is still no vaccine available to prevent leishmaniasis in humans, even after more than 5 decades of scientific research on the field is probably a consequence of a combination of factors, such as the adoption of empirical approaches, or the use of potentially inadequate animal models that together may be a barrier for the necessary translation to clinical context (Cecilio *et al*, 2018). The adoption of rational strategies in anti-*Leishmania* vaccine development, is the best way to *a priori* overcome potential limitations and potentially assure a parallel considering the pre-clinical and clinical contexts (Rueckert and Guzman, 2012).

The main objective of this dissertation was the rational development of anti-*Leishmania* vaccine strategies for humans:

i. Because leishmaniasis is a vector-borne disease, an important part of our rational was the dual focus on *Leishmania* parasites and phlebotomine sand flies, both considering vaccine design and vaccine testing.

ii. Because the strategies were idealized for humans, the target population was always accounted for.

Consequently the development/optimization of the vaccine approaches, and the characterization of their vaccine potential (immunogenicity and/or safety and/or efficacy) are an important major part of the work. Still, and additionally, the vaccine approaches we explored allowed the exploitation of different questions related with:

i. The potential impact of vector- and parasite- antigens on each other in the context of vaccination.

ii. The relevance of the vaccines' spectrum.

2. Results

2.1. Pre-clinical antigenicity studies of an innovative multivalent vaccine for human visceral leishmaniasis

The notion that previous infection by *Leishmania* spp. in endemic areas leads to robust anti-*Leishmania* immunity, supports vaccination as a potentially effective approach to prevent disease development. Nevertheless, to date there is no vaccine available for human leishmaniasis.

We optimized and assessed *in vivo* the safety and immunogenicity of an innovative vaccine candidate against human visceral leishmaniasis (VL), consisting of Virus-Like Particles (VLP) loaded with three different recombinant proteins (LJL143 from *Lutzomyia longipalpis* saliva as the vector-derived (VD) component, and KMP11 and LeishF3+, as parasite-derived (PD) antigens) and adjuvanted with GLA-SE, a TLR4 agonist.

No apparent adverse reactions were observed during the experimental time-frame, which together with the normal hematological parameters detected seems to point to the safety of the formulation. Furthermore, measurements of antigen-specific cellular and humoral responses, generally higher in immunized *versus* control groups, confirmed the immunogenicity of the vaccine formulation. Interestingly, the immune responses against the VD protein were reproducibly more robust than those elicited against leishmanial antigens, and were apparently not caused by immunodominance of the VD antigen. Remarkably, priming with the VD protein alone and boosting with the complete vaccine candidate contributed towards an increase of the immune responses to the PD antigens, assessed in the form of increased *ex vivo* CD4+ and CD8+ T cell proliferation against both the PD antigens and total *Leishmania* antigen (TLA).

Overall, our immunogenicity data indicate that this innovative vaccine formulation represents a promising anti-*Leishmania* vaccine whose efficacy deserves to be tested in the context of the “natural infection”.

Reprinted from PLoS Neglected Tropical Diseases 2017; 11(11): e0005951. doi:10.1371/journal.pntd.0005951.

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RESEARCH ARTICLE

Pre-clinical antigenicity studies of an innovative multivalent vaccine for human visceral leishmaniasis

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Citation: Cecílio P, Pérez-Cabezas B, Fernández L, Moreno J, Carrillo E, Requena JM, et al. (2017) Pre-clinical antigenicity studies of an innovative multivalent vaccine for human visceral leishmaniasis. PLoS Negl Trop Dis 11(11): e0005951. https://doi.org/10.1371/journal.pntd.0005951

Editor: Hechmi Louzir, Institut Pasteur de Tunis, TUNISIA

Received: March 16, 2017

Accepted: September 11, 2017

Published: November 27, 2017

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The research leading to these results received funding from the European Community's Seventh Framework Programme under grant agreement No.603181 [Clinical Studies on a Multivalent Vaccine for Human Visceral Leishmaniasis (MuLeVaClin)] and from the project NORTE-01-0145-FEDER-000012, supported by

Abstract

The notion that previous infection by *Leishmania* spp. in endemic areas leads to robust anti-*Leishmania* immunity, supports vaccination as a potentially effective approach to prevent disease development. Nevertheless, to date there is no vaccine available for human leishmaniasis. We optimized and assessed *in vivo* the safety and immunogenicity of an innovative vaccine candidate against human visceral leishmaniasis (VL), consisting of Virus-Like Particles (VLP) loaded with three different recombinant proteins (LJL143 from *Lutzomyia longipalpis* saliva as the vector-derived (VD) component, and KMP11 and LeishF3+, as parasite-derived (PD) antigens) and adjuvanted with GLA-SE, a TLR4 agonist. No apparent adverse reactions were observed during the experimental time-frame, which together with the normal hematological parameters detected seems to point to the safety of the formulation. Furthermore, measurements of antigen-specific cellular and humoral responses, generally higher in immunized *versus* control groups, confirmed the immunogenicity of the vaccine formulation. Interestingly, the immune responses against the VD protein were reproducibly more robust than those elicited against leishmanial antigens, and were apparently not caused by immunodominance of the VD antigen. Remarkably, priming with the VD protein alone and boosting with the complete vaccine candidate contributed towards an increase of the immune responses to the PD antigens, assessed in the form of increased *ex vivo* $CD4^+$ and $CD8^+$ T cell proliferation against both the PD antigens and total *Leishmania* antigen (TLA). Overall, our immunogenicity data indicate that this innovative vaccine formulation represents a promising anti-*Leishmania* vaccine whose efficacy deserves to be tested in the context of the "natural infection".

PLOS | NEGLECTED TROPICAL DISEASES

Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). Additionally, this study was funded in part by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases (JGV, SK, FO). PC and BPC were supported by fellowships from the European Community's Seventh Framework Programme under grant agreements No. 603181 (Project MuLeVaClin) and No. 603240-2 (Project NMTryPI), respectively. PC was also supported by Foundation for Science and Technology (FCT), Portugal, through the individual grant SFRH/BD/121252/2016. JM and JMR are supported by a grant from Fondo de Investigaciones Sanitarias (ISCIII-RETICRD16/0027/0008-FEDER). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Author summary

Although vaccination is accepted as a potentially effective approach to prevent leishmaniasis, to date there is no vaccine available for human disease. The research on the topic is therefore extremely important, and the design and testing of new vaccine approaches, as well as non-traditional immunization schemes continues to be as relevant as before. This study proposes an innovative vaccine approach for human visceral leishmaniasis, not only due to its multi-antigen nature which contemplates both parasite and vector derived proteins, but also because it explores the possibility of the use of *Influenza* virosomes as antigen-delivery vehicles. A strong TLR-4 agonist completes the vaccine formulation. Here we show the rationale-behind this vaccine approach, the safety of all the vaccine components in our *in vivo* context, and immunogenicity studies of the optimized vaccine candidate in mice that explored the contribution of the virosome to the antigen-elicited immune responses. Additionally, we tested an unusual immunization scheme that potentiated the final vaccine-elicited immune responses. This prime-boost immunization approach gives relevance to the use of both parasite and vector derived antigens together as an anti-*Leishmania* vaccine, and proposes a new strategy for vaccination in endemic areas, where people are constantly exposed to sand fly bites.

Introduction

Leishmaniasis is a spectrum of pathological outcomes caused by different *Leishmania spp*., intracellular parasites with a complex life cycle requiring a susceptible host and a permissive vector [1]. Visceral leishmaniasis, the most severe form of the disease, fatal if untreated, is caused by *L. donovani* and *L. infantum*, parasite species that migrate to the liver, spleen and bone marrow [2–4]. It has a worldwide distribution, being endemic in 74 countries, representing more than 37% of the total Earth terrestrial area [5]. Every year an estimated 0.2 to 0.4 million new VL cases occur and more than 20 000 people die, mostly in developing nations where access to healthcare is limited [6]. Furthermore, scarce and sometimes ineffective treatment options challenge leishmaniasis control [7].

Vaccination is considered one of the most cost/effective ways to control *Leishmania* infection. However, no human leishmaniasis vaccine is currently available. Several candidates have been proposed during the past few decades [8]. Some were shown to be immunogenic and have conferred protection against *Leishmania* in rodent models. Nevertheless, most of them were discarded after proving to be ineffective in large animals [8, 9]. Furthermore, most of these studies shared a limitation which may have been responsible for the overestimation of the vaccine candidates effectiveness: they had a binomial focus (host-parasite) and disregarded the contribution of the vector, essential in vaccine efficacy determination as highlighted by Peters *et al* who showed the loss of protection of a potentially-good vaccine candidate when tested in the context of vector-transmitted leishmaniasis [10].

Leishmania parasites are transmitted by sand flies from the genera *Lutzomyia* and *Phlebotomus* in a specific vector-*Leishmania spp*. pairing [11]. During the sand fly blood meal, parasites together with vector derived factors, including saliva, are introduced into host skin [11–13]. Previous exposure to sand fly salivary components has been shown to confer protection against vector-transmitted *Leishmania* [14, 15]. Furthermore, in recent studies, protection against natural transmission of *Leishmania* has been attained by vaccination with defined salivary molecules in animal models for both cutaneous leishmaniasis and VL [16, 17].

Interestingly, these proteins were shown to improve the protection induced by live anti-*Leishmania* vaccines [18, 19]. Fundamentally, the Th1 immune response elicited against a salivary molecule can adversely impact parasite establishment in the host.

This study proposes a novel vaccine candidate based on defined antigens of both parasite (KMP11 and LeishF3+, the latter a fusion protein consisting of Nucleoside hydrolase, Sterol 24-c-methyltransferase and Cysteine protease B), and sand fly vector (salivary protein LJL143) origins, formulated into *Influenza* virosomes and adjuvanted with GLA-SE, a TLR-4 agonist. The sand fly antigen LJL143 was shown to produce a long lasting Th1 immune response in dogs, which impacted parasite growth *in vitro* [20]. One of the parasite-derived antigens, KMP-11, was already demonstrated to be individually effective against VL in the pre-clinical context [21], as were each of the individual components of the second parasite-derived antigen, the fusion protein LeishF3+ [22–24]. Additionally, LeishF3+ predecessor antigen (a fusion protein consisting of Nucleoside hydrolase and Sterol 24-c-methyltransferase, but not Cysteine protease B), was considered safe and immunogenic in the clinical context (Phase I trial) [25], as were also both the adjuvant and the virosomes [26, 27]. *Influenza* virosomes represent a unique vaccine delivery system, flexible but robust, that allows loading of a wide variety of antigens [28, 29]. The VLP-based antigen formulation has the potential to generate both $CD4^+$ and $CD8^+$ specific memory T cells, the latter due to the potentiation of cross-presentation events [30]. The immune response elicited by the immunization should induce a Th1 phenotype due to the adjuvant chosen and the presence of the sand fly salivary antigen [26, 31]. In theory, an immunized individual bitten by an infected sand fly and exposed to parasites and vector saliva, will quickly mount both a strong Th1 anti-*Leishmania*, and a strong Th1-DTH anti-sand fly saliva immune responses, resulting in prevention of infection establishment.

Here, we explore the safety and antigenicity of the vaccine candidate, using *ex-vivo* and *in-vivo* approaches.

Materials and methods

Ethics statement

Animal experiments were performed in accordance with the IBMC.INEB Animal Ethics Committee and the Portuguese National Authorities for Animal Health guidelines (directive 2010/63/EU). BPC and ACdS are accredited for animal research (Portuguese Veterinary Direction —DGAV, Ministerial Directive 113/2013). DGAV approved the animal experimentation presented in this manuscript under the license number 0421/000/000/2013.

The study with human Peripheral Blood Mononuclear Cells (PBMCs) was approved by the Hospital de Fuenlabrada (Madrid, Spain) Ethics and Research Committee (protocols APR12-65 and APR14-64), and all participants gave written informed consent to be involved.

Antigens and Adjuvant

KMP11. The *L. infantum* gene coding for KMP11 (LinJ.35.2260) was cloned in the vector pET-28b for expression in *E. coli* BL21 (DE3). Briefly, the gene was amplified from *Leishmania* genomic DNA (strain JPC) using the oligonucleotides 5'-CCATGGCCAC CACGTACGAG G (Fw; underlined is the *Nco*I restriction site) and 5'-GGATCCTTAC TTGGACGGGT ACTGCG (Rv; underlined is the *Bam*HI restriction site), and subcloned into pET-28b using a NcoI/BamHI restriction approach. The final construct (pET-LiKMP11; confirmed by DNA sequencing) was then transformed into *E. coli* BL21 (DE3) by electroporation. Protein expression was achieved by aired incubation of a bacterial suspension (OD600 0.6–0.8) for 4 hours at 37˚C, under selective pressure (25 μg/ml of kanamycin) and IPTG induction (1 mM). Protein was purified first by stepwise ammonium sulfate precipitation (KMP11 did not precipitate

until protein solution arose an 80% ammonium sulfate saturation) and afterwards by anion-exchange chromatography (DEAE-Sephacel; KMP11 eluted in 10 mM Tris/HCl (pH = 8,5), 150 mM NaCl). Finally, the protein was passed through a polymyxin-B agarose matrix (Sigma-Aldrich, MO, USA) for endotoxin removal. The purity was higher than 95%, as determined by SDS-PAGE and Coomassie staining.

LeishF3 and LeishF3+. The fusion protein LeishF3, used only during the optimization process of the vaccine candidate, was produced and purified as described elsewhere [25]. The upgraded version of LeishF3, LeishF3+ was originated by the fusion of an additional *Leishmania* antigen (CPB) to the two that constitute LeishF3 (NH and SMT), in a way to increase epitope diversity and consequently enhance human T cell recognition. Production and purification processes used are similar to the ones used previously [25].

LJL143. The sandfly salivary protein used during the optimization process of the vaccine candidate (his-tagged) was produced using a mammalian expression system, as explained elsewhere [20]. LJL143 used in the antigenicity pre-clinical assay *per-se* (non his-tagged) was obtained using a yeast expression system (more cost-effective). Briefly, DNA coding for LJL143 without the signal peptide was codon optimized based on *Pichia pastoris* usage preference and subcloned into Pichia secretory expression vector pPICZαA (Invitrogen) using EcoRI/XbaI restriction sites. The correct insert sequence and reading frame of recombinant plasmid was confirmed by double-stranded sequencing using vector flanking primers α-factor and 3'AOX-1 and then transformed into *Pichia pastoris* X-33 by electroporation. The expression of LJL143 was induced with 0.5% methanol at 30˚C for 72 hours and the highest expression clone was chosen for making seed stock with 20% glycerol. Large-scale expression of LJL143 was induced with methanol in 10L fermentation. Coomassie G-250 (Simply Blue) stained NuPAGE Bis-Tris gels (Invitrogen) were used to assess the purity of the recombinant protein.

Adjuvant. The synthetic TLR-4 agonist Glucopyranosyl Lipid A (GLA-SE) was produced and provided by IDRI, as previously reported [32].

VLP-based antigen formulation process

Four different virosomal preparations have been specifically designed for this study, three of them containing each of the individual antigens, and one containing all the three antigens together. Briefly, a solution containing 1 mg of inactivated *Influenza* virus A/H1N1/California was pelleted at 286 000g for 1 hour, dissolved in presence of 0.5 ml of PBS containing 0.1 M of Octaethyleneglycol mono (n-dodecyl) ether (OEG; Sigma Aldrich, MO, USA), and then mixed with 32 mg of phosphatidylcholine (Lipoid Ag, Steinhausen, Switzerland) dissolved in 1.5 ml of PBS-OEG 0.1 M. The mixture was centrifuged at 100 000g for 30 min and the supernatant containing Haemagglutinin and Neuraminidase was recovered. For the individual virosomal formulations, the obtained supernatant was then mixed with 2 mg of Leish-F3 (or Leish-F3+), or KMP11 or LJL143 in presence of detergent. Virosomes were then formed by detergent removal and sterile-filtered. The virosome particles containing the mixture of the three antigens were produced similarly, from 1 mg of starting influenza protein mixed with 1 mg of each antigen (Leish F3+, KMP11 and LJL143). Size determination and distribution of the particle population was performed using a Zetasizer Nano instrument (Malvern Instruments, Malvern, UK). Parasite derived and/or VD proteins content in virosome particle was determined by SDS-PAGE Coomassie Stained.

Human PBMCs *ex vivo* assays

Subjects included in this study were residents of a *L. infantum* post-outbreak area. Up to 14 healthy endemic individuals (theoretically never exposed to *Leishmania*), 11 asymptomatic

subjects (positivity to the *in vitro* PBMC proliferation assay to soluble *Leishmania* antigen) and 21 cured VL patients (clinically diagnosed with VL; presence of *Leishmania* confirmed in blood by PCR; three months after successful treatment with liposomal amphotericin B) were included in the antigenicity assays. Blood samples were collected at the hospital blood bank and the internal medicine department (Hospital of Fuenlabrada, Madrid). PBMCs were prepared by density gradient centrifugation of heparinized blood samples (Lymphocyte Isolation Solution, RAFER, Spain). PBMCs were adjusted up to 2×10^6 cells/ml in complete medium (RPMI 1640 supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2mM L-glutamine, 25mM HEPES and 10% heat inactivated fetal calf serum), and cultured in 96-well plates at a density of 2×10^5 cells per well for 5 days with either KMP11 (10 μg/ml), LeishF3 (10 μg/ml), LeishF3+ (10 μg/ml), LJL143 (10 μg/ml), soluble leishmanial antigen—SLA (10 μg/ml) or PHA-M (5 μg/ml) in a final volume of 200 μl per well. The supernatants of the *in vitro* cell cultures were collected and stored at -20˚C for cytokine quantification. Interferon-γ, granzyme B, TNF-α, and IL-10, were quantified in culture supernatants, using the BD Cytometric Bead Array Human Flex Set (BD Biosciences, NJ, USA) following the manufacturer's instructions. Data were acquired using a FACSCalibur flow cytometer and analyzed using the Flow Cytometric Analysis Program Array (BD Biosciences, NJ, USA).

Mice

Six to eight weeks old male BALB/c mice (Charles River Laboratories, France) were maintained under specific-pathogen free conditions at the IBMC facilities, with water and food *ad libitum*.

General *in vivo* experimental layout

Animals were immunized intramuscularly with a maximum of 50 μl of the respective formulation in the thigh. The volumes administered were based on the concentration of the antigen/adjuvant preparations, and adjusted to equivalent final volumes with PBS. Unless otherwise stated, BALB/c mice were immunized three times at four weeks intervals, in the two thighs alternately. Four weeks after the last immunization, animals were euthanized by cervical dislocation, under volatile anesthesia (Isoflurane, Piramal healthcare, Northumberland, UK).

Mice immunizations

Two major *in vivo* experimental set ups originated this work, the first one for optimization purposes, and the second one as the actual pre-clinical trial.

Optimization of the vaccine candidate: Determination of the best antigen and adjuvant doses. Seven BALB/c mice/group were immunized with 1 μg or 5 μg of adjuvant and 1 μg or 5 μg of each individual virosomal preparations of LJL143, KMP11 and LeishF3 (formulated-antigens, VPA). In parallel, fourteen BALB/c mice received adjuvanted non-formulated antigens (PA) in the same two doses (1 and 5 μg). One μg of non-adjuvanted antigens (P) or PBS were injected in control animals.

Optimized-vaccine pre-clinical trials. Seven BALB/c mice/group were immunized with adjuvanted non-formulated proteins [1 μg of each component; PA (1+1+1)] or with adjuvanted formulated antigens (VPA). Two different VPA combinations were tested regarding antigen quantities: 1+1+1 or 1+5+5 indicate the administered dosages in μg of LJL143, KMP11 and LeishF3+ (individual virosome formulations). VPA (Mix) refers to the third virosome formulation tested, in which three antigens (1 μg, each) were simultaneously formulated in the same virosome. In parallel, an extra group received adjuvanted non-formulated proteins (GLA-SE+KMP11+LJL143+LeishF3+; 1 μg each), three weeks after priming with 1 μg of non-

adjuvanted LJL143 (Pre-LJL PA). Only the adjuvant (A) or the adjuvanted empty-virosome (VA) were injected in the negative control groups. The experimental timeline is represented in S2 Fig.

Mice whole blood/sera collection

Blood from mice was collected through intracardiac puncture under isoflurane anesthesia. One hundred μl of blood were immediately dispensed to a pre-heparinized tube to be used for general hematological determinations. The remaining volume was left to clot, and serum was then collected and stored at -80˚C for posterior immunoglobulin titration.

Splenic aseptic collection and processing

Mice were disinfected using 70% ethanol. Thereafter, abdominal skin was cut with sterile scissors and removed to expose the abdomen. Peritonea were then opened using a new pair of sterile scissors and tweezers, and the spleens harvested to pre-weighed 15 mL falcon tubes containing 5 ml of complete RPMI (Lonza, Switzerland) [10% heat-inactivated FBS (Lonza, Switzerland), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (BioWhittaker, Walkersville, MD, USA)] supplemented with 50 μM 2-mercaptoethanol (Sigma-Aldrich, MO, USA). Falcon tubes were re-weighed to obtain spleen masses. Splenic single cell suspensions were then obtained using Falcon™ Cell Strainers (Fisher Scientific, MA, USA), and their concentrations determined using an EVE™ automatic cell counter (NanoEntek, Seoul, Korea).

Cellular proliferation assays

Ten million cells per animal were pelleted, washed twice with PBS and stained for 10 minutes at 37˚C with CFSE (1 μM; in 1 ml of PBS; Thermo Fisher Scientific, MA, USA). Complete RPMI was then added to cell suspensions to stop the reaction, that were then pelleted, re-suspended in complete RPMI and incubated at 4˚C for 5 minutes. Afterwards, cells were once more centrifuged (5 min, 350 g), resuspended in complete RPMI supplemented with 50 μM 2-mercaptoethanol (Sigma-Aldrich, MO, USA), and plated into u-bottom 96-well plates at the final amount of 2.5 x 10^5 cells per well. After plating, different stimuli were added, depending on the experiment: individual non-formulated antigens (KMP11, LJL143 or LeishF3(+); 10 μg/ml), a pool of the three recombinant antigens (KMP11+LJL143+LeishF3+; 10 μg/ml each), and total *Leishmania* antigen (TLA; equivalent to 10 parasites per cell). Concanavalin A (3 μg/ml) and complete RPMI medium were added as positive and negative controls, respectively. From each animal, a single CFSE staining was performed, being only then the cells divided and stimulated. This warrants that any proliferating cells, regardless the condition, comes from the same initial suspension. Cells were incubated (37˚C and 5% CO_2) for three (positive controls) or four days (remaining stimuli), and then the originated cell culture supernatants were collected and stored at -80˚C for cytokine quantification. Each determination was performed in duplicate. Proliferating $CD4^+$ and $CD8^+$ cell populations were determined by Flow Cytometry, based on the premise that CFSE intensity gradually decreases after each cell division.

Flow cytometry

The anti-mouse monoclonal antibodies used to perform this study were all purchased from BioLegend (CA, USA) unless otherwise stated: FITC labeled anti-MHC-II(I-Ad) (AMS-32.1, BD Biosciences, NJ, USA), anti-IFN-γ (XMG1.2) and anti-IL-17A (TC11-18H10.1); PE labeled anti-CD8 (53–6.7, BD), anti-Siglec-F (E50-2440, BD), anti-IL-4 (11B11) and anti-IL-6 (MP5-20F3); PerCP-Cy5.5 labeled anti-Ly6C (HK1.4) and anti-TNFα (MP6-XT22); PE-Cy7 labeled

anti-CD3 (HA2) and anti-CD11b (M1/70); APC-Cy7 labeled anti-CD11c (N418); APC labeled anti-CD19 (6D5), anti-IL-5 (TRFK5) and anti-IL-10 (JES5-16E3); BV510 labeled anti-CD4 (RM4-5) and Pacific Blue™ labeled anti-Ly6G (1A8).

To analyze lymphoid and myeloid cell populations, two panels of antibodies were designed. The lymphoid panel was composed of anti-CD8, -CD3, -CD4, and -CD19. The Myeloid panel comprised anti-CD11b, -CD11c, -Siglec-F, -Ly6C, -Ly6G and -MHC-II. Surface staining of splenic cells was performed in PBS + 0.5% BSA (20 min, 4˚C) followed by 15 min fixation with 2% PFA. For intracellular staining (non-specific cytokine production), splenocytes were cultured for 2h with PMA/Ionomycin (50/500 ng/ml) and then Brefeldin A (10μg/mL) was added for 2 additional hours. Cells were surface stained, fixed and permeabilized with 1% saponin (Sigma-Aldrich, MO, USA) and then intracellularly stained [33]. Samples were acquired in a FACSCanto (BD) and analyzed with FlowJo software v10 (TreeStar, OR, USA).

An initial gate plotting FSC-A versus SSC-A was performed. Afterwards, singlets were selected by plotting FSC-A versus FSC-H and the remaining cell populations were resolved. T lymphoid cell populations were defined as $CD3^+/CD4^+$ and $CD3^+/CD8^+$ while B cells were defined as $CD19^+$. Non-specific cytokine production by T cells was assessed within $CD3^+/CD4^+$ and $CD3^+/CD8^+$ cells. Myeloid cell populations were gated as eosinophils (Siglec-F^+/SSC-$H^{int/high}$), neutrophils ($CD11b^{high}$/$Ly6G^{high}$/Siglec-F^-), DCs ($CD11c^+$/MHC-$II^{int/high}$) and monocytes/macrophages ($CD11b^+$/$CD11c^-$/$Ly6G^-$/Siglec-F^-). Proliferating T cells ($CD4^+$ or $CD8^+$) were defined as $CFSE^{int/low/neg}$ (FITC channel), always comparing each condition with the respective negative control.

Determination of mouse cytokines by ELISA

Cytokines were quantified, according to the manufacturer, using the commercial kits: Mouse IL-10 DuoSet ELISA, (R&D Systems, MN, USA), IL-12p70, IL-4 and IFN-γ ELISA MAX Deluxe (BioLegend, CA, USA).

Hematological parameters determination

Uncoagulated murine blood samples were used to obtain a complete blood evaluation, including haemoglobin and hematocrit levels, and total red blood cell, white blood cell, and platelet counts, using an automated blood cell counter (Sysmex K1000, Hamburg, Germany).

Serum immunoglobulins titration by ELISA

For specific immunoglobulin titration assays, high protein binding 96-well plates were coated overnight at 4˚C, individually with each one of the three antigens comprising the vaccine formulation (1 μg/ml), with a pool of the three antigens (1 μg/ml each), or with soluble *Leishmania* antigen (SLA; 1 μg/ml); all solutions were prepared in NaHCO3 0.1 M. Additionally, total IgG levels were also determined, using as a coating agent α-mouse IgG (1 μg/ml; Southern Biotech, AL, USA). Plates were then washed with PBS Tween 0.1%, blocked with 1% gelatin in PBS (blocking buffer) for 1 hour at 37˚C and re-washed. Each serum was then serially diluted (twofold, 7 dilutions) in blocking buffer. Wells filled with just blocking buffer were used as blanks. Plates were incubated for 1 hour at 37˚C and re-washed. Afterwards IgG and isotypes, IgM and IgE were detected using horseradish peroxidase (HRP) coupled α-mouse antibodies [diluted 1:5000 (IgM, IgE, IgG1, IgG3, IgG2b and IgG2a; Southern Biotech, AL, USA) or 1:8000 (IgG; Southern Biotech, AL, USA) in blocking buffer; incubated for 30 minutes, at 37˚C]. The plates were washed for a last time, and the substrate (orthophenyldiamine (OPD) in citrate buffer) was added for 10 minutes, time after which the reaction was stopped with

HCl 3 N. Absorbance values were determined at 492 nm in a Synergy™ 2 Multi-Mode Reader (BioTek instruments, VT, USA).

The value of the last dilution factor for which the corrected optical density was equal or higher than 0.1 was the defined titer of the antibody (endpoint titer), as has been previously described [34].

Statistical analysis

Results are generally expressed per individual animals/samples, with a representation of the group mean value ± standard deviation. Statistical differences were analyzed using GraphPad Prism v6.01 (CA, USA). Mice experimental groups were compared using either the one-way ANOVA or the unpaired t-test. Comparisons between human samples were performed using Mann–Whitney test.

Results

Rationale behind the vaccine formulation and optimization steps

Optimal antigen/adjuvant doses. To determine if the specific responses elicited by vaccination are dependent on the doses of antigens/adjuvants, two different antigen/adjuvant amounts were tested *in vivo*. Initially, vaccine-elicited specific humoral and cellular immune responses were evaluated in mice immunized with either 1 μg or 5 μg of adjuvant/formulated-antigens (VPA) and compared with those obtained for control groups that received either non-adjuvanted formulated antigens (VP; 1 μg of each antigen) or PBS. The specific $CD4^+$ and $CD8^+$ T cells proliferation levels determined against LJL143, as well as the IgG antibody titers detected were higher in animals immunized with the adjuvanted (VPA; 1 μg of antigen) in comparison with animals immunized with the non-adjuvanted (VP; 1 μg of antigen) formulations (Fig 1i and 1ii; $p \leq 0.05$, $p \leq 0.001$ and $p \leq 0.01$, respectively), indicating the necessity of the adjuvant inclusion in the vaccine formulation. Interestingly, the $CD4^+$ proliferating T cell levels in response to both LJL143 and LeishF3 were higher in animals that received 1 μg of formulated-antigens/adjuvant in comparison with those that received 5 μg (Fig 1i; $p \leq 0.0001$). The same response was observed for $CD8^+$ proliferating T cell levels against LJL143 ($p \leq 0.01$), while no differences between groups were detected in response to LeishF3 (Fig 1i). Of note, the responses against LeishF3 were weaker compared to those mounted against LJL143, and the magnitude of the immune response was higher for $CD4^+$ than $CD8^+$ T cells (Fig 1i). There was no difference in the proliferation of either $CD4^+$ or $CD8^+$ T cells between groups in response to KMP11 (Fig 1i). The picture concerning the elicited specific humoral immune response is reversed. The detected IgG titers specific for LJL143 were higher in animals that received 5 μg of formulated-antigens/adjuvant, compared with ones that received 1 μg ($p \leq 0.05$) (Fig 1ii). Humoral reactivity was not detected in this experiment against either LeishF3 or KMP11.

A similar experimental layout was used to assess responses in animals that received adjuvanted non-formulated antigens (PA) in the same two doses (1 and 5 μg), in comparison with control groups that received either non-adjuvanted antigens (P; 1 μg of each antigen) or PBS. An adjuvant effect on the antigen-elicited immune responses was observed for LJL143 and LeishF3 (S1i and S1ii Fig; PA *versus* P; $p \leq 0.05$–0,001). Furthermore, without formulation, LJL143 retained its higher immunogenicity compared to LeishF3 and KMP11 (S1i and S1ii Fig). Moreover, the two doses of antigens induced similar $CD4^+$ and $CD8^+$ T cell responses against LJL143; the responses were higher in animals that received 1 μg compared to 5 μg for LeishF3, and were similar to controls for KMP11 (S1i Fig). In line with the verified for formulated antigens, the detected specific humoral responses against both LJL143 and LeishF3 were

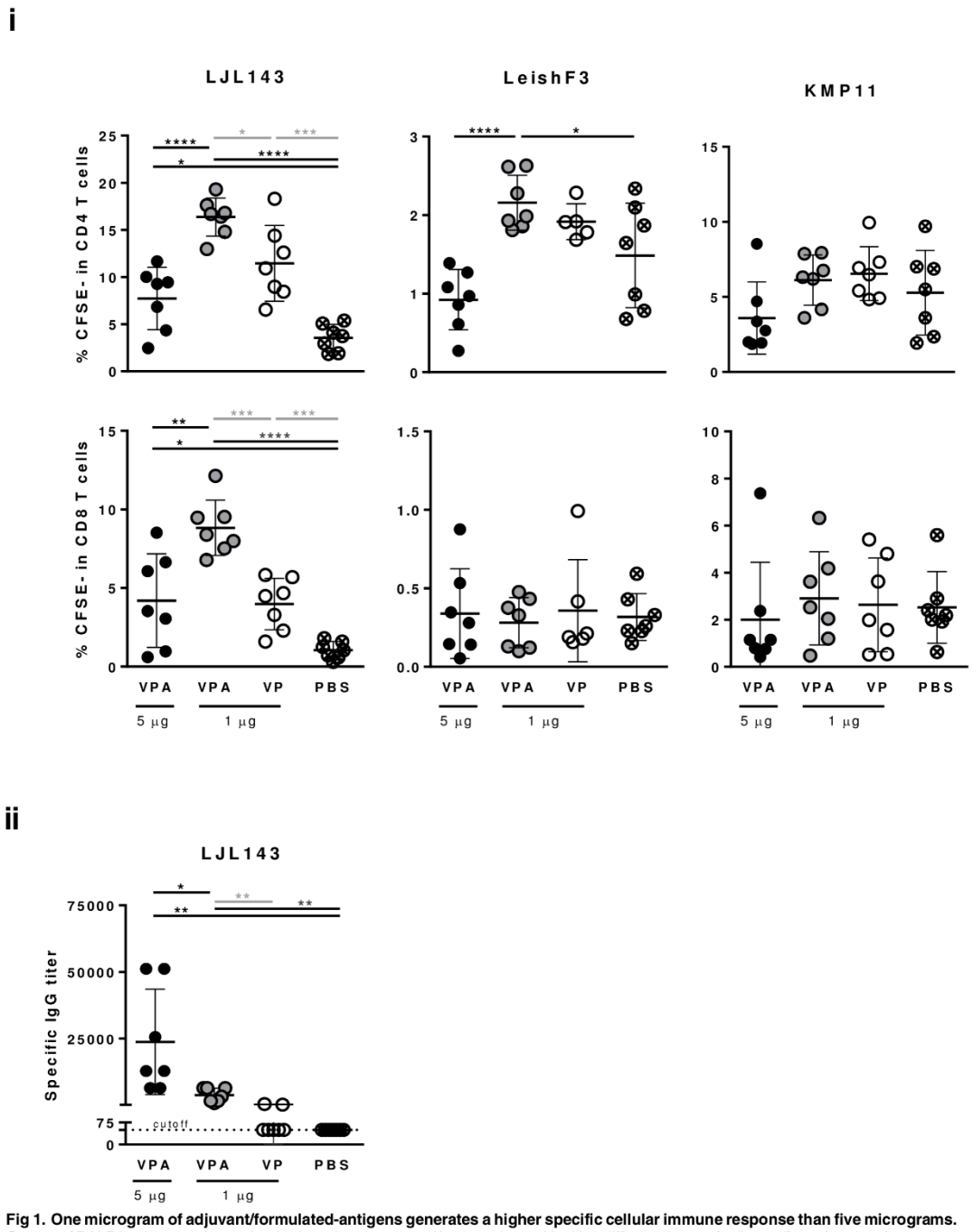


Fig 1. One microgram of adjuvant/formulated-antigens generates a higher specific cellular immune response than five micrograms. Groups of BALB/c mice were immunized 3 times i.m. (separated by 4 weeks each) with 2 different doses of adjuvant/formulated-antigens: 1 µg

and 5 μg of each individual component (VPA). Non-adjuvanted formulated antigens (VP; 1 μg of each antigen) or PBS were injected in animals from the 2 control groups. Four weeks after the last immunization, animals were euthanized and their spleens and sera collected. (i) Specific $CD4^+$ and $CD8^+$ T cell proliferation was assessed by Flow Cytometry four days after CFSE-stained splenocytes-culture in the presence of LJL143, LeishF3 or KMP11 (10 μg/ml). (ii) Serum antigen specific IgG titers for LJL143 were determined by ELISA. Each dot represents one animal. Average and SD of the values within each group are shown. Statistical differences are properly identified (Unpaired t-test: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$).

https://doi.org/10.1371/journal.pntd.0005951.g001

higher in animals that received 5 μg of antigens/adjuvant and were not detected in any of the KMP11-immunized groups (S1ii Fig).

Immune recognition of the antigens by individuals from a VL-endemic area in Spain. The different immune response-magnitudes induced by the antigens in mice indicated that a final step to assess the immunogenicity of each antigen in humans, *ex-vivo*, was needed. Individual specific recognition of LJL143, LeishF3 and KMP11, was evaluated in PBMCs from asymptomatic and cured VL patients from a leishmaniasis high-incidence area (Madrid, Spain), by measuring the specific cytokine profile on supernatants after five days of stimulation. In parallel, PBMCs from endemic healthy individuals were used as controls. Remarkably, stimulation with KMP11 induced a better response. Samples from cured VL and asymptomatic patients, significantly ($p \leq 0.05$) and tendentiously secreted more IFN-γ than the controls (Fig 2i). Additionally, TNF-α and IL-10 responses generated against KPM11 were also tendentiously higher comparing infected (symptomatic and cured) with controls, while Granzyme B responses (excluding some outliers) were generally comparable among groups (Fig 2i). On the other hand, the responses detected against LeishF3 were lower in magnitude, and generally similar comparing the infected (asymptomatic and cured) with the controls (Fig 2i). Curiously, PBMCs from some individuals of the three different groups (Old World human samples) responded to LJL143, a salivary protein from *L. longipalpis*, the vector of VL in the New World (Fig 2i).

Due to the weak immunogenic response obtained against LeishF3 in humans, the immunogenicity of an improved version of this antigen, named LeishF3+ was assessed through the same *ex vivo* stimulation approach. Basically, to enhance human T cell recognition we increased epitope diversity by fusing an additional antigen, CPB, to LeishF3, to generate LeishF3+. As a mean of comparison, stimulation with LeishF3 and LeishF3+ was performed in parallel. Interestingly, the responses detected against LeishF3+, in terms of IFN-γ, TNF-α and Granzyme B were significantly improved, only in the group of samples from asymptomatics, in comparison with the ones induced by LeishF3 (Fig 2ii; $p \leq 0.01$, $p \leq 0.001$ and $p \leq 0.05$, respectively). On average, the IFN-γ, TNF-α, IL-10 and Granzyme B responses of samples from asymptomatic patients detected against LeishF3+ were 50, 25, 15 and 10 fold higher than the responses obtained for control samples (Fig 2ii).

Vaccine pre-clinical antigenicity tests in mice

Different experimental groups were designed for the pre-clinical tests of the optimized vaccine candidate in mice to assess, besides the safety of the vaccine components, the influence of several variables in the final outcome of the immunization, such as the contribution of the virosome to the induction of immunogenicity or the possibility of immunodominance of the sand fly salivary protein (S2 Fig). In parallel, the effect of a prime with the sand fly-salivary protein in the final vaccine-elicited immune response was evaluated (S2 Fig). Defined above as essential to increase the antigens immunogenicity, the adjuvant (GLA-SE) was administered to all groups. Human immunogenicity studies dictated the replacement of LeishF3 by LeishF3+ as one of the three antigens of the optimized-vaccine.

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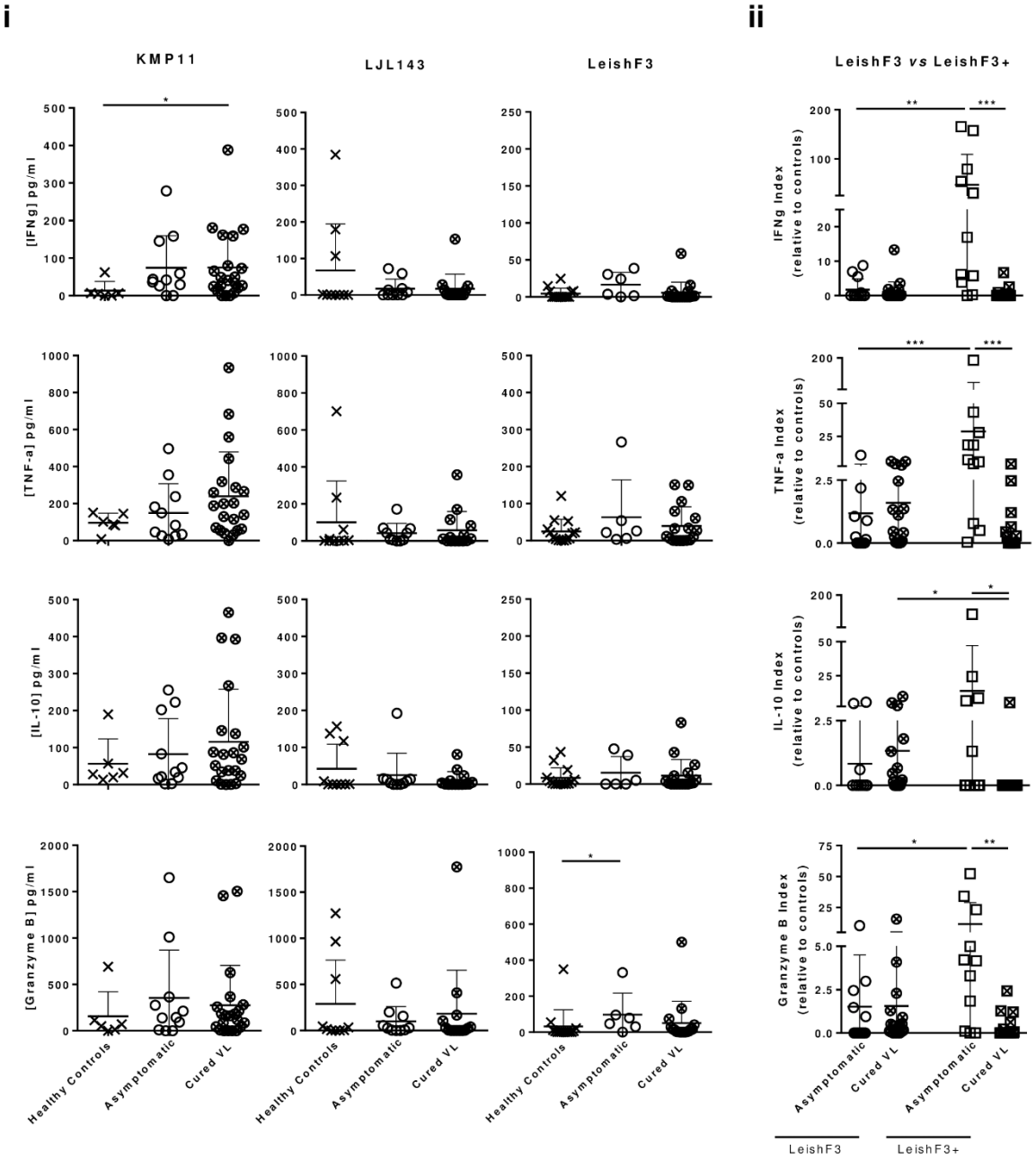


Fig 2. Antigen recognition in patients from a *L. infantum* endemic area in Spain. PBMCs were purified from peripheral blood collected from asymptomatic and cured VL patients or matched endemic controls. (i) Two hundred thousand cells were stimulated for 5 days with either KMP11 (10 μg/ml), LeishF3 (10 μg/ml) or LJL143 (10 μg/ml). Specific cell responses were identified through quantification of IFN-γ, TNF-α, IL-10 and Granzyme B in the resulting culture supernatants. (ii) A similar proliferation experiment was performed to compare human responses induced by the same amount (10 μg/ml)

of LeishF3, and of its improved version LeishF3+; presented data resulted from the normalization of the determined cytokine absolute values, in relation to the average values determined for endemic controls. Each dot represents one individual. Average and SD of the values within each group are shown. Statistical differences are properly identified (Mann–Whitney test: * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$).

https://doi.org/10.1371/journal.pntd.0005951.g002

Safety profile of the vaccine components. The safety profile of the different components was extrapolated by the determination of general hematological parameters in whole blood, antibody-specific IgE titers in serum, and cellular composition of the splenic cell compartment. The hematological parameters determined were comparable among groups and were within their normal ranges (Table 1). The spleen weights and cell numbers were also comparable among groups (S3i Fig). Consequently, the determined absolute numbers of lymphoid and myeloid splenic cell populations, were also in the magnitude of the normal ranges, and overall comparable among groups (S3ii and S3iii Fig). Furthermore, the responsiveness of $CD4^+$ T cells, evaluated through cytokine production after non-specific stimulation, was similar among groups (polyfunctional $CD4^+$ T cells; S3iii Fig), and no antigen-specific IgE titers were detected (S3iv Fig). Overall, the full panel of results points to the safety of all the vaccine components.

Immunogenic profile of the optimized vaccine. To determine the immunogenicity of the optimized vaccine (normal immunization scheme), specific-elicited humoral and cellular immune responses were characterized.

Antigen-specific humoral responses were determined in sera of vaccinated animals (normal immunization scheme) by ELISA. All groups showed specific responses against the pool of antigens, with comparable magnitudes among groups, and a mixed IgG1/IgG2a response (Fig 3i and 3ii). In contrast, the magnitude of antibody responses detected against individual antigens was distinct; the one against LeishF3+ was generally the highest and the one against KMP11 was non-detectable (Fig 3iii). Curiously, the virosome formulations promoted stronger humoral responses, specifically against LeishF3+ (Fig 3iii). To note, the best single-antigen specific isotype response was detected in the group of animals immunized with VPA (1+5+5) that showed a prevalence of IgG2a α-LJL143 and a mixed IgG1/IgG2a response against LeishF3+ (Fig 3iv).

Antigen-specific cellular immune responses were determined through properly controlled cell proliferation assays using splenocytes. Overall, all the groups of vaccinated animals (normal immunization scheme) showed specific cell proliferation against the pool of antigens, at levels comparable among groups for proliferating $CD4^+$ T cells (Fig 4i; $p \leq 0.0001$ *versus* controls), and with some minor differences among groups for $CD8^+$ T cells (Fig 4i). Looking at the results of individual proliferation against each of the antigens, T cell responses to LJL143 were slightly stronger than those detected for LeishF3+, while no significant responses were detected against KMP11 (Fig 4ii). However, these differences in response-magnitude seem not to be due to an immunodominance of the sand fly-derived antigen, because results of the groups that received VPA (1+1+1) and VPA (1+5+5) are overall comparable (Fig 4ii). Interestingly, and in line with the observed response of human PBMCs, the responses obtained against LeishF3+ were higher in magnitude, than the ones previously obtained against LeishF3 (Fig 4ii *versus* S1i Fig; PA (1+1+1) *versus* PA). To note, both the $CD4^+$ and $CD8^+$ T cell specific responses were higher in the groups that received either PA (1+1+1) or VPA (Mix) (Fig 4i and 4ii).

Cytokines quantified in the resulting supernatants from antigen-specific cell proliferation assays align with the cell proliferation results. All the groups of vaccinated animals (normal immunization scheme) showed specific production of both pro-inflammatory and regulatory/anti-inflammatory cytokines against the pool of antigens, at levels comparable among groups (Fig 5i; at least $p \leq 0.01$ comparing vaccinated groups with controls). The regulatory cytokine

Table 1. Hematological profile determined in the optimized vaccine pre-clinical trials.

	Erythrocytes (x 10^12 cells/L)	Hemoglobin (g/dL)	Hematocrit (%)	Leukocytes (x 10^9 cells/L)	Platelets (x 10^9 cells/L)
VPA (1+1+1)	8.299 ± 0.5661	13.51 ± 0.7712	40.54 ± 2.989	5.129 ± 1.810	568.6 ± 113.1
VPA (1+5+5)	8.539 ± 0.6421	13.76 ± 0.8404	41.14 ± 3.392	4.714 ± 1.593	507.6 ± 97.93
VPA (Mix)	9.014 ± 0.1952	13.87 ± 0.4424	45.49 ± 1.463	5.514 ± 1.389	517.6 ± 59.46
VA	8.356 ± 0.4527	13.40 ± 0.5538	40.61 ± 2.389	5.929 ± 1.665	415.9 ± 144.9
A	8.917 ± 0.8441	13.89 ± 1.051	44.50 ± 4.941	6.586 ± 1.710	389.9 ± 55.54
PA (1+1+1)	8.713 ± 0.3155	13.51 ± 0.4706	43.63 ± 2.094	4.771 ± 1.627	510.4 ± 157.8
Pre-LJL PA	8.681 ± 0.5582	13.40 ± 0.7141	42.80 ± 3.180	7.8 ± 2.998	386.1 ± 131.5

The values shown represent the mean ± standard deviation. Reference intervals, according to Charles River Laboratories (8–10 week male mice) are: 6.93–12.24 x 10^{12} cells/L for erythrocytes, 12.6–20.5 g/dl for hemoglobin, 42.1–68.3% for hematocrit, 3.48–14.03 x 10^{9} cells/L for leukocytes and 420–1698 x 10^{9} cells/L for platelets.

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IL-10 was detected at higher levels, followed by IFN-γ, IL-4 and TNF-α, four days after splenocytes stimulation with the pool of antigens (Fig 5i). The cytokine response detected against each of the individual antigens was also a mirror of the cell proliferation results (Fig 5ii; S4 Fig). Of note, a comparable or relatively higher IFN-γ *versus* IL-10 response against LJL143 was detected in the groups that received PA (1+1+1) and VPA (MIX), or VPA (1+1+1) and VPA (1+5+5), respectively (Fig 5ii). LeishF3+ induces both IFN-γ and IL-10 responses in lower magnitudes, compared to the responses observed for LJL143, with the IFN-γ/IL-10 balance being higher in the group that received the non-formulated antigens (Fig 5ii). Additionally, and although unexpectedly high, the IFN-γ response detected against KMP11 was non-specific in nature (magnitude similar between vaccinated and control groups; Fig 5ii).

The cellular response of vaccinated mice against total parasite extract (Total *Leishmania* Antigens–TLA) was also evaluated through cell proliferation assays. While the proliferation levels of $CD8^+$ T cells remained at the basal level, a tendency of increased $CD4^+$ T cells proliferation was observed at comparable levels for all immunized groups, compared with controls (Fig 6i). Nevertheless, comparing the proliferation levels against TLA with the proliferation levels against the individual vaccine antigens, they were generally low (Fig 4ii *versus* Fig 6i). Cytokine production in response to TLA stimulation followed the same pattern: generally absent in the control groups *versus* variable in immunized groups (Fig 6ii; no statistically significant differences). A predominance of pro-inflammatory cytokines (mainly IFN-γ) were detected over regulatory/anti-inflammatory ones (Fig 6ii).

Priming with the sand fly salivary protein LJL143 enhances the response generated against parasite-derived antigens and produces a significant response to TLA. Previous observations [18, 19, 35, 36] support that a priming with a sand fly salivary protein alone and boosting with the sand fly protein together with other *Leishmania* antigens, may positively influence the outcome of posterior immunizations with complex vaccine formulations. To understand its potential, we tested within the antigenicity clinical trial, the effect of a previous administration of non-adjuvanted LJL143 on the response elicited by the non-formulated optimized vaccine.

In terms of the specific humoral immune response, priming with the salivary protein promoted an overall decrease in specific IgG titers against the pool of antigens with a prevalence of IgG1 isotype titers, in comparison with their counterparts (Fig 3i and 3ii; Pre-LJL PA versus PA (1+1+1); $p \leq 0.05$).

Interestingly, while the specific cellular response detected against the pool of proteins or the sand fly salivary protein were comparable between the primed and non-primed groups, and

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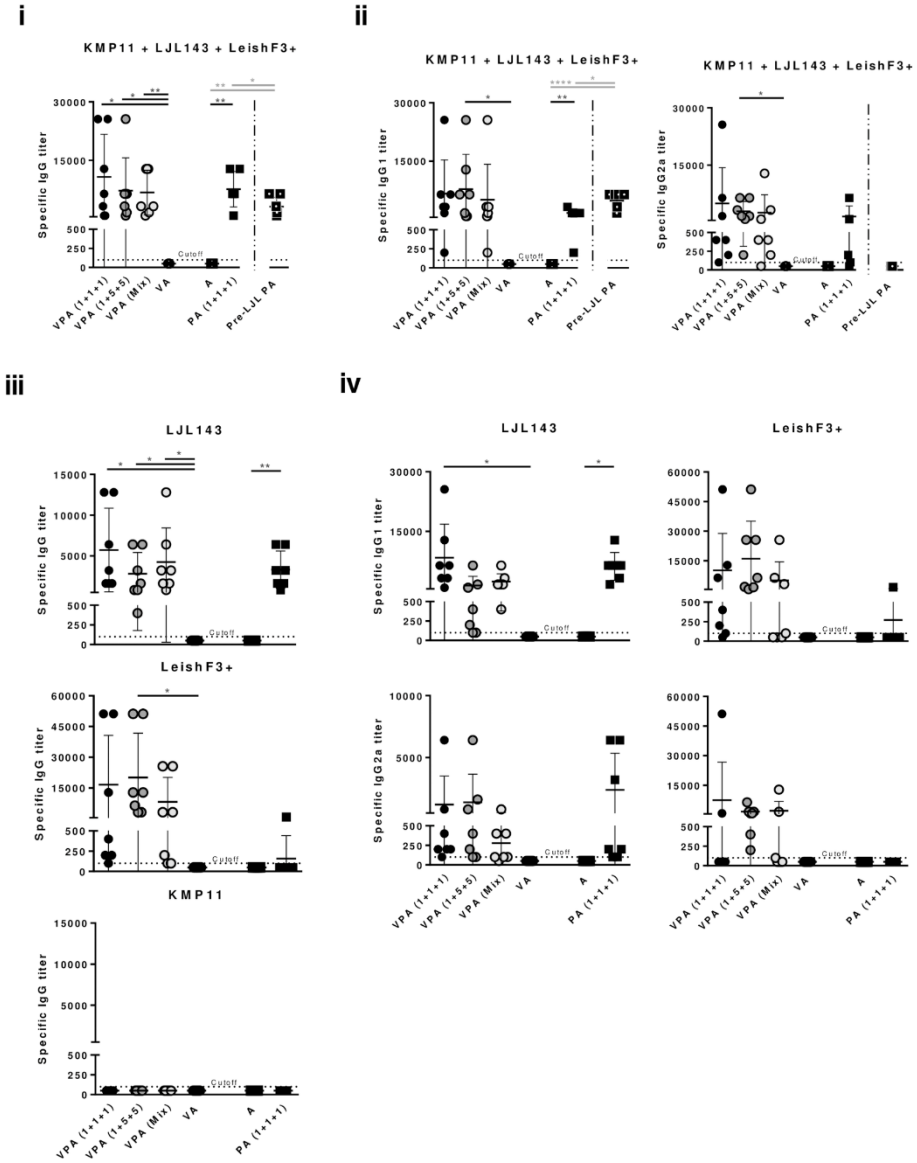


Fig 3. Antigen-specific vaccine-elicited humoral responses. Different experimental groups were designed for the development of the pre-clinical trials of the vaccine candidate in mice. Two groups represent negative controls: one composed by animals which received only the adjuvant (A), and other composed by animals that received the adjuvanted empty virosome (VA). The third group received non-formulated proteins with adjuvant in the dosage of 1 μg of each component [PA (1+1+1)]. The fourth was immunized with the same non-formulated proteins, but was primed with non-adjuvanted LJL143 three weeks before the first immunization (Pre-LJL PA). The three remaining groups received different formulations of adjuvanted formulated antigens (VPA). Two different VPA combinations were tested regarding antigen quantities: 1+1+1 or 1+5+5 indicate the administered dosages of formulated LJL143, KMP11 and LeishF3+ (individual virosome formulations). VPA (Mix) refers to the third virosome formulation tested, in which the three antigens (1μg each) were simultaneously formulated in the same virosome. Mice were immunized 3 times i.m. (separated by 4 weeks each),

euthanized 4 weeks after the last immunization, and their sera collected. Specific IgG or IgG1 and IgG2a titers against the pool of antigens (i or ii, respectively), and against each individual antigen (iii or iv, respectively) were determined by ELISA. Each dot represents one animal. Average and SD of the values within each group are shown. Statistical differences are properly identified (One-Way ANOVA or Unpaired t-test (for comparison between primed and non-primed animals): * $p \leq 0.05$, ** $p \leq 0.01$ and **** $p \leq 0.0001$).

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significantly different from the controls (Fig 4i; $p \leq 0.0001$), higher levels of proliferating $CD4^+$ T cells against LeishF3+ and KMP11 or TLA were significantly, or tendentiously detected in the group of animals that was primed with the salivary protein LJL143 (Fig 4ii and Fig 6i; Pre-LJL PA *versus* PA (1+1+1); $p \leq 0.05$). Regarding $CD8^+$ T cells, an apparent improvement of response was observed only against TLA when comparing primed with non-primed groups (Fig 6i). Cytokines quantified in the resulting supernatants from cell proliferation assays showed a robust production of IFN-γ against the pool of antigens in both primed and non-primed animals that was higher in the latter (Fig 5i; Pre-LJL PA *versus* PA (1+1+1); $p \leq 0.01$). In contrast, higher levels of IL-10 were detected against the pool of antigens in the group of animals that received the priming with LJL143 in comparison with its counterpart (Fig 5i;

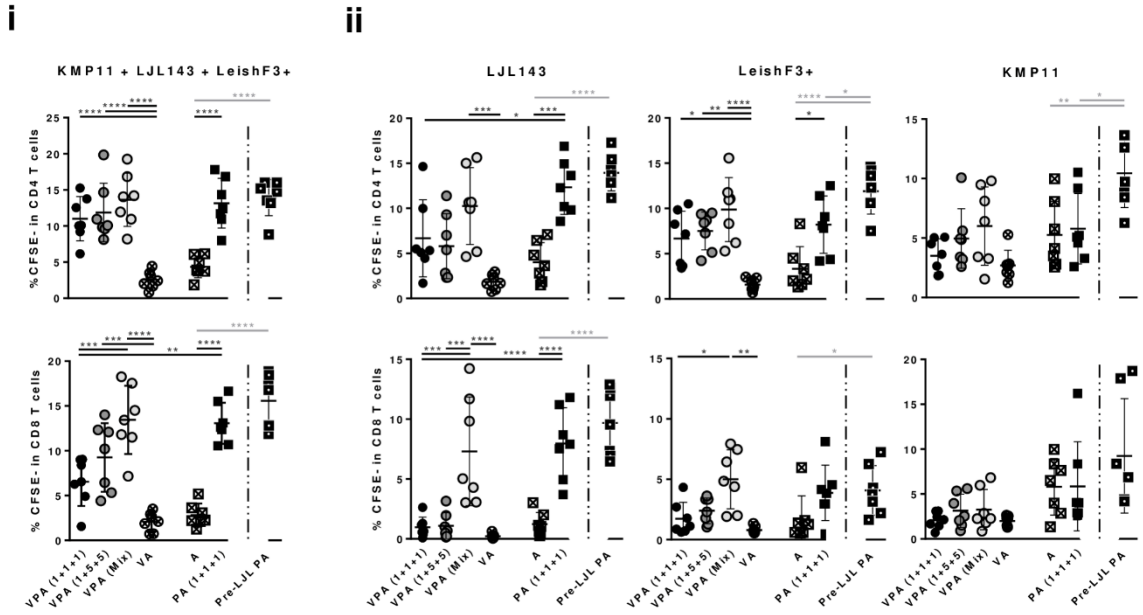
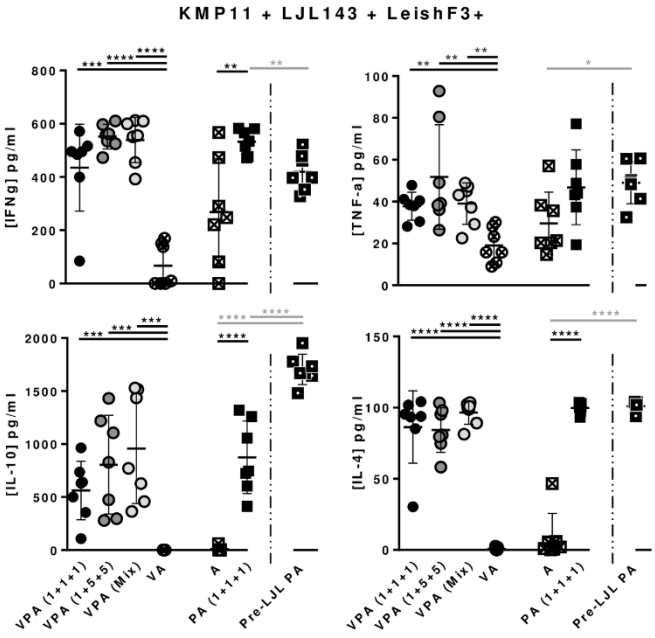


Fig 4. Antigen-specific vaccine-elicited T cell proliferation. Different experimental groups were designed for the development of the pre-clinical trials of the vaccine candidate in mice. Two groups represent negative controls: one composed by animals which received only the adjuvant (A), and other composed by animals that received the adjuvanted empty-virosome (VA). The third group received non-formulated proteins with adjuvant in the dosage of 1 μg of each component [PA (1+1+1)]. The fourth received the same non-formulated proteins, but was primed with non-adjuvanted LJL143 three weeks before the first immunization (Pre-LJL PA). The three remaining groups received different formulations of adjuvanted formulated antigens (VPA). Two different VPA combinations were tested regarding antigen quantities: 1+1+1 or 1+5+5 indicate the administered dosages of formulated LJL143, KMP11 and LeishF3+ (individual virosome formulations). VPA (Mix) refers to the third virosome formulation tested, in which the three antigens (1μg each) were simultaneously formulated in the same virosome. Mice were immunized 3 times i.m. (separated by 4 weeks each), euthanized 4 weeks after the last immunization, and their spleens collected and processed. Frequencies of proliferating splenic T cells ($CD4^+$ and $CD8^+$) were determined by flow cytometry, after four days of culture with the pool of antigens (10 μg/ml each) (i), or each of the individual antigens (10 μg/ml) (ii). Each dot represents one animal. Average and SD of the values within each group are shown. Statistical differences are properly identified (One-Way ANOVA or Unpaired t-test (for comparison between primed and non-primed animals): * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$).

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i


KMP11 + LJL143 + LeishF3+
[IFNg] pg/ml
[TNF-a] pg/ml
[IL-10] pg/ml
[IL-4] pg/ml
VPA (1+1+1)
VPA (1+5+5)
VPA (Mix)
VA
A
PA (1+1+1)
Pre-LJL PA

ii

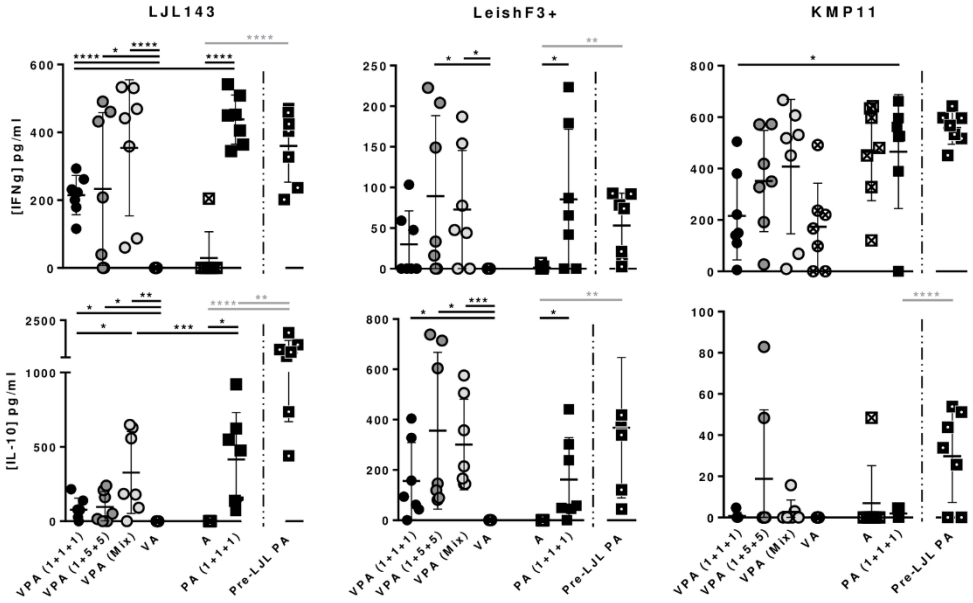

LJL143
LeishF3+
KMP11
[IFNg] pg/ml
[IL-10] pg/ml
VPA (1+1+1)
VPA (1+5+5)
VPA (Mix)
VA
A
PA (1+1+1)
Pre-LJL PA

Fig 5. Antigen-specific vaccine-elicited cellular cytokine responses. Different experimental groups were designed for the development of the pre-clinical trials of the vaccine candidate in mice. Two groups represent negative controls: one composed by animals which received only the adjuvant (A), and other composed by animals that received the adjuvanted empty-virosome (VA). The third group received non-formulated proteins with adjuvant in the dosage of 1 μg of each component [PA (1+1+1)]. The fourth was immunized with the same non-formulated proteins, but was primed with non-adjuvanted LJL143 three weeks before the first immunization (Pre-LJL PA). The three remaining groups received different formulations of adjuvanted formulated antigens (VPA). Two different VPA combinations were tested regarding antigen quantities: 1+1+1 or 1+5+5 indicate the administered dosages of formulated LJL143, KMP11 and LeishF3+ (individual virosome formulations). VPA (Mix) refers to the third virosome formulation tested, in which the three antigens (1 μg each) were simultaneously formulated in the same virosome. Mice were immunized 3 times i.m. (separated by 4 weeks each), euthanized 4 weeks after the last immunization, and their spleens collected. Typically pro-inflammatory and regulatory/anti-inflammatory cytokines were quantified by ELISA in the supernatants resultant from cellular proliferation assays against the pool of antigens (10 μg/ml each) (i). In parallel IFN-γ, and IL-10 levels were quantified by ELISA in the supernatants resultant from cellular proliferation assays against each of the individual antigens (10 μg/ml) (ii). Average and SD of the values within each group are shown. Statistical differences are properly identified (One-Way ANOVA or Unpaired t-test (for comparison between primed and non-primed animals): * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$).

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$p \leq 0.0001$). Comparing primed with non-primed groups, cytokine-secretion tendencies were maintained in response to LJL143, with a tendentiously lower production of IFN-γ and a significantly higher production of IL-10 (Fig 5ii; Pre-LJL PA *versus* PA (1+1+1); $p \leq 0.01$). A similar cytokine profile was detected in response to LeishF3+, while once more, the high IFN-γ levels detected in response to KMP11 were non-specific in origin (Fig 5ii). Remarkably, the cytokine response detected against TLA was higher in primed compared with non-primed groups [Fig 6ii; Pre-LJL PA *versus* PA (1+1+1)]. Of note, the production of IFN-γ in this group, in response to TLA, was five times higher in average than the secretion of IL-10 (Fig 6ii).

Discussion

Because in *Leishmania* spp. endemic areas the majority of infected persons do not develop clinical symptoms and previous infection leads to robust immunity against the parasite, vaccination is considered as one of the most viable ways to control *Leishmania* infection. However, to date there is no anti-*Leishmania* vaccine available for humans [3, 8]. This work proposes an innovative vaccine concept, consisting on Virus-Like Particles (VLP) loaded with 3 different antigens, two from the parasite and one from the sand fly vector, adjuvanted with a TLR4 agonist, as a strong candidate to fill in the existing gap in terms of human anti-*Leishmania* vaccines.

Although already demonstrated as a useful adjuvant in the context of anti-*Leishmania* vaccination, we considered it essential to determine the effect of GLA-SE on vaccine-elicited immune responses, mainly because the vaccine candidate we propose is much more complex than the one previously tested (single recombinant fusion protein) [25], with a multi-antigen nature and a virosomal component, which may itself have an adjuvant effect [37]. As expected, the adjuvant generally improved the antigen-elicited immune response, both in terms of specific cellular and humoral responses elicited by non-formulated antigens (S1i and S1ii Fig; PA *versus* P). Furthermore, similar results were obtained for virosome-formulated proteins (Fig 1i and 1ii; VPA *versus* PA) indicating, on one hand, the essentiality of the adjuvant in this vaccination context, and on the other that the *Influenza* VLP are working mainly as vehicles, and not as adjuvants in this context.

For almost two decades in vaccinology, the effect of the antigen dosage in the final outcome of the immunization has been studied and discussed, always in parallel with the concept of antigen affinity [38, 39]. Here, in order to define the optimal vaccine composition, based on the specific responses elicited, we tested two doses of antigens and adjuvant. A lower antigen/adjuvant dose, although is worse regarding the humoral immune response elicited (Fig 1ii, S1ii

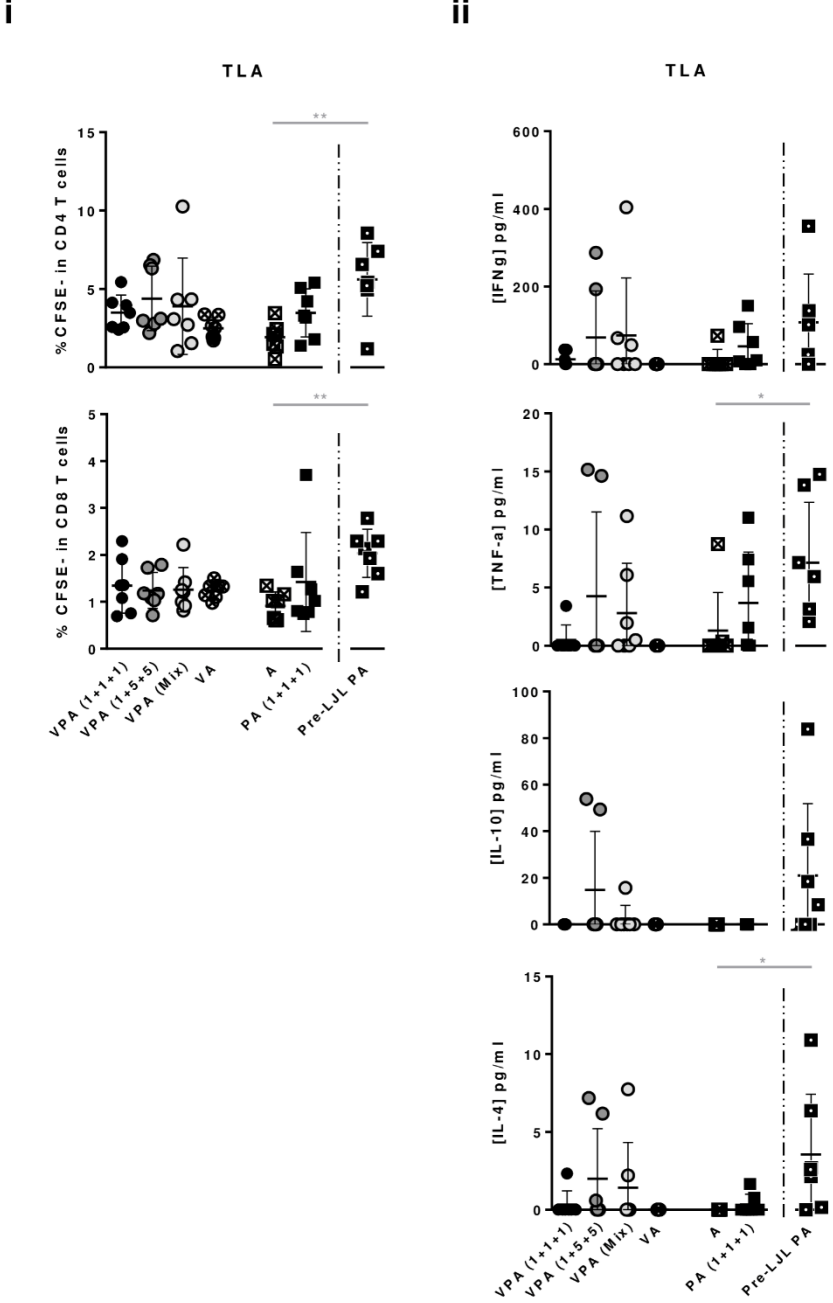


Fig 6. Determination of vaccine-elicited cellular immune responses against total parasite extract. Different experimental groups were designed for the development of the pre-clinical trials of the vaccine candidate in mice. Two groups represent negative controls: one composed by animals which received only the

adjuvant (A), and other composed by animals that received the adjuvanted empty virosomes (VA). The third group received non-formulated proteins with adjuvant in the dosage of 1 μg of each component [PA (1+1+1)]. The fourth received the same non-formulated proteins, but was primed with non-adjuvanted LJL143 three weeks before the first immunization (Pre-LJL PA). The three remaining groups received different formulations of adjuvanted formulated antigens (VPA). Two different VPA combinations were tested regarding antigen quantities: 1+1+1 or 1+5+5 indicate the administered dosages of formulated LJL143, KMP11 and LeishF3+ (individual virosome formulations). VPA (Mix) refers to the third virosome formulation tested, in which the three antigens (1μg each) were simultaneously formulated in the same virosome. Mice were immunized 3 times i.m. (separated by 4 weeks each), euthanized 4 weeks after the last immunization, and their spleens collected. (i) Frequencies of splenic proliferating T cells were determined by flow cytometry, after four days of culture with Total *Leishmania* Antigen (TLA; equivalent to 10 parasites/cell). (ii) Cytokines were quantified by ELISA in the supernatants from the cellular proliferation assays. Average and SD of the values within each group are shown. Statistical differences are properly identified (One-Way ANOVA or Unpaired t-test (for comparison between primed and non-primed animals): * $p \leq 0.05$ and ** $p \leq 0.01$).

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Fig), promotes a stronger specific cellular immune response against both LJL143 and LeishF3 (Fig 1i, S1i Fig). These results point therefore to the idea that "less is more", once it is generally accepted that cellular immunity is essential for *Leishmania* elimination [8], and justify the choice of the lower antigen/adjuvant dosages used in the pre-clinical trials *per se*.

In agreement with previous observations [40–42], we demonstrated the immunogenicity of KMP11 in humans. In fact, KMP11 was the antigen that generated a better response in the VL patients PBMCs stimulation experiments, with a significant increase in IFN-γ production by cells collected from cured VL patients compared with cells from matching endemic controls (Fig 2i). Such an observation was paramount to the final decision to include this particular antigen as a component of the innovative vaccine candidate. A possible explanation for the weak KMP11 immunogenicity detected in mice, which contrasts with previous studies in animals, is the use of the recombinant protein in opposition with the use of different DNA-based or heterologous recombinant live-vaccine approaches [21, 43–45].

On the other hand, the non-expressive response obtained in human *ex vivo* immunogenicity studies against LeishF3 (results generally similar between VL patients and controls; Fig 2i) was unexpected. A previous study showed the individual immunogenicity of NH and SMT, the two components of the fusion protein LeishF3, and successfully defined it as immunogenic and safe in a Phase I human clinical trial [25]. However, while the *ex vivo* immunogenicity assessment done by Coler and colleagues [25] was performed in a cohort from a *L. donovani* endemic area in Bangladesh, ours was performed using a cohort from a *L. infantum* endemic area in Spain, which may explain the lower-than-expected reactivity detected. These results led to the characterization of the immunogenicity of a LeishF3 "upgraded version" named LeishF3 + using the same human cohort, and the final substitution of LeishF3 by LeishF3+ in the vaccine formulation due to the observed improvement of the detected responses (Fig 2ii).

Interestingly, PBMCs from some individuals of the three different studied groups, including the controls (Old World human samples) responded to LJL143 (Fig 2i), a salivary protein from *Lutzomyia longipalpis*, the vector of VL in the New World. The sand fly salivary Lufaxin-like proteins are found in both the New and Old Worlds sand flies [46]. Within this family, LJL143 from *L. longipalpis* and PpeSP06, the homologous salivary protein from *P. perniciosus*, the main vector of VL in the Mediterranean Basin, share an amino acid sequence conservation of 45%. In line with this evidence, the reactivity, equally detected in samples from infected and non-infected individuals, is a potential indicator of immune cross-recognition of LJL143, with which in theory, the studied population has not been in contact before. These results further support the inclusion of this antigen in the vaccine formulation, stressing the sand fly salivary protein LJL143 as a potential "broad-spectrum antigen".

All the above mentioned justifies the final composition of the optimized innovative vaccine used in the definitive pre-clinical trials in mice for extrapolation of its safety profile and characterization its in-depth immunogenic profile. Furthermore, several variables in the final outcome of the immunization, such as the contribution of the virosome to the induction of immunogenicity, or the possibility of immunodominance of the sand fly salivary protein, were considered.

The values of hematological studies, splenic cell populations, $CD4^+$ T cell non-specific reactivity and IgE specific titers (Table 1, S3i–S3iv Fig), determined in the pre-clinical trials, potentially indicate the safety of each vaccine component (proteins, adjuvant and virosome). The absence of specific IgE titers deserves to be highlighted, due to the correlation of antigen-specific IgE and vaccine-associated anaphylatic reactions development, shown particularly, but not exclusively for anti-*Influenza* vaccines [47]. To further explore the safety of the vaccine candidate, a repeated dose toxicity study in rabbits, complying with the WHO Expert Committee on Biological Standardization [48] is ongoing.

The different optimized formulations tested are indeed immunogenic, eliciting overall significant specific humoral and cellular immune responses (Figs 3i–3iv and 4i and 4ii). Regarding the humoral responses detected, they were generally mixed in nature (IgG1/IgG2a), indicating a mixed Th1/Th2 phenotype. Furthermore, the improvement of the specific humoral response against LeishF3+ induced by the VLP-based antigen formulations (Fig 3iii) deserves to be highlighted, as a possible advantage of the use of formulated antigens without forgetting, however, the debatable relevance of the humoral immune responses in the context of VL [49]. In respect to cellular immune responses detected, they shown distinct magnitudes, depending on each of the individual antigens. Reproducibly, the sand fly-derived antigen induced a more robust response than the parasite-derived ones (LJL143 $\geq$ LeishF3+ $>$ KMP11; Fig 4ii). Of note, the responses obtained against LeishF3+ were higher than those previously obtained against LeishF3 (Fig 4ii *versus* S1i Fig; PA (1+1+1) *versus* PA). This difference in the magnitude of the responses detected against the three different antigens seems not to be an immunodominance problem, since both cellular and humoral immune responses detected against LeishF3+ (the parasite derived antigen showing significant responses) were similar for the groups that received VPA (1+1+1) and VPA (1+5+5) (doses of LJL143, KMP11 and LeishF3+, respectively; Figs 3iii and 4ii).

Although it is a dogma that the protection against *Leishmania spp*. requires antigen-specific $CD4^+$ and $CD8^+$ T cell responses, the correlates of immunity to human VL are yet to be completely understood [50]. Therefore, the vaccine correlates of protection are still a debatable issue that takes bigger proportions when we add the translatability of animal pre-clinical trials to the equation. This said, the balance between specific IFN-γ and IL-10 production, has been used as predictive of vaccine efficacy in mice [51, 52]. In our study, through cell proliferation assays we detected in non-primed groups, a higher production of IL-10 than IFN-γ in response to the pool of antigens or to LeishF3+ alone, an either comparable or prevalent IFN-γ over IL-10 response against LJL143 (depending on the experimental group; Fig 5i and 5ii) and a limited but prevalent IFN-γ over IL-10 response against total parasite antigens (TLA; Fig 6ii). The Th1 directed response induced by stimulation with TLA, the experimentally closest experimental set up to the infectious process (deposition of whole parasites in the skin) is a promising indication of vaccine effectiveness. Nevertheless, we cannot ignore the apparent main Th2 response induced by the pool of antigens and LeishF3+, and either mixed or Th1 responses induced by LJL143. One curious observation is that, while the IL-10 levels quantified in the cell proliferation against the pool of antigens are 1.5 fold higher than the sum of levels determined in the cell proliferation against the individual antigens (excluding KMP11), the same comparison gives similar IFN-γ (Fig 5i and 5ii). This particular observation, together with evidence

showing that stimulation with high antigen doses leads to enhanced IL-10 production by Th1 $CD4^+$ cells [53], makes us speculate on the occurrence of a possible regulatory mechanism *in vitro* as a way to control a vigorous immune response and prevent inflammation-mediated damage.

In parallel, in the pre-clinical trial, we evaluated the effect of priming with the sand fly salivary protein in the final vaccine-elicited immune responses. Interestingly, the previous administration of the sand fly saliva derived antigen may be beneficial for the generation of a better response against the parasite-derived antigens, particularly in terms of cellular immunity (higher $CD4^+$ T cell proliferation against LeishF3+, KMP11 and TLA; Figs 4ii and 6i). Nevertheless, the IL-10 response detected, particularly against the pool of antigens and LJL143, was higher in the primed animals (Fig 5i and 5ii). On the other hand, the specific IFN-γ response generated by TLA increased tendentiously comparing primed with non-primed animals, and was 5 fold higher than the TLA induced IL-10 response (Fig 6ii), making this vaccination approach interesting to be tested in terms of anti-*Leishmania* effectiveness, in the context of natural infection (parasites delivered by the sand fly in the presence of salivary proteins).

Overall our results indicate that the innovative vaccine candidate tested here represents a promising anti-*Leishmania* vaccine. Some questions remain that need to be further explored, such as the potential benefits or implications of the predicted constant vector exposure in endemic countries, as well as a probable exposure to *Influenza* virus, to the final vaccine-induced responses.

Supporting information

S1 Fig. Effect of antigen/adjuvant doses in the response generated by non-formulated proteins. Groups of BALB/c mice were immunized 3 times i.m. (separated by 4 weeks each) with 2 different doses of adjuvant/antigens: 1 μg and 5 μg of each individual component (PA). Non-adjuvanted antigens (P; 1 μg of each antigen) or PBS were injected in controls. Four weeks after the last immunization, animals were euthanized and their spleens and sera collected. Specific $CD4^+$ and $CD8^+$ (i) T cell proliferation was assessed by Flow Cytometry four days after CFSE-stained splenocytes culture in the presence of each of the single non-formulated antigens (10 μg/ml). (ii) Serum antigen specific IgG titers for each of the antigens were determined by ELISA. Each dot represents one animal. Average and SD of the values within each group are shown. Statistical differences are properly identified (Unpaired t-test: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$).
(TIF)

S2 Fig. Representation of the pre-clinical trial *per-se* timeline. M represents month. W represents week. V represents virosome. A represents adjuvant. P represents proteins or antigens. Numbers 1+1+1 or 1+5+5 in brackets represent the administered doses in μg of LJL143, LeishF3+ and KMP-11, respectively. VPA(Mix) represents one formulation in which the three antigens (1μg each) were simultaneously formulated in the same virosome, contrarily to the other two VPA formulations that are mixtures of individual virosomal antigen preparations.
(TIF)

S3 Fig. Extrapolation of the safety profile of the vaccine components. Different experimental groups were designed for the development of the pre-clinical trials of the vaccine candidate in mice. Two groups represent negative controls: one composed by animals which received only the adjuvant (A), and other composed by animals that received the adjuvanted empty-virosome (VA). The third group received non-formulated proteins with adjuvant in the dosage of 1 μg of each component [PA (1+1+1)]. The fourth received the same non-formulated

proteins, but was primed with non-adjuvanted LJL143 three weeks before the first immunization (Pre-LJL PA). The three remaining groups received different formulations of adjuvanted formulated antigens (VPA). Two different VPA combinations were tested regarding antigen quantities: 1+1+1 or 1+5+5 indicate the administered dosages of formulated LJL143, KMP11 and LeishF3+ (individual virosome formulations). VPA (Mix) refers to the third virosome formulation tested, in which the three antigens (1μg each) were simultaneously formulated in the same virosome. Mice were immunized 3 times i.m. (separated by 4 weeks each), euthanized 4 weeks after the last immunization, and their spleens and sera collected. (i) Spleen weights and total cell numbers were determined. Myeloid (ii) and lymphoid (iii) splenic cell populations frequencies were determined by Flow Cytometry, and translated to absolute numbers. (iv) Antigen-specific IgE titers were determined by ELISA (individually against LJL143, LeishF3 + and KMP-11). Average and SD of the values within each group are shown. Statistical differences are properly identified (One-Way ANOVA: * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$). (TIF)

S4 Fig. Specific vaccine-elicited cellular cytokine responses. Different experimental groups were designed for the development of the pre-clinical trials of the vaccine candidate in mice. Two groups represent negative controls: one composed by animals which received only the adjuvant (A), and other composed by animals that received the adjuvanted empty virosome (VA). The third group received non-formulated proteins with adjuvant in the dosage of 1 μg of each component [PA (1+1+1)]. The fourth received the same non-formulated proteins, but was primed with non-adjuvanted LJL143 three weeks before the first immunization (Pre-LJL PA). The three remaining groups received different formulations of adjuvanted formulated antigens (VPA). Two different VPA combinations were tested regarding antigen quantities: 1+1+1 or 1 +5+5 indicate the administered dosages of formulated LJL143, KMP11 and LeishF3+ (individual virosome formulations). VPA (Mix) refers to the third virosome formulation tested, in which the three antigens (1μg each) were simultaneously formulated in the same virosome. Mice were immunized 3 times i.m. (separated by 4 weeks each), euthanized 4 weeks after the last immunization, and their spleens collected. TNF-α, and IL-4 levles were quantified by ELISA in the supernatants resultant from cellular proliferation assays against each of the individual antigens. Data presented refers only to non-primed animals. Each dot represents one animal. Average and SD of the values within each group are shown. Statistical differences are properly identified (One-Way ANOVA: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$). (TIF)

Acknowledgments

We greatly appreciate all of the human subjects for their cooperation.

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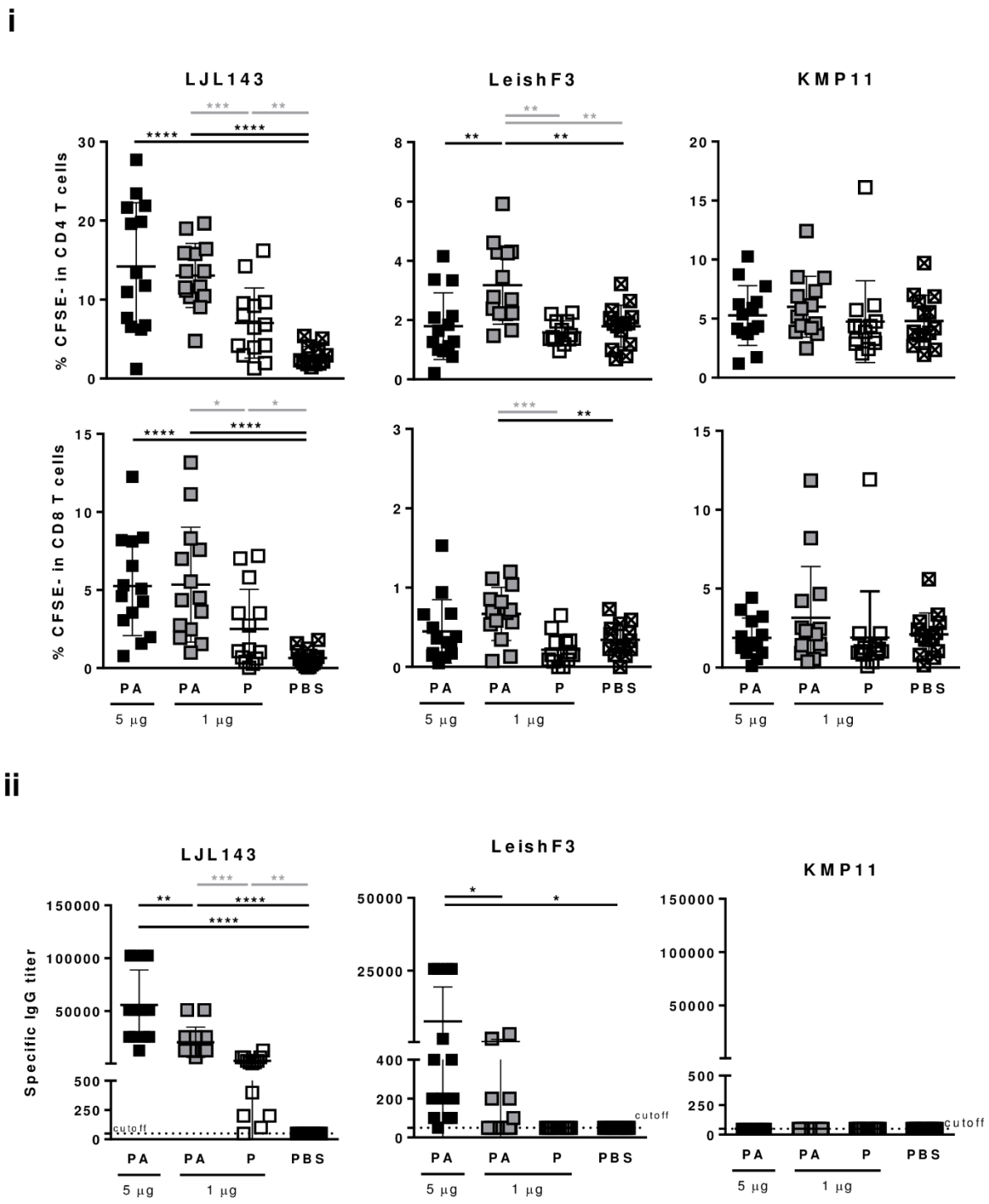
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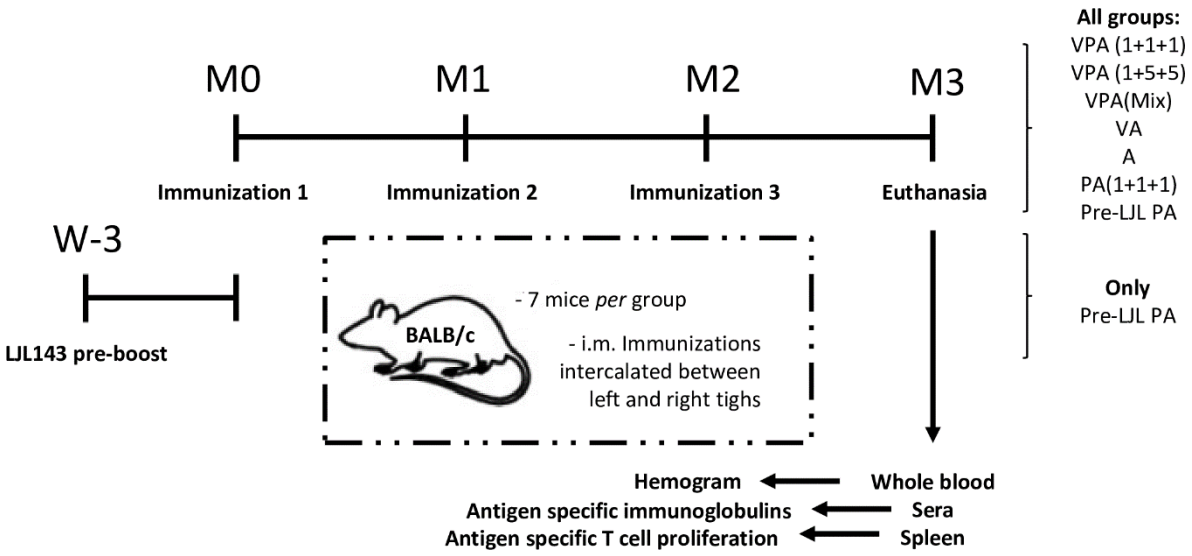
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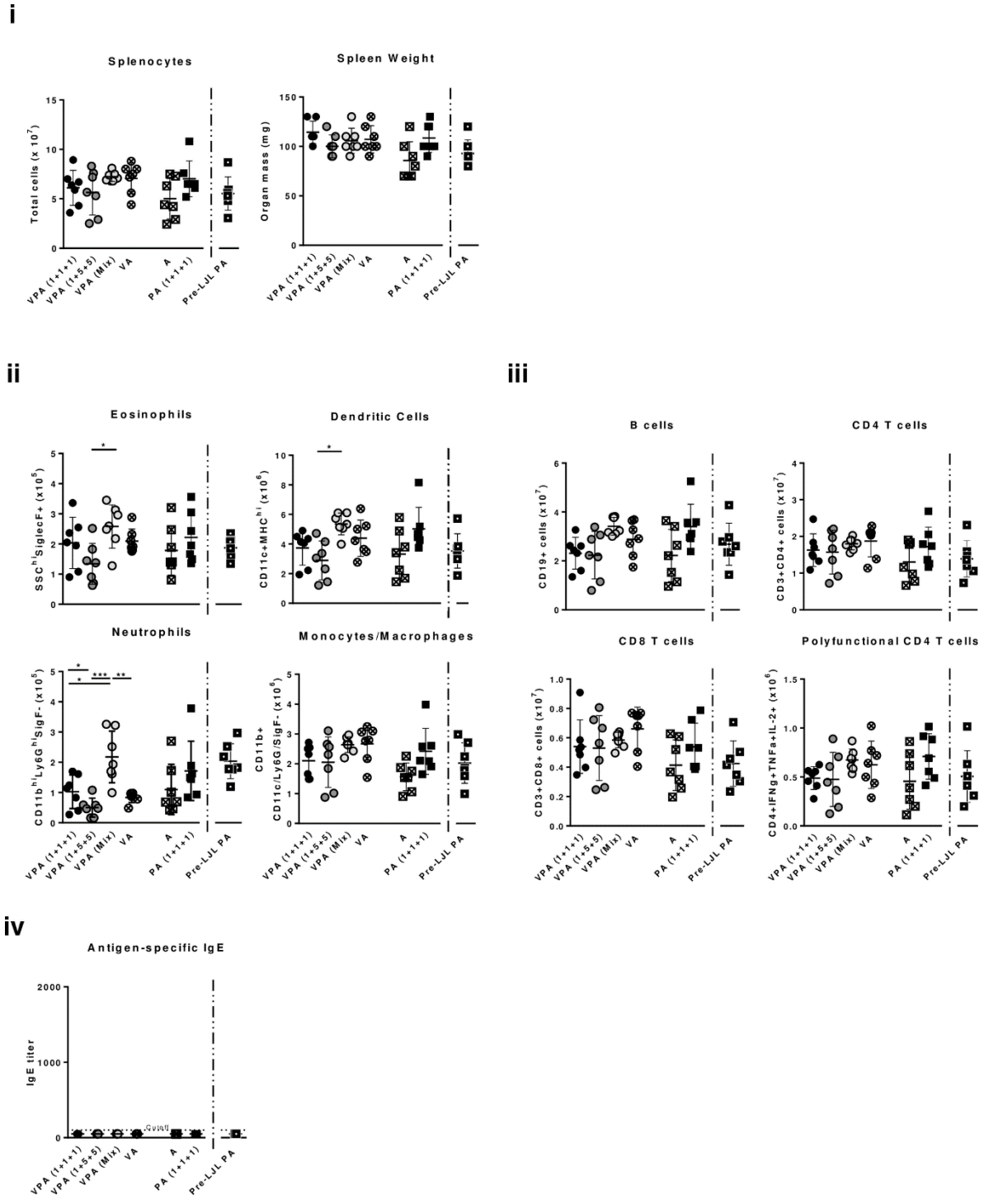


S1 Fig. Effect of antigen/adjuvant doses in the response generated by non-formulated proteins. Groups of BALB/c mice were immunized 3 times i.m. (separated by 4 weeks each) with 2 different doses of adjuvant/antigens: 1 µg and 5 µg of each individual component (PA). Non-adjuvanted antigens (P; 1 µg of each antigen) or PBS were injected in controls. Four weeks after the last immunization, animals were euthanized and their spleens and sera

collected. Specific $CD4^+$ and CD8+ (i) T cell proliferation was assessed by Flow Cytometry four days after CFSE-stained splenocytes-culture in the presence of each of the single non-formulated antigens (10 µg/ml). (ii) Serum antigen specific IgG titers for each of the antigens were determined by ELISA. Each dot represents one animal. Average and SD of the values within each group are shown. Statistical differences are properly identified (Unpaired t-test: * p≤0.05, ** p≤0.01, *** p≤0.001 and **** p≤0.0001).

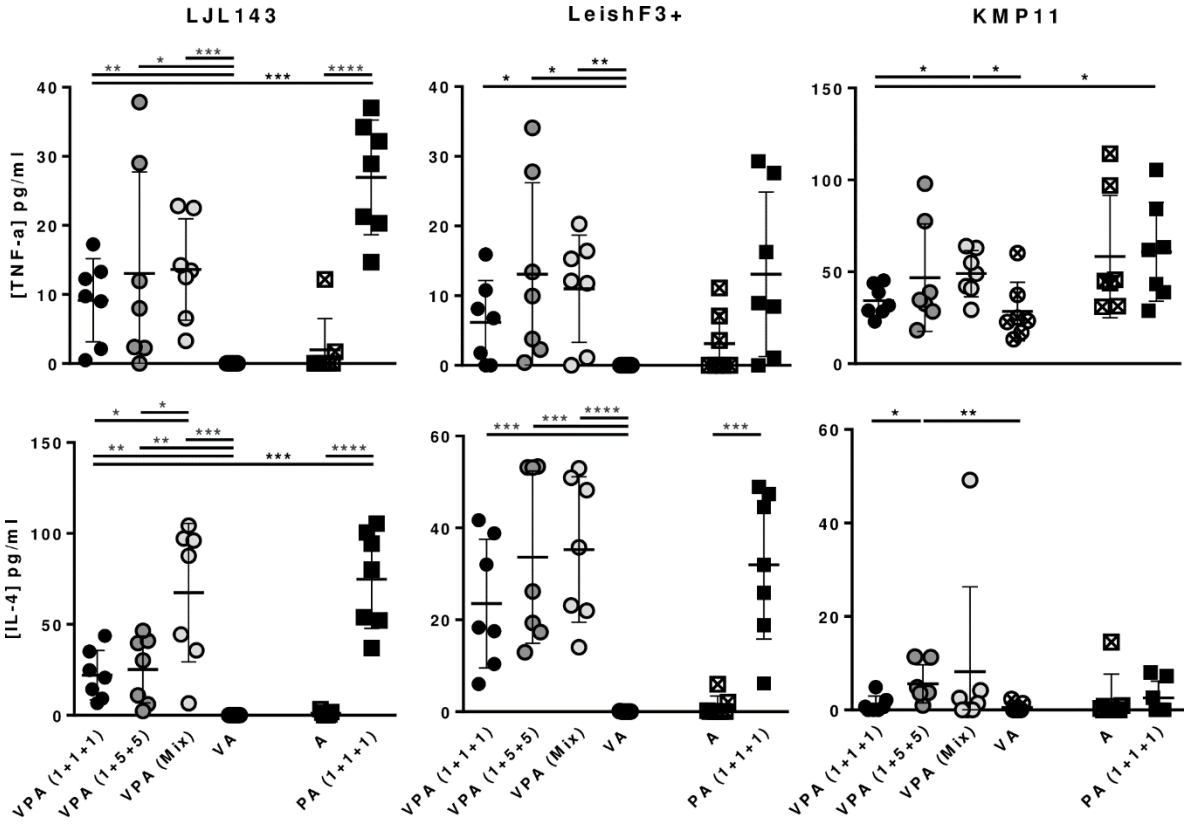


S2 Fig. Representation of the pre-clinical trial *per-se* timeline. M represents month. W represents week. V represents virosome. A represents adjuvant. P represents proteins or antigens. Numbers 1+1+1 or 1+5+5 in brackets represent the administered doses in µg of LJL143, LeishF3+ and KMP-11, respectively. VPA(Mix) represents one formulation in which the three antigens (1µg each) were simultaneously formulated in the same virosome, contrarily to the other two VPA formulations that are mixtures of individual virosomal antigen preparations.



S3 Fig. Extrapolation of the safety profile of the vaccine components. Different experimental groups were designed for the development of the pre-clinical trials of the vaccine candidate in mice. Two groups represent negative controls: one composed by animals which received only the adjuvant (A), and other composed by animals that received the adjuvanted empty-virosome (VA). The third group received non-formulated proteins with

adjuvant in the dosage of 1 µg of each component [PA (1+1+1)]. The fourth received the same non-formulated proteins, but was primed with non-adjuvanted LJL143 three weeks before the first immunization (Pre-LJL PA). The three remaining groups received different formulations of adjuvanted formulated antigens (VPA). Two different VPA combinations were tested regarding antigen quantities: 1+1+1 or 1+5+5 indicate the administered dosages of formulated LJL143, KMP11 and LeishF3+ (individual virosome formulations). VPA (Mix) refers to the third virosome formulation tested, in which the three antigens (1µg each) were simultaneously formulated in the same virosome. Mice were immunized 3 times i.m. (separated by 4 weeks each), euthanized 4 weeks after the last immunization, and their spleens and sera collected. (i) Spleen weights and total cell numbers were determined. Myeloid (ii) and lymphoid (iii) splenic cell populations-frequencies were determined by Flow Cytometry, and translated to absolute numbers. (iv) Antigen-specific IgE titers were determined by ELISA (individually against LJL143, LeishF3+ and KMP-11). Average and SD of the values within each group are shown. Statistical differences are properly identified (One-Way ANOVA: * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$).



S4 Fig. Specific vaccine-elicited cellular-cytokine responses. Different experimental groups were designed for the development of the pre-clinical trials of the vaccine candidate in mice. Two groups represent negative controls: one composed by animals which received only the adjuvant (A), and other composed by animals that received the adjuvanted empty-virosome (VA). The third group received non-formulated proteins with adjuvant in the dosage of 1 µg of each component [PA (1+1+1)]. The fourth received the same non-formulated proteins, but was primed with non-adjuvanted LJL143 three weeks before the first immunization (Pre-LJL PA). The three remaining groups received different formulations of adjuvanted formulated antigens (VPA). Two different VPA combinations were tested regarding antigen quantities: 1+1+1 or 1+5+5 indicate the administered dosages of formulated LJL143, KMP11 and LeishF3+ (individual virosome formulations). VPA (Mix) refers to the third virosome formulation tested, in which the three antigens (1µg each) were simultaneously formulated in the same virosome. Mice were immunized 3 times i.m. (separated by 4 weeks each), euthanized 4 weeks after the last immunization, and their spleens collected. TNF-α, and IL-4 levels were quantified by ELISA in the supernatants resultant from cellular proliferation assays against each of the individual antigens. Data presented refers only to non-primed animals. Each dot represents one animal. Average and SD of the values within each group are shown. Statistical differences are properly identified (One-Way ANOVA: * p≤0.05, ** p≤0.01, *** p≤0.001 and ****

2.2. Engineering a vector-based pan-*Leishmania* vaccine for humans: proof of principle

A vaccine for human leishmaniasis is considered an achievable goal, justified by the protection observed following leishmanization campaigns, and a central tool to meet the ultimate target of disease control. However, to date we still do not have a vaccine available for humans, a consequence (amongst other factors) of a lack of pre-clinical to clinical translatability. We know today that the memory immune response against vector saliva negatively impacts infection, making sand fly salivary proteins attractive anti-*Leishmania* vaccine candidates. However, these proteins on one hand are not innocuous molecules, and on the other may have a limited vaccine spectrum, being effective only in the geographical region where the vector species is present. With our work, we propose an approach to design rationally a vector-based vaccine without the abovementioned limitations, and with a higher potential of pre-clinical to clinical translation. We show that this strategy works, at least considering the *in vivo* maintenance of the immunogenic potential of two distinct sand fly salivary proteins already demonstrated as potentially effective anti-*Leishmania* vaccines, and propose it as a proof of principle for a future vector-derived pan-*Leishmania* vaccine: a single antigen, constructed modularly with fractions of distinct salivary proteins, and rationally using reverse vaccinology.

Submitted

Engineering a vector-based pan-*Leishmania* vaccine for humans: proof of principle

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Short title: Exploring vector saliva to generate a pan-*Leishmania* vaccine

Data availability: All relevant data are within the paper and its supplementary materials.

Keywords: *Leishmania*; Cutaneous Leishmaniasis; Visceral Leishmaniasis; Sand fly salivary proteins; Vaccine; Bioinformatics; Multi-epitope; Chimera

ABSTRACT

Leishmaniasis is a spectrum of diseases transmitted by sand fly vectors that deposit *Leishmania* spp. parasites in the host skin during blood feeding. Currently, available treatment options are limited, associated with high toxicity and emerging resistance. Even though a vaccine for human leishmaniasis is considered an achievable goal, to date we still do not have one available, a consequence (amongst other factors) of a lack of pre-clinical to clinical translatability. Pre-exposure to uninfected sand fly bites or immunization with defined sand fly salivary proteins was shown to negatively impact infection. Still, cross-protection reports are rare and dependent on phylogenetic proximity of the sand fly species, meaning that a the applicability of a sand fly saliva-based vaccine will be limited to a defined geography, one parasite species and one form of leishmaniasis. As a proof of principle of a future vector saliva-based pan-*Leishmania* vaccine, we engineered through a reverse vaccinology approach that maximizes translation to humans, a fusion protein consisting of immunogenic portions of PdSP15 and LJL143, sand fly salivary proteins demonstrated as potential vaccine candidates against cutaneous and visceral leishmaniasis, respectively. The *in silico* analysis was validated *ex vivo*, through T cell proliferation experiments, proving that the fusion protein (administered as a DNA vaccine) retained the immunogenicity of both PdSP15 and LJL143. Additionally, while no significant effect was detected in the context of *L. major* transmission by *P. duboscqi*, this DNA vaccine was defined as partially protective, in the context of *L. major* transmission by *L. longipalpis* sand flies. Importantly, a high IFNγ response alone was not enough to confer protection, that mainly correlated with low T cell mediated *Leishmania*-specific IL-4 and IL-10 responses, and consequently with high pro/anti-inflammatory cytokine ratios. Overall our immunogenicity data suggests that to design a potentially safe vector-based pan-*Leishmania* vaccine, without geographic restrictions and against all forms of leishmaniasis is an achievable goal. This is why we propose our approach as a proof-of principle, perhaps not only applicable to the anti-*Leishmania* vector-based vaccines' field, but also to other branches of knowledge that require the design of multi-epitope T cell vaccines with a higher potential for translation.

INTRODUCTION

Leishmaniasis is a spectrum of diseases caused by at least 20 different species belonging to the *Leishmania* genus (Kinetoplastida; Trypanosomatida)[1]. These parasites have a complex life-cycle that depends on two different organisms to be completed: an arthropod vector (phlebotomine sand flies) and a vertebrate host (from reptiles to mammals)[1]. Furthermore, considering the parasite-host binomium: most human *Leishmania* pathogens also infect animals (zoonosis)[1,2]; disease development is dependent on the evasion/manipulation of the host immune response[3]; and the disease forms usually correlate with specific parasite species[1]. Additionally, considering the parasite-vector interface, although experimentally some vectors were shown to be permissive to different *Leishmania* spp., in nature ecological and molecular factors typically restrict vector-*Leishmania* spp. pairings[1,4,5]. All of the abovementioned considerations make the control of leishmaniasis an extremely challenging task, requiring a trinomial focus on the infected human individuals; on the vectors; and on the different animal reservoirs[6,7].

On opposite sides of the leishmaniasis disease spectrum are the cutaneous and visceral forms, both very relevant, due to the number of cases recorded worldwide annually (and DALYs associated), considering the former[8,9], or due to the fatality rate (over 95%, if untreated), considering the latter[9]. Together, visceral and cutaneous leishmaniases are endemic in 97 countries (co-endemic in 65 of them) and are responsible for up to one million new cases and more than 20000 deaths, estimated to occur annually[9,10]. Although visceral leishmaniasis was defined as potentially controllable in the short-term, today this is only hypothetically achievable in the Indian sub-continent where the disease is of anthroponotic origin[7,11]. Despite several attempts to prevent the disease, with numerous candidates tested along the years, today there is still no vaccine available for human leishmaniasis[12]. The absence of prophylaxis makes disease control dependent on the treatment of active cases. However, not only is a vaccine for leishmaniasis considered an achievable goal, justified by the protection observed following leishmanization campaigns[12], but also an essential tool to meet the ultimate target of disease control and even elimination, considering a long-term perspective[7].

One of the most recently explored possibilities of “anti-*Leishmania*” vaccines are the vector-based ones. An anti-vector-saliva immune response, elicited by exposure to uninfected sand fly bites, conferred protection against vector-transmitted cutaneous disease[13], opening the door to testing defined sand fly-salivary proteins as vaccines that demonstrated

effectiveness in the context of cutaneous[14-18] or visceral[19] disease. Protection is thought to be mediated by saliva-specific IFN-γ secretion by CD4+ T cells at the bite site, early after transmission, via the generation of a robust Th1 delayed-type hypersensitivity (DTH) reaction, that negatively (and indirectly) affects parasites' establishment[20]. Still, cross-protection reports are rare and dependent on phylogenetic proximity of the sand fly species[21] (less probable to occur between we think in New World *versus* Old World vector species), meaning that most of the sand fly salivary proteins will probably work as effective vaccines only in a defined geography, against one parasite species and consequently one form of leishmaniasis[22]. Additionally, most of these proteins have potent biological activities[23], which may pose a barrier considering the safety requirements for approval of a human pharmaceutical[24].

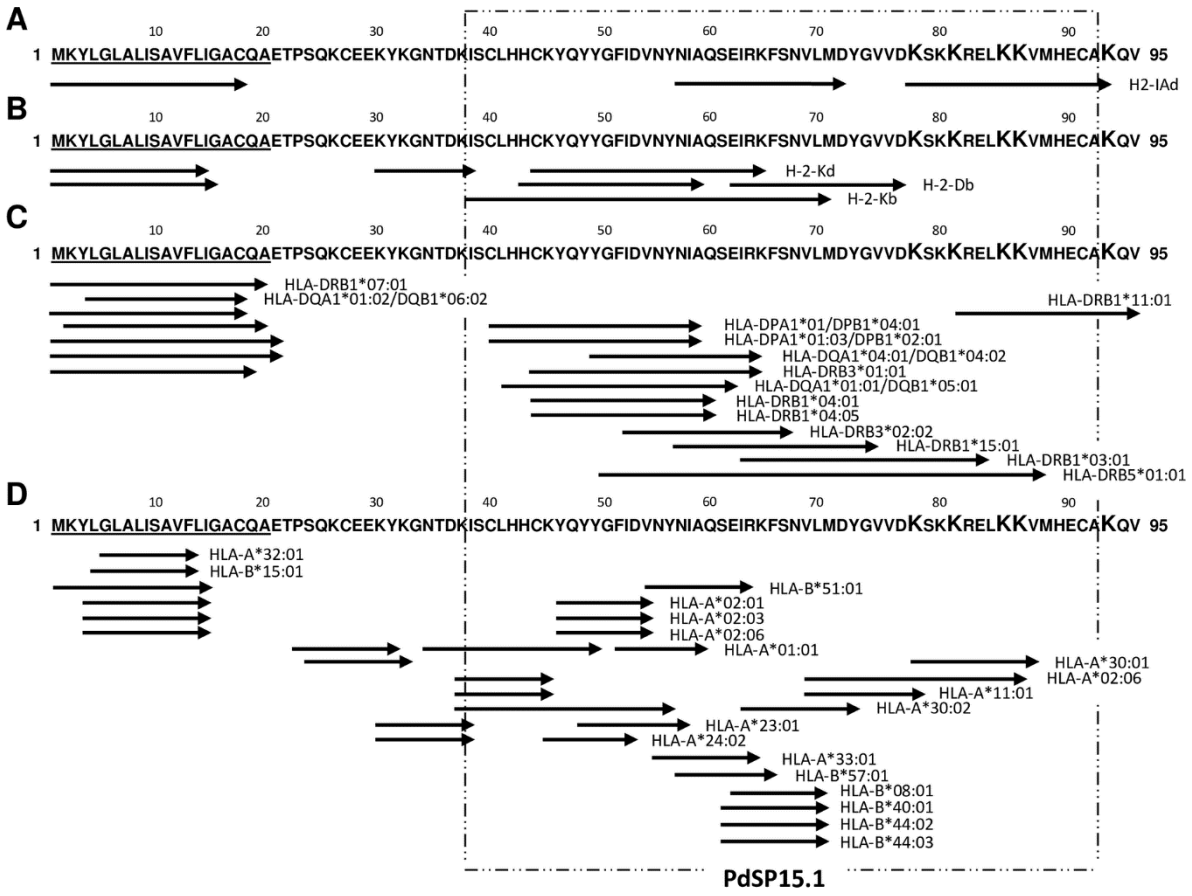
Having in mind the abovementioned limitations, we engineered a chimeric antigen that includes *in silico*-determined immunogenic T-cell epitopes from two salivary proteins whose anti-*Leishmania* vaccine potential was at least partially demonstrated: PdSP15 from *Phlebotomus duboscqi*, the main West African vector of cutaneous leishmaniasis, a protein defined as an effective anti-*Leishmania* vaccine in a non-human primate model[18]; and LJL-143 from *Lutzomyia longipalpis*, the main South American vector of visceral leishmaniasis, an antigen demonstrated to negatively impact *L. infantum* parasites *ex vivo*[25], and to potentially work as "adjuvant-like" in a prime-boost immunization strategy[26]. We further validated the overall bioinformatics predictions *ex-vivo* and show that this neo-antigen, administered as a DNA vaccine, generates specific T cell reactivity against salivary gland extracts from both *P. duboscqi and L. longipalpis*, indicating a potential broadening of the spectrum. Finally, we tested this new potential vaccine in the context of *L. major* transmission by *P. duboscqi or L. longipalpis*, and propose our strategy as a proof of principle for a future vector-derived pan-*Leishmania* vaccine: a single antigen, constructed modularly with fractions of distinct antigens, and rationally using reverse vaccinology.

RESULTS

Epitope mapping of the sand fly salivary proteins. Going towards a reverse vaccinology approach we predicted CD4+ and CD8+ T cell epitopes considering both murine and human alleles for two distinct sand fly salivary proteins previously studied in the context of anti-*Leishmania* vaccines, PdSP15 from *P. duboscqi* and LJL-143 from *L. longipalpis,* in a way to disclose their immunogenic potential considering cell-mediated responses. We performed this mouse/human analysis to bridge pre-clinical and clinical contexts. Additionally, we also mapped CD4+ T cell epitopes, considering only human alleles, for two known homologs of the abovementioned proteins, PpeSP06 and PpSP15 respectively, to assess the potential for cross-reactivity.

Figure 1 illustrates the overall snapshot obtained for PdSP15, where there are highlighted the protein regions containing epitopes predicted to bind to MHC-II murine alleles (A and A´), MHC-I murine alleles (B and B'), MHC-II human alleles (C and C´ – only very high affinity binders, with a percentile ranking ≤ 2.5, are represented) and MHC-I human alleles (D and D´). With respect to MHC-II binding analysis against murine alleles, the predictions were negative for two of the three MHC molecules studied: H2-IE^{d}, representative of BALB/c mice with the lowest percentile rank determined of 17.39, and H2-IA^{b} representative of C57BL/6 mice with the lowest percentile rank determined of 18.55. Only for the allele H2-IA^{d} from BALB/c, 14 epitopes were predicted as good binders (percentile rank ≤ 10; Supplementary data 1), 4 of which within the signal peptide sequence, and the remaining in three different regions of the protein, represented in Figure 1A/A´. Regarding binding predictions against MHC-I murine alleles, several 8-14 MER peptides were determined to bind efficiently (percentile rank ≤ 1) to four of the five alleles explored, two from BALB/c (H-2-D^{d} and H-2-K^{d}, but not H-2-L^{d}; Supplementary data 2) and the other two from C57BL/6 (H-2-D^{b} and H-2-K^{b}; Supplementary data 3) mice, mainly within two regions we define as "murine CD8+ T cell epitopes' rich": between residues 40 and 80 (Figure 1B), and the C-terminal portion, starting from residue 100 (Figure 1B´). The analysis against MHC-II human alleles also revealed, apart from several hits in the signal peptide region, two "T cell epitopes' rich" protein regions (Figure 1C/C'). Considering only the determined very high affinity binders (percentile ranking ≤ 2.5), peptides comprised between residues 40 and 80 were predicted to bind against 11 different human alleles, while peptides comprised between residues 110 and 130 were predicted to bind against 4 different human alleles (Figure 1C/C'; Supplementary data 4, represented in dark green). If we extend this analysis to the peptides

with determined percentile ranks between 2.5 and 7.5, these two “highly immunogenic” regions (as predicted *in silico* considering cellular immunity) are still noticeable (Supplementary Figure 2A/A’; Supplementary data 4, represented in light green). Considering this analysis, the same two regions now contain peptides predicted to bind to 17 and 12 different human alleles, respectively (Supplementary Figure 2A/A’). Importantly, only for 3 of the 27 human MHC-II molecules studied, no CD4+ T cell epitopes were predicted, if we exclude the signal peptide sequence: HLA-DQA1*03:01/DQB1*03:02; HLA-DQA1*05:01/DQB1*03:01 and HLA-DRB1*12:01 (Supplementary data 4). The same human MHC-II binding analysis was performed for the PdSP15 homologue, PpSP15, with a similar determined overall picture (Supplementary Figure 3A/A’; Supplementary data 5). Briefly, excluding the signal peptide sequence, two similar “highly immunogenic” regions were detected: considering all hits with determined percentile rankings ≤ 7.5, peptides comprised between residues 40 and 80 were predicted to bind against 22 different human alleles (Supplementary Figure 3A; Supplementary data 5), while peptides comprised between residues 110 and 130 were predicted to bind against 6 different human alleles (Supplementary Figure 3A’; Supplementary data 5). Finally, regarding PdSP15 epitope mapping considering human MHC-I alleles, the analysis revealed many different epitopes, covering almost the entire protein sequence. Still, most of these epitopes were localized within the same abovementioned two regions, with combined determined good binders (percentile rank ≤ 1) against 20 different human alleles (Figure 1D/D’; Supplementary data 6). From the remaining 7 alleles, no predicted binders were found for HLA-A*26:01, HLA-A*68:02, HLA-B*07:02, HLA-B*35:01 and HLA-B*53:01 (Supplementary data 6).


A
B
C
D
1 MKYLGLALISAVFLIGACQAETPSQKCEEKYKGNTDKISCLHHCKYQYYGFIDVNYNIAQSEIRKFSNVLMDYGVVDKSKKRELKKVMHECAKQV 95
H2-IAd
H-2-Kd
H-2-Db
H-2-Kb
HLA-DRB1*07:01
HLA-DQA1*01:02/DQB1*06:02
HLA-DRB1*11:01
HLA-DPA1*01/DPB1*04:01
HLA-DPA1*01:03/DPB1*02:01
HLA-DQA1*04:01/DQB1*04:02
HLA-DRB3*01:01
HLA-DQA1*01:01/DQB1*05:01
HLA-DRB1*04:01
HLA-DRB1*04:05
HLA-DRB3*02:02
HLA-DRB1*15:01
HLA-DRB1*03:01
HLA-DRB5*01:01
HLA-A*32:01
HLA-B*15:01
HLA-B*51:01
HLA-A*02:01
HLA-A*02:03
HLA-A*02:06
HLA-A*01:01
HLA-A*30:01
HLA-A*02:06
HLA-A*11:01
HLA-A*30:02
HLA-A*23:01
HLA-A*24:02
HLA-A*33:01
HLA-B*57:01
HLA-B*08:01
HLA-B*40:01
HLA-B*44:02
HLA-B*44:03
PdSP15.1

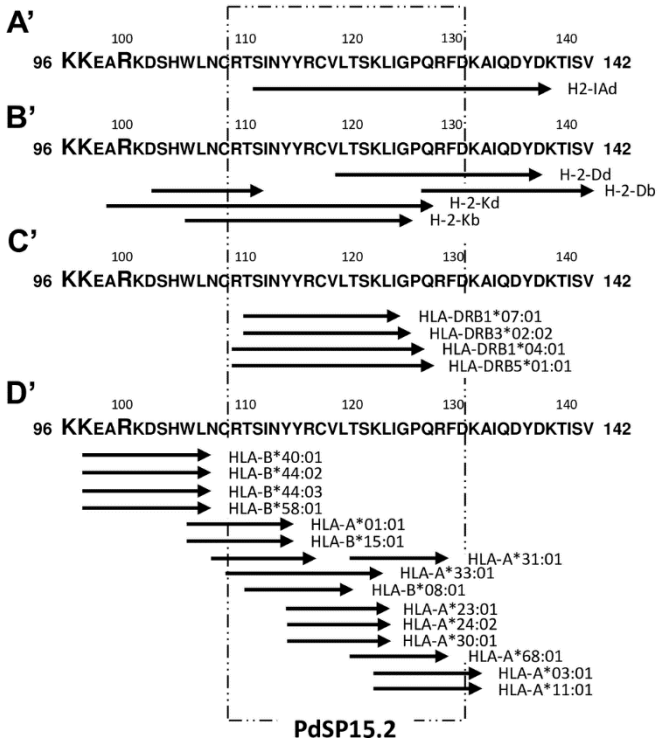
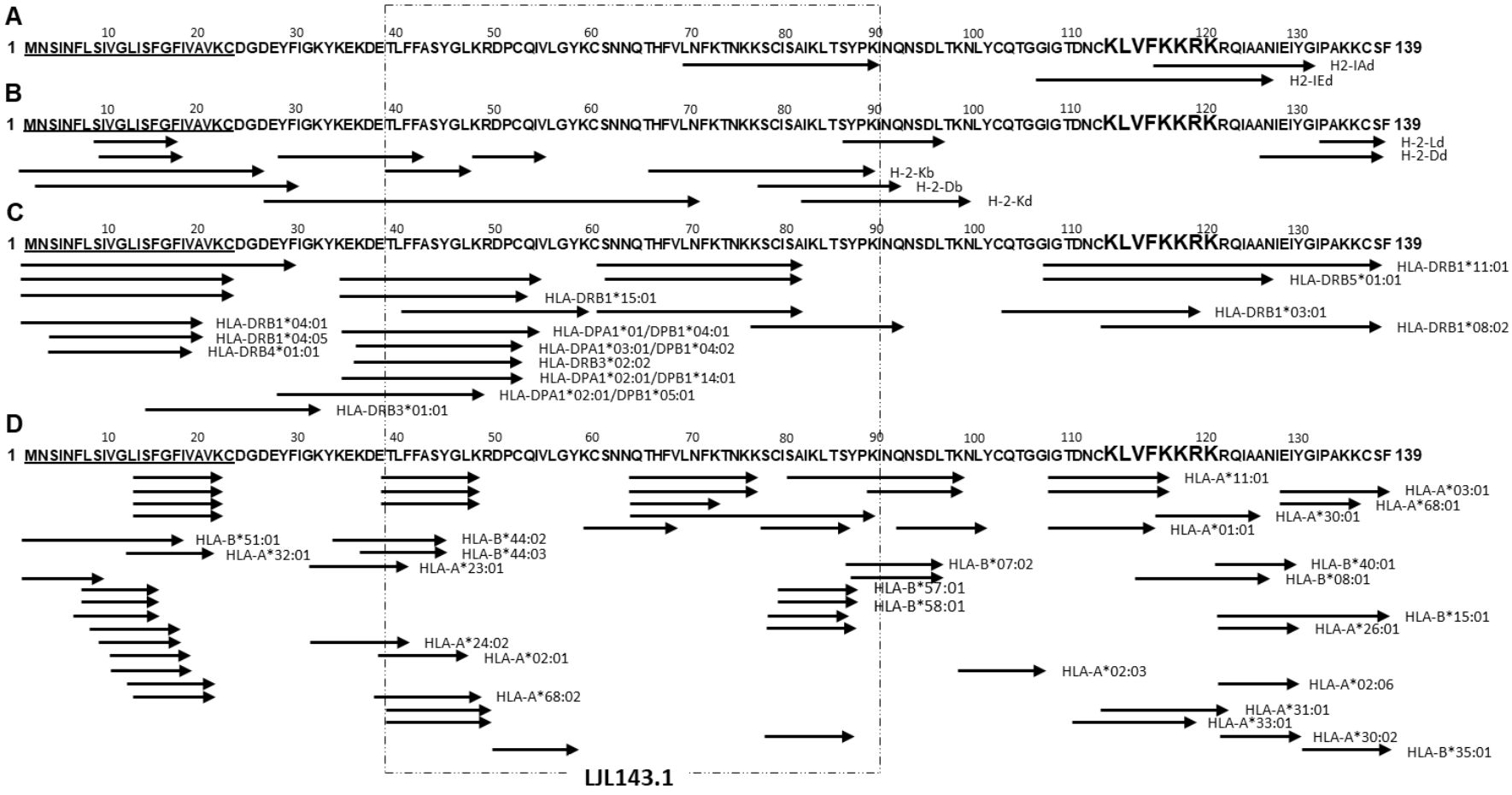

A'
B'
C'
D'
96 KKEARKDSHWLNCRTSINYYRCVLTSKLIGPQRFDKAIQDYDKTISV 142
H2-IAd
H-2-Dd
H-2-Db
H-2-Kd
H-2-Kb
HLA-DRB1*07:01
HLA-DRB3*02:02
HLA-DRB1*04:01
HLA-DRB5*01:01
HLA-B*40:01
HLA-B*44:02
HLA-B*44:03
HLA-B*58:01
HLA-A*01:01
HLA-B*15:01
HLA-A*31:01
HLA-A*33:01
HLA-B*08:01
HLA-A*23:01
HLA-A*24:02
HLA-A*30:01
HLA-A*68:01
HLA-A*03:01
HLA-A*11:01
PdSP15.2

Figure 1. PdSP15 T cell epitope mapping: overall picture of the *in silico* analysis performed against murine and human MHC-I and MHC-II molecules. PdSP15 (*GenBank acc. no. ABI15933*) T cell epitope mapping was performed using the IEDB Analysis Resource considering 3 murine and 27 human MHC-II alleles and 5 murine and 27 human MHC-I alleles. Translated results of the predictions of murine MHC-II restricted epitopes (A and A'), murine MHC-I restricted epitopes (B and B'), human MHC-II restricted epitopes (C and C') and human MHC-I restricted epitopes (D and D') are represented. Results are shown by allele. Each arrow represents one or more (contiguous) predicted epitopes: the top 1% hits for MHC-I molecules and the top 2.5% (very high affinity binders) or 10% hits for MHC-II human and murine alleles, respectively. Underlined protein residues represent the signal peptide sequence. Magnified protein residues are potentially important for protein biological activity. Dashed boxes represent the convergent analysis of the different *in silico* determinations that enabled the selection of two protein portions to be included in the final chimeric sand fly salivary antigen.

Similarly, Figure 2 illustrates the results obtained for LJL-143. In the binding analysis against murine MHC-II alleles, peptides were predicted to bind against the two BALB/c mice alleles screened (Supplementary data 7) and against the C57BL/6 mice allele studied (Supplementary data 8). In theory, the chances of immunogenicity (considering CD4+ T cell responses) will be higher in the BALB/c background, with five immunogenic regions determined, comparing with the C57BL/6 background, with only one region determined between residues 139 and 156 (Figure 2A/A'; Supplementary data 7 and 8). Regarding the murine MHC-I restricted epitope mapping, several hits were determined to bind against the five alleles studied (Supplementary data 9 and 10 represent the results obtained for BALB/c and C57BL/6 alleles, respectively). Considering the five alleles together, the protein coverage in terms of predicted epitopes is almost integral, excluding the region between residues 100 and 130 (Figure 2B/B'). With respect to the *in silico* analysis for human MHC-II alleles, and considering only the very high affinity binders (percentile rank ≤ 2.5), at least one epitope was predicted for 23 of the 27 alleles studied (Supplementary data 11, dark green highlighted cells). This analysis showed once more an almost complete protein coverage of human MHC-II restricted epitopes (Figure 2C/C'). Nevertheless, the region comprised between residues 60 and 160 seems to be less enriched in human CD4+ T cell epitopes with very high affinity binders determined only for 5/27 alleles (Figure 2C/C'). If we extend the analysis and consider the peptides with determined percentile ranks between 2.5 and 7.5 (Supplementary data 11, light green highlighted cells), the in silico predictions

suggest that LJL-143 is pan-immunogenic, with at least one epitope was predicted for the each of the 27 human alleles studied (that theoretically ensure >99% of human population coverage). This extended analysis, translated into Supplementary Figure 4A/A' shows a complete protein coverage of human CD4+ T cell epitopes, with the maintenance of the region "less enriched", now smaller and comprised between residues 90 and 140. The same human MHC-II binding analysis was performed for the LJL-143 homologue, PpeSP06 (Supplementary data 12; Supplementary Figure 5A/A'). Similar to what we observed for LJL-143 almost the full protein sequence contains human MHC-II restricted epitopes, considering a percentile rank threshold of 7.5 (Supplementary Figure 5A/A'). Additionally, and also in line with the observed for the PpeSP06 homologue, more hits were found in the signal peptide and c-terminal regions, while less hits were found in the region comprised between residues 90 and 140 (Supplementary Figure 5A/A'). Overall, the analysis performed shows that PpeSP06 contains human MHC-II restricted epitopes for 25 of the 27 molecules studied, excluding the signal peptide sequence (no hits found for the alleles HLA-DQA1*05:01/DQB1*03:01 and HLA-DQA1*01:02/DQB1*06:02; Supplementary data 12). Finally, regarding LJL-143 epitope mapping considering human MHC-I alleles, the analysis shows once more an almost complete coverage of the protein sequence, this time homogeneously (no "rich or poor epitope regions"; Figure 2D/D'). Interestingly, and as was observed in the analysis against human MHC-II alleles, at least one peptide was defined as a good binder for all the 27 alleles studied (Supplementary data 13).

In summary, *in silico* predictions indicate that both proteins have a good potential for immunogenicity, particularly considering human alleles.


A
1 MNSINFLSIVGLISFGFIVAVKCDGDEYFIGKYKEKDETLFFASYGLKRDPCQIVLGYKCSNNQTHFVLNFKTNKKSCISAIKLTSYPKINQNSDLTKNLYCQTGGIGTDNCKLVFKKRKRQIAANIEIYGIPAKKCSF 139
H2-IAd
H2-IEd
B
H-2-Ld
H-2-Dd
H-2-Kb
H-2-Db
H-2-Kd
C
HLA-DRB1*11:01
HLA-DRB5*01:01
HLA-DRB1*15:01
HLA-DRB1*03:01
HLA-DRB1*04:01
HLA-DRB1*04:05
HLA-DRB4*01:01
HLA-DPA1*01/DPB1*04:01
HLA-DPA1*03:01/DPB1*04:02
HLA-DRB3*02:02
HLA-DPA1*02:01/DPB1*14:01
HLA-DRB1*08:02
HLA-DPA1*02:01/DPB1*05:01
HLA-DRB3*01:01
D
HLA-A*11:01
HLA-A*03:01
HLA-A*68:01
HLA-A*30:01
HLA-A*01:01
HLA-B*51:01
HLA-A*32:01
HLA-B*44:02
HLA-B*44:03
HLA-A*23:01
HLA-B*07:02
HLA-B*40:01
HLA-B*08:01
HLA-B*57:01
HLA-B*58:01
HLA-B*15:01
HLA-A*26:01
HLA-A*24:02
HLA-A*02:01
HLA-A*02:03
HLA-A*02:06
HLA-A*68:02
HLA-A*31:01
HLA-A*33:01
HLA-A*30:02
HLA-B*35:01
LJL143.1

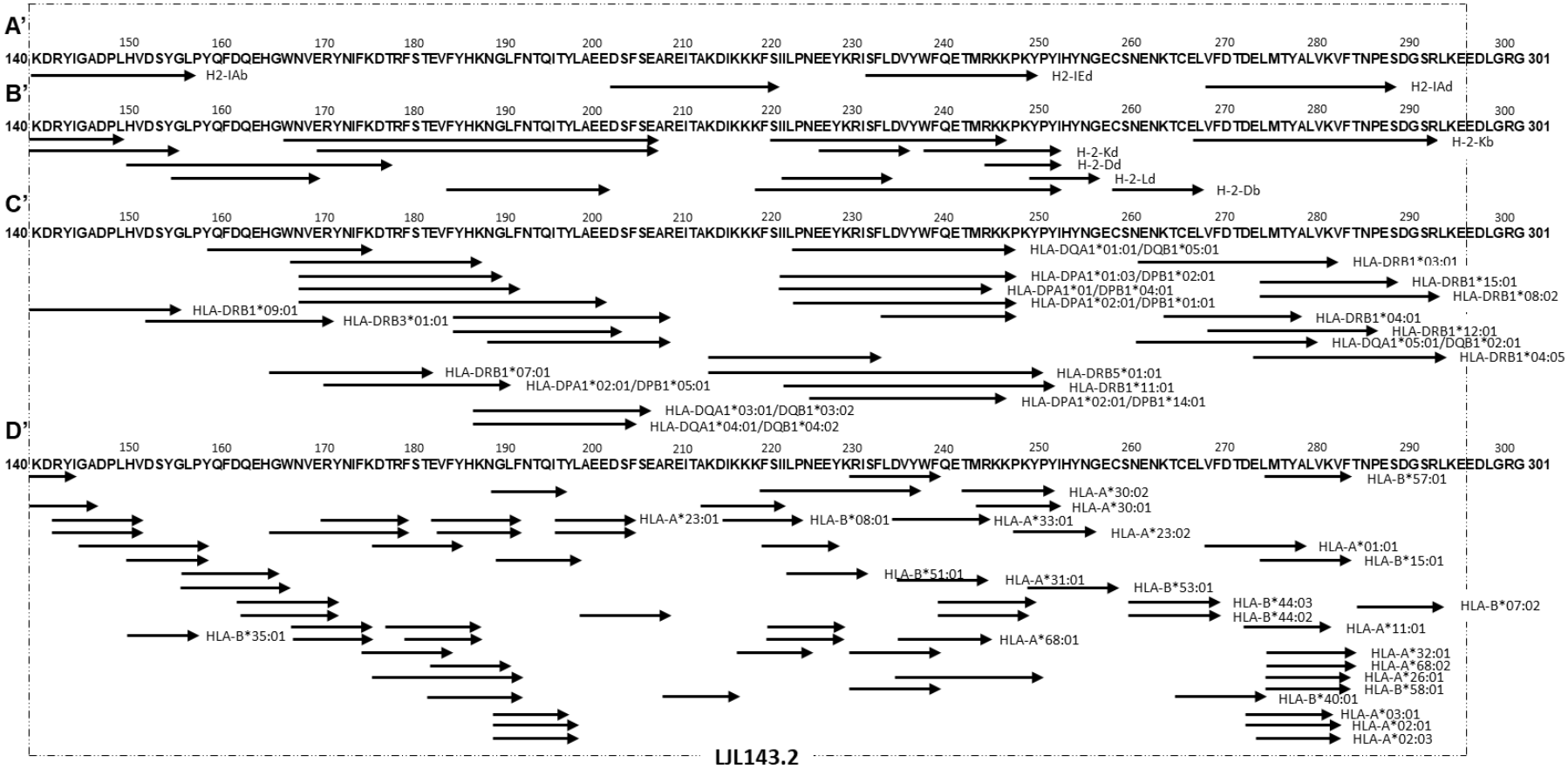

A'
B'
C'
D'
140 KDRYIGADPLHVDSYGLPYQFDQEHGWNVERYNIFKDTRFSTEVFYHKNGLFNTQITYLAEEDSFSEAREITAKDIKKKFSIILPNEEYKRISFLDVYWFQETMRKKPKYPYIHYNGECSNENKTCELVFDTDELMTYALVKVFTNPESDGSRLKEEDLGRG 301
H2-IAb
H2-IEd
H2-IAd
H2-Kb
H2-Kd
H2-Dd
H2-Ld
H2-Db
HLA-DQA1*01:01/DQB1*05:01
HLA-DRB1*03:01
HLA-DPA1*01:03/DPB1*02:01
HLA-DRB1*15:01
HLA-DPA1*01/DPB1*04:01
HLA-DRB1*08:02
HLA-DRB1*09:01
HLA-DPA1*02:01/DPB1*01:01
HLA-DRB3*01:01
HLA-DRB1*04:01
HLA-DRB1*12:01
HLA-DQA1*05:01/DQB1*02:01
HLA-DRB1*04:05
HLA-DRB1*07:01
HLA-DRB5*01:01
HLA-DPA1*02:01/DPB1*05:01
HLA-DRB1*11:01
HLA-DPA1*02:01/DPB1*14:01
HLA-DQA1*03:01/DQB1*03:02
HLA-DQA1*04:01/DQB1*04:02
HLA-B*57:01
HLA-A*30:02
HLA-A*30:01
HLA-A*23:01
HLA-B*08:01
HLA-A*33:01
HLA-A*23:02
HLA-A*01:01
HLA-B*15:01
HLA-B*51:01
HLA-A*31:01
HLA-B*53:01
HLA-B*44:03
HLA-B*07:02
HLA-B*44:02
HLA-A*11:01
HLA-B*35:01
HLA-A*68:01
HLA-A*32:01
HLA-A*68:02
HLA-A*26:01
HLA-B*58:01
HLA-B*40:01
HLA-A*03:01
HLA-A*02:01
HLA-A*02:03
LJL143.2

Figure 2. LJL-143 T cell epitope mapping: overall picture of the *in silico* analysis performed against murine and human MHC-I and MHC-II molecules. LJL-143 (GenBank acc. no. AAS05319) T cell epitope mapping was performed using the IEDB Analysis Resource considering 3 murine and 27 human MHC-II alleles and 5 murine and 27 human MHC-I alleles. Translated results of the predictions of murine MHC-II restricted epitopes (A and A'), murine MHC-I restricted epitopes (B and B'), human MHC-II restricted epitopes (C and C') and human MHC-I restricted epitopes (D and D') are represented. Results are presented by allele. Each arrow represents one or more (contiguous) predicted epitopes: the top 1% hits for MHC-I molecules and the top 2.5% (very high affinity binders) or 10% hits for MHC-II human and murine alleles, respectively. Underlined protein residues represent the signal peptide sequence. Magnified protein residues are potentially important for protein biological activity. Dashed boxes represent the convergent analysis of the different *in silico* determinations that enabled the selection of two protein portions to be included in the final chimeric sand fly salivary antigen.

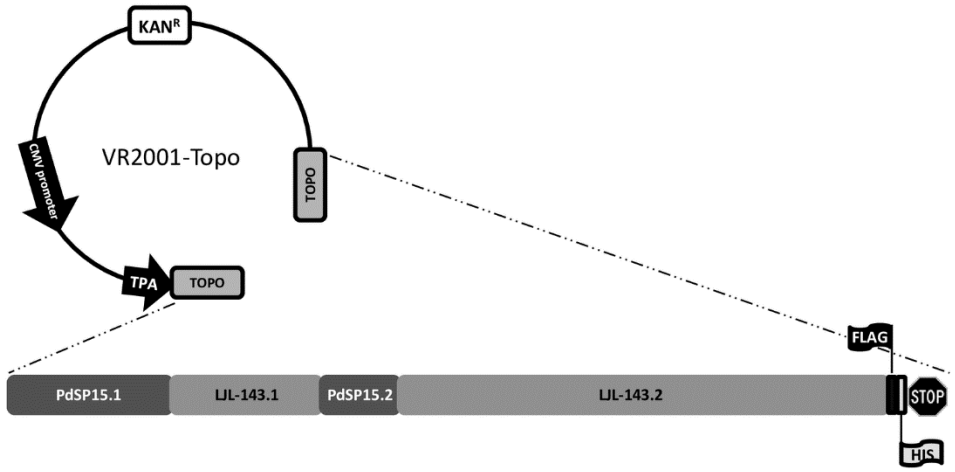
Engineering a multi-sand fly species based anti-Leishmania vaccine: proof of principle. Focusing on the *in silico* determinations described above we performed a convergent analysis to define the protein regions of both PdSP15 and LJL-143 with the "best immunogenic potential": i) containing both MHC-I and MHC-II restricted epitopes for different human alleles (to maximize population coverage); ii) containing conserved epitopes (comparing protein homologs, to maximize potential cross-protective potential); and iii) containing both murine MHC-I and MHC-II restricted epitopes (to *maximize the possibility of translation of pre-clinical studies to the clinical context*). Regarding PdSP15, this convergent analysis revealed two protein portions, PdSP15.1 and PdSP15.2, consisting of 55 and 22 amino acids, respectively (Figure 1; dashed boxes). Although these portions comprise 54% or 63% excluding the signal peptide sequence, of the full-length protein, they overall contain the majority of the murine and human MHC-I and –II restricted epitopes predicted *in silico* (Figure 1; Supplementary Figure 2). Additionally, they are 61.8 % and 78.3 % conserved, comparing with PpSP15, the PdSP15 homologue studied (Supplementary Figure 3), and exclude the signal peptide sequence, as well as 4 of the 8 basic residues thought to be important for substrate interaction (e.g. heparin), and consequently for the PdSP15 anti-haemostatic properties[55]. Similarly, for LJL-143 two protein portions were defined, LJL143.1 and LJL143.2, consisting of 50 and 155 amino acids, respectively (Figure 2). Although visually less clear, the convergent analysis (dashed boxes) led us to exclude, in addition to the signal peptide, the region between residues 90 and 140, less enriched in human MHC-II restricted epitopes (Figure 2; Supplementary Figure 4). LJL143.1 and LJL143.2 together represent 68% or 74% excluding the signal peptide sequence, of the full-length protein and are 38.5 and 50% conserved, comparing with PpeSP06, the LJL-143 homologue studied (Supplementary Figure 5).

These rationally chosen protein portions were then used as a template to design a single antigen (fusion protein) that in theory would maintain the individual immunogenic properties of each of the single sand fly salivary proteins, but not their biological activities. To maximize the potential loss of the known anti-haemostatic properties of PdSP15 and LJL-143[55,56], we designed the fusion antigen in an interpolated manner (Figure 3A). We did not use any linkers, and added a dual tag at the C-terminal (FLAG-tag followed by HIS-tag; Figure 3A), to allow protein purification and traceability, as well as tags removal (the FLAG-tag contains an enterokinase cleavage site). The resulting sequence coding for this neo-antigen was codon optimized to the mammalian codon usage preference (Figure 3B), synthetized and cloned into the VR2001-TOPO vector, immediately downstream of a sequence coding for

the tissue plasminogen activator signal peptide, that ensures protein secretion (Figure 3A). The VR2001-TOPO vector was previously used both in the context of DNA vaccination and of *in vitro* protein expression using a mammalian expression system[25,47]. Furthermore, we submitted the fusion protein sequence to additional bioinformatics determinations using the available online tools SCRATCH and AlgPred. This engineered neo-antigen was defined as potentially immunogenic (with a probability of 89.9%, Figure 3C). No transmembrane domains were predicted within the protein sequence, while 4 disulphide bonds were predicted to be formed, due to the presence of 8 cysteines (Figure 3C). Additionally, the fusion protein was predicted to be insoluble under overexpression conditions, and a non-allergen, due to the 3 of 4 negative results given by the AlgPred algorithm (Figure 3C).

In theory, the abovementioned approach (we propose as a proof of principle of a future vector-derived pan anti-*Leishmania* vaccine) warrant the broadening of the vaccine spectrum of any individual sand fly salivary protein candidate, taking in consideration the geography (Old and New worlds), target population (endemics and non-endemics) and disease manifestation.

A



B

```
   I  S  C  L  H  H  C  K  Y  Q  Y  Y  G  F  I  D  V  N  Y  N  I  A  Q  S  E  I  R  K  F  S  N  V  L  M  D  Y  G  V
1  ATCAGCTGTCTGCACCACTGCAAGTACCAGTACTACGGCTTCATCGACGTGAACTACAATATCGCCCAGAGCGAGATCCGGAAGTTCAGCAACGTGCTGATGGACTACGGCGTG  114

   V  D  K  S  K  K  R  E  L  K  K  V  M  H  E  C  A  L  F  F  A  S  Y  G  L  K  R  D  P  C  Q  I  V  L  G  Y  K  C
115 GTGGACAAGAGCAAGAAGCGCGAGCTGAAGAAAGTGATGCACGAGTGCGCCCTGTTCTTCGCCAGCTATGGCCTGAAGAGAGATCCCTGCCAGATCGTGCTGGGCTACAAGTGC  228

   S  N  N  Q  T  H  F  V  L  N  F  K  T  N  K  K  S  C  I  S  A  I  K  L  T  S  Y  P  K  I  R  T  S  I  N  Y  Y  R
229 AGCAACAATCAGACCCACTTCGTCCTGAACTTCAAGACCAACAAGAAGTCCTGCATCAGCGCCATCAAGCTGACAAGCTACCCCAAGATCCGGACCAGCATCAACTACTACAGA  342

   C  V  L  T  S  K  L  I  G  P  Q  R  F  D  K  D  R  Y  I  G  A  D  P  L  H  V  D  S  Y  G  L  P  Y  Q  F  D  Q  E
343 TGCGTGCTGACCAGCAAGCTGATCGGCCCTCAGAGATTCGACAAGGACCGGTATATCGGCGCTGACCCTCTGCACGTGGACTCTTACGGACTGCCCTACCAGTTCGACCAAGAG  456

   H  G  W  N  V  E  R  Y  N  I  F  K  D  T  R  F  S  T  E  V  F  Y  H  K  N  G  L  F  N  T  Q  I  T  Y  L  A  E  E
457 CACGGCTGGAACGTGGAACGGTACAACATCTTCAAGGACACCCGGTTCAGCACCGAGGTGTTCTACCACAAGAACGGCCTGTTCAACACCCAGATCACCTACCTGGCCGAAGAG  570

   D  S  F  S  E  A  R  E  I  T  A  K  D  I  K  K  K  F  S  I  I  L  P  N  E  E  Y  K  R  I  S  F  L  D  V  Y  W  F
571 GACAGCTTCAGCGAGGCCAGAGAGATCACCGCCAAGGACATCAAGAAGAAGTTCTCCATCATCCTGCCGAACGAAGAGTACAAGCGGATCTCCTTCCTGGACGTGTACTGGTTC  684

   Q  E  T  M  R  K  K  P  K  Y  P  Y  I  H  Y  N  G  E  C  S  N  E  N  K  T  C  E  L  V  F  D  T  D  E  L  M  T  Y
685 CAAGAGACAATGCGGAAGAAGCCTAAGTACCCCTACATCCACTACAACGGCGAGTGCTCCAACGAGAACAAGACCTGCGAGCTGGTGTTCGACACCGACGAGCTGATGACATAC  798

   A  L  V  K  V  F  T  N  P  E  S  D  G  S  R  L  K  E  D  Y  K  D  D  D  D  K  H  H  H  H  H  H  *
799 GCCCTGGTCAAGGTGTTCACAAACCCCGAGTCCGACGGCAGCAGACTGAAAGAGGACTACAAGGACGACGACGACAAGCACCACCACCATCACCACTGA  837
                                                                                  FLAG-tag      HIS-tag
```

C

***in silico* determinations**

Prediction	Result		Tool
	Qualitative	Probability	
Antigenicity	/	0.899527	
Transmembrane domains	Absent	0.987357	
α-helical transmembrane protein	No	0.0103291	
β-barrel transmembrane protein	No	0.0023138	SCRATCH *
Dissulfide bonds	4 predicted: Cys(247-254); Cys(54-68); Cys(94-115); Cys(3-7)	/	
Solubility upon overexpression	Insoulble	0.699259	
Allergenic potential	Non-Allergen	/	
motiff alignment	No hits found	/	
mapping of IgE epitopes	The protein sequence does not contain experimentally proven IgE epitopes	/	AlgPred &
allergen representative peptides	No hits found	/	
SVM analysis based on AA composition	Allergen (PPV 47.13%; NPV 89.71%)	/	

Figure 3. Engineering a multi-sand fly species based anti-*Leishmania* vaccine. A single DNA-based vaccine was engineered, considering a reverse vaccinology approach applied

to the two salivary proteins this study focuses on: PdSP15 and LJL-143. The scheme of the final DNA vaccine, consisting of the VR2001-TOPO vector backbone and the fusion salivary antigen coding sequence, composed by the selected immunogenic portions of the two salivary proteins joined contiguously in an interpolated manner, a FLAG-tag, a HIS-tag and a stop codon is represented (A). The final sequence coding for the fusion salivary antigen (codon optimized for the mammalian codon usage bias), used in the cloning approach, as well as the respective translation, is shown (B). Underlined nucleotides code for the portions of PdSP15, while non-underlined ones code for the portions of LJL-143. FLAG- and HIS-tags coding sequences are highlighted. The fusion protein amino-acid sequence was subjected to *in silico* predictions of antigenicity, solubility upon overexpression, presence of transmembrane domains, disulphide bonds formation and allergenic potential, whose quantitative and/or qualitative results are represented (C).

Ex vivo cell proliferation experiments attest the immunogenicity of the engineered multi-sand fly species single-antigen and validate in silico determinations. To understand if the immunogenic properties of PdSP15 and LJL-143 were retained by the fusion antigen, we performed a DNA vaccination-based immunogenicity trial. Briefly, BALB/C mice, background we chosen based on *in silico* predictions, were immunized intradermally in the right ear three times at two weeks intervals with 5 µg of VR-2001-SfSPChimera plasmid, or with either *P. duboscqi* or *L. longipalpis* Salivary Gland Homogenates (SGH) - the equivalent of 1 sand fly salivary gland pair. Control animals received the same volume of the vehicle solution (Phosphate-Buffered Saline - PBS). One month after the last immunization, we evaluated T cell proliferation in the context of recall experiments using a co-culture of CFSE-stained splenocytes from immunized animals, and naïve BMDCs pulsed with *P. duboscqi, L. longipalpis, P. papatasi or P. pernicious* SGH at a final concentration of 3 sand fly salivary gland pairs/ml. Co-cultures of splenocytes and non-pulsed BMDCs, either non-stimulated or stimulated with the mitogen Concanavalin A, were used as experimental negative and positive controls, respectively. Importantly these controls revealed that the splenic T cells' basal proliferation levels (Supplementary Figure 6A), and proliferative capacity (Supplementary Figure 6B) were comparable among the different groups. When we used *P. duboscqi* SGH as recall agent, we detected significantly higher specific T cell proliferation (CD3+) in the groups immunized with either *P. duboscqi* SGH or the chimeric DNA vaccine, compared to control animals (Figure 4A; $p \leq 0.01$ and $p \leq 0.05$, respectively), and to animals that received *L. longipalpis* SGH (Figure 4A; $p \leq 0.05$). This picture was overall reproduced when we analysed specific T helper (CD3+/CD4+) and

T cytotoxic (CD3+/CD8+) cells' proliferation separately, although more consistently for the first T cell subset. All statistically significant differences were maintained when we analysed proliferation within CD3+/CD4+ cells (controls *versus P. duboscqi* SGH and VR-2001-SfSPChimera groups, p≤0.001 and p≤0.05, respectively; *P. duboscqi* SGH group *versus L. longipalpis SGH group, p≤0.01),* while regarding CD3+/CD8+ cells' proliferation the statistical relevance comparing VR-2001-SfSPChimera and control groups was not observed (Figure 4A). Importantly, when we used *L. longipalpis SGH* as a stimulus, we detected specific T cell proliferation (CD3+) in the group of animals immunized with *L. longipalpis SGH,* and once again in animals that received the chimeric DNA vaccine (Figure 4B). Relevantly, the proliferation levels were similar for these two groups, and significantly higher than both controls, and animals immunized with *P. duboscqi* SGH (Figure 4B; at least p≤0.01). Once more, this result was overall recapitulated when we analysed CD3+/CD4+ and CD3+/CD8+ populations individually (Figure 4B). However CD3+/CD8+ T cell proliferation in response to L. longipalpis was not significantly different comparing the groups that received *L. longipalpis and P. duboscqi SGH (Figure 4B).* On the other hand, the responses detected against SGH from the vectors *P. papatasi and P. perniciosus,* that contain the homologous proteins PpSP15 and PpeSP06, respectively were not as striking. In response to P. papatasi SGH, a higher proliferation of CD4+ T cells was observed in the group of animals that was immunized with *P. duboscqi* SGH, in comparison to controls and to animals that received *L. longipalpis* SGH (Supplementary Figure 6C; p≤0.05), but not compared to animals immunized with the chimeric DNA vaccine. In respect to the T cell proliferation detected in response to *P. perniciosus* SGH no major differences were observed (Supplementary Figure 6D).

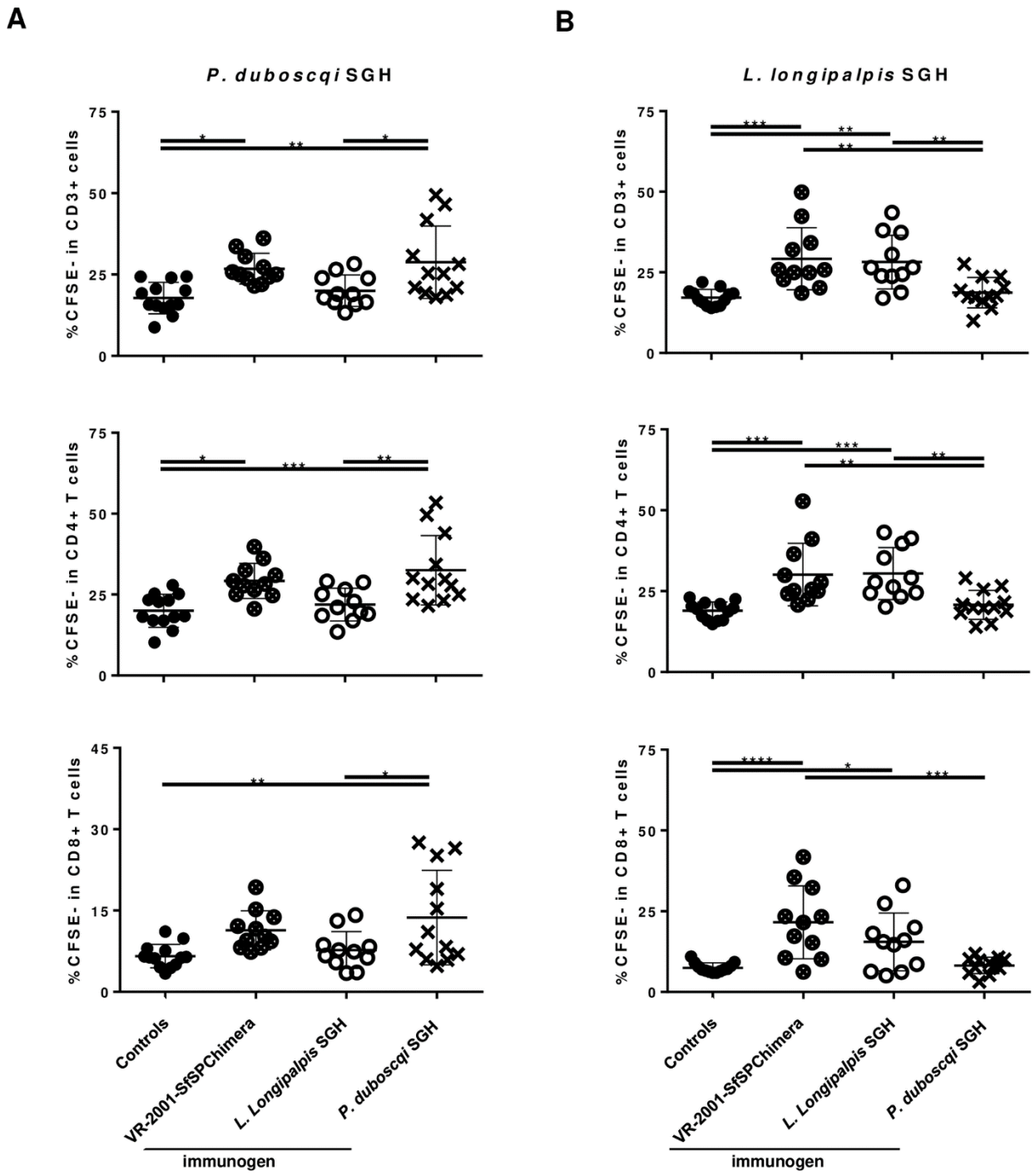


Figure 4. Vaccination with the sand fly-derived DNA chimeric vaccine elicit specific T cell responses against both *P. duboscqi and L. longipalpis saliva.* *BALB/C mice were immunized* intradermally in the right ear three times at two weeks intervals with *5 µg* of VR-2001-SfSPChimera plasmid (crossed circles), or with either *L. longipalpis (white circles)* or *P. duboscqi (crosses)* SGH - the equivalent of 1 sand fly salivary gland pairs. Control animals received the same volume of the vehicle solution (PBS; black circles).

One month after the last immunization, animals were euthanized, their spleens collected and processed to obtain CFSE-stained splenocytes suspensions. Frequencies of proliferating splenic T cells (total CD3+ and CD3+/CD4+ or CD3+/CD8+) were determined by flow cytometry after four days of culture *in the presence of BMDCs (*5:1 ratio) *pulsed with P. duboscqi (A) or L. longipalpis (B)* SGH (final concentration of 3 sand fly salivary gland pair/ml). Results from three independent experiments are shown. Each dot represents one animal. Average and SD of the values within each group are shown. Statistical differences are properly identified (One-Way ANOVA with Tukey´s *post hoc* analysis: * p≤0.05, ** p≤0.01, *** p≤0.001 and **** p≤0.0001).

Protection from sand fly-transmitted infection is dependent on high IFN-γ/IL-10 and IFN-γ/IL-4 ratios. The abovementioned immunogenic potential of the engineered chimeric antigen that indicates a potential "vaccine-spectrum enlargement", led us to conduct a preliminary efficacy trial in the context of vector transmitted infections. First, we explored the efficacy of our chimeric DNA vaccine in the context of cutaneous leishmaniasis, using C57BL/6 mice, the murine model that parallels human disease. Briefly, mice were immunized intradermally in the right ear, three times at two week intervals with 5 µg of either VR-2001-SfSPChimera or the empty vector, and challenged one month later on the contralateral ear via sand fly bites: 10 *P. duboscqi* sand flies (per animal) carrying mature *L. major* infections (Figure 5A). Ear lesion progression was assessed weekly, and total parasite burdens were evaluated at two and six weeks post challenge (Figure 5A). In this context, no differences were detected between vaccinated and control animals in both pathology, assessed by ear thickness measurements (Figure 5B), and total ear parasite burden determined at the two and six week time-points (Figure 5C). These results dictated the use of a different model. For that, the overall experimental time-line adopted was similar, but the murine strain used was different (BALB/c), as was the vector parasite-combination (*L. longipalpis – L. major*, typically a VL vector, transmitting a CL causative parasite species) - Figure 5D. In this context, lesion development was also similar comparing vaccinated with control animals up to week 4 post-challenge (Figure 5E). From then on, the average lesion diameter of control animals increased in a higher proportion comparing with animals immunized with VR-2001-SfSPChimera, though the differences were not statistically significant. These results aligned with the total ear parasite burdens determined. While at two weeks post-challenge no differences were noted, at six weeks post-infection the VR-2001-SfSPChimera vaccinated animals showed significantly lower parasite burdens in comparison with the animals that received the empty vector (p≤0.05; Figure 5E).

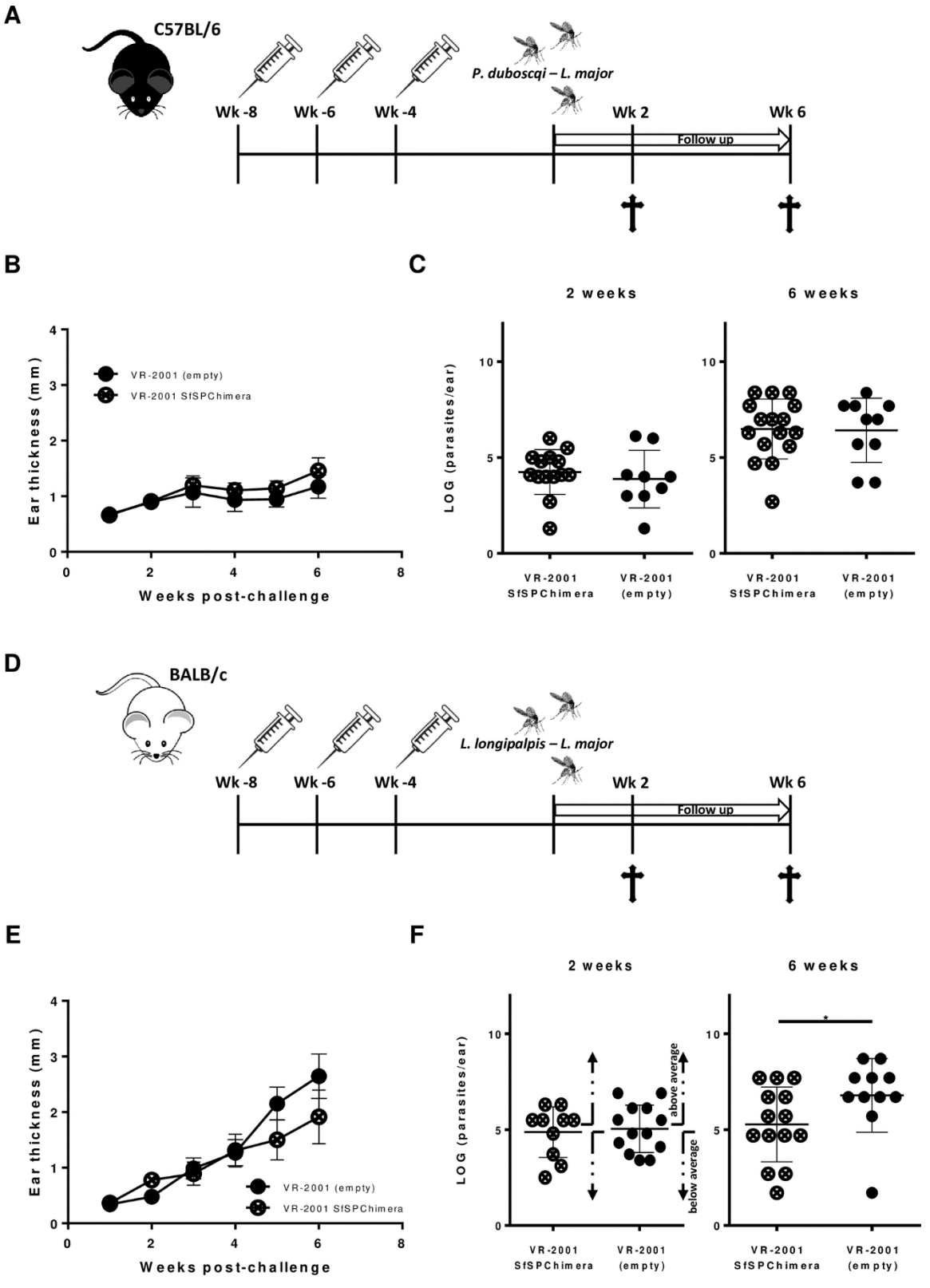

A
C57BL/6
P. duboscqi – L. major
Wk -8
Wk -6
Wk -4
Wk 2
Wk 6
Follow up
B
Ear thickness (mm)
VR-2001 (empty)
VR-2001 SfSPChimera
Weeks post-challenge
C
2 weeks
6 weeks
LOG (parasites/ear)
VR-2001 SfSPChimera
VR-2001 (empty)
D
BALB/c
L. longipalpis – L. major
E
F
above average
below average
*

Figure 5. Sand fly-derived DNA chimeric vaccine preliminary efficacy trial. C57BL/6 or *BALB/C mice were immunized* intradermally in the right ear three times at two weeks intervals with *5 µg* of VR-2001-SfSPChimera plasmid (crossed circles), or with the empty vector (control animals; black circles). C57BL/6 mice were challenged one month later on the contralateral ear via sand fly bites - 10 *P. duboscqi sand flies* carrying mature *L. major* infections were used per animal. Ear pathology was assessed weekly, and experiments were terminated at either two or six weeks post challenge for parasite burden evaluation (A). Ear thickness measurements are represented for both VR-2001-SfSPChimera immunized and control animals (B). Total ear parasite burdens at two and six weeks post-challenge are also shown (C). BALB/c mice were also challenged one month after the last immunization on the contralateral ear via sand fly bites, using a different species - 10 *L. longipalpis sand flies* carrying mature *L. major* infections were used per animal (D). The follow up and endpoints used were the same as abovementioned (D). Ear thickness measurements are once more represented for both VR-2001-SfSPChimera immunized and control animals (E), as are the determined parasite burdens at two and six weeks post-challenge (F). Results from at least two independent experiments are represented in each graph. Each dot represents one animal. Average and SEM or SD of the values within each group are shown for ear thickness or parasite burden measurements, respectively. Each group represented in the 2 weeks' time-point of panel F was subdivided in two groups based on parasite burden values: below and above average. Statistical differences are properly identified (Mann-Whitney test: * $p \leq 0.05$).

Considering that the phenotype observed at six weeks post-infection in BALB/c mice must be a consequence of an immune response initiated earlier, we decided to look at local anti-*Leishmania* immune responses early after infection (2 weeks' time-point) using an ex-vivo approach. Briefly, cytokine secretion by ear CD4+ T cells was evaluated after an overnight culture of total ear cells in the presence of SLA. Since at two weeks post-challenge the determined parasite burdens were similar, comparing vaccinated with control animals, the first step of our analysis was the correlation of those parasite burdens with the respective *Leishmania*-specific cytokine responses. Curiously, while no correlation was observed between *Leishmania*-specific IFN-γ secretion by CD4+ T cells and parasite burdens, a positive correlation was defined between parasite burdens and both *Leishmania*-specific IL-4 and IL-10 secretion by CD4+ T cells ($p \leq 0.05$; Figure 6A). To understand if this correlation was a result of the natural heterogeneity of our *in vivo* model, or a consequence of a vaccine-induced effect, we further investigated these *Leishmania*-specific cytokine responses, now in the context of a divergent analysis. For this we divided both vaccinated and control animals in two sub-groups (total of 4 groups) - the ones with determined parasite burdens

below average, and the above average counterparts (Figure 5F). This analysis revealed first that the *Leishmania*-specific IFN-γ levels did not differ comparing the sub-groups, considering both animals that received VR-2001-SfSPChimera and the ones that received the empty vector, and were significantly higher comparing all the vaccinated animals with all the controls ($p \leq 0.01$; Figure 6B). Importantly, for both IL-4 and IL-10 secretion by CD4+ T cells in response to *Leishmania* antigens, the picture was different. While no differences were detected comparing all the VR-2001-SfSPChimera vaccinated animals with all the controls, when we looked at sub-groups, no differences were detected within the controls, while a significant higher frequency of *Leishmania*-specific IL-4 and IL-10 secreting CD4+ T cells was detected comparing the vaccinated animals showing parasite burdens above average, with the ones with determined parasite burdens below average ($p \leq 0.05$; Figure 6B). Consequently, when we looked at the pro/anti-inflammatory cytokine ratios, evident differences were observed, only within the VR-2001-SfSPChimera vaccinated sub-groups (Figure 6C). Considering both IFN-γ/IL-10 and IFN-γ/IL-4 ratios, they were significantly higher in the vaccinated animals with determined parasite burdens below average, in comparison with the ones showing parasite burdens above average ($p \leq 0.05$ and $p \leq 0.01$, respectively) (Figure 6C). Indeed, when we correlated these ratios with parasite burdens, now only for the VR-2001-SfSPChimera immunized animals, robust negative correlations were established ($p \leq 0.01$ / r = -08649 and $p \leq 0.05$ / r = -0.7494, respectively).

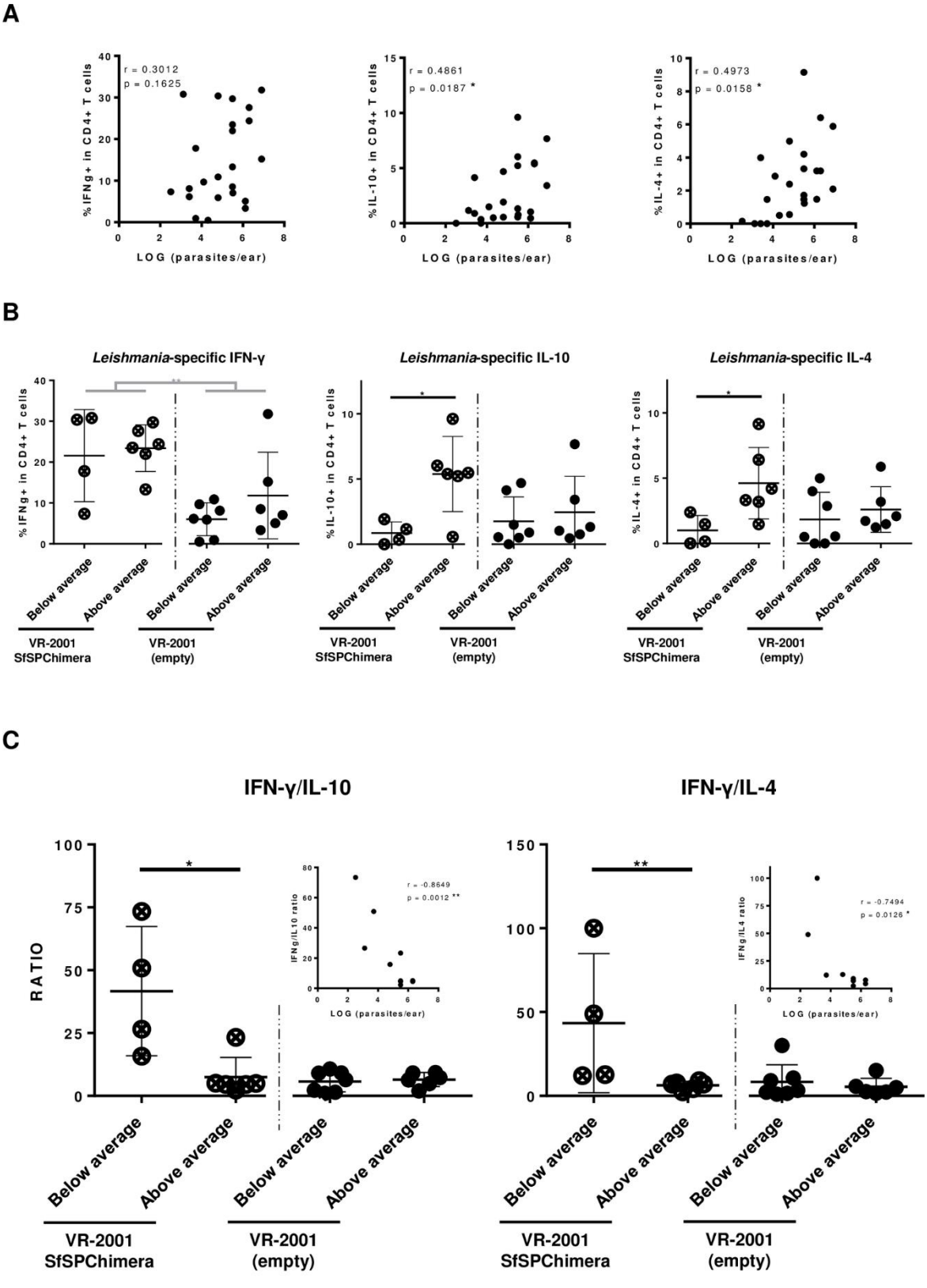

A
r = 0.3012
p = 0.1625
% IFNg+ in CD4+ T cells
LOG (parasites/ear)
r = 0.4861
p = 0.0187 *
% IL-10+ in CD4+ T cells
LOG (parasites/ear)
r = 0.4973
p = 0.0158 *
% IL-4+ in CD4+ T cells
LOG (parasites/ear)
B
Leishmania-specific IFN-γ
Leishmania-specific IL-10
Leishmania-specific IL-4
% IFNg+ in CD4+ T cells
% IL-10+ in CD4+ T cells
% IL-4+ in CD4+ T cells
Below average
Above average
VR-2001 SfSPChimera
VR-2001 (empty)
C
IFN-γ/IL-10
IFN-γ/IL-4
RATIO
r = -0.8649
p = 0.0012 **
IFNg/IL10 ratio
r = -0.7494
p = 0.0126 *
IFNg/IL4 ratio
LOG (parasites/ear)
Below average
Above average
VR-2001 SfSPChimera
VR-2001 (empty)

Figure 6. Protection against *L. longipalpis* sand fly-transmitted *L. major* infection in BALB/c mice is dependent on high IFN-γ/IL-10 and IFN-γ/IL-4 ratios. *BALB/C mice were immunized* intradermally in the right ear three times at two weeks intervals with *5 µg* of VR-2001-SfSPChimera plasmid (crossed circles), or with the empty vector (control animals; black circles). One month after the last immunization mice were challenged on the contralateral ear via sand fly bites - 10 *L. longipalpis sand flies* carrying mature *L. major* infections were used per animal. Two weeks later animals were euthanized and their ears collected and processed. Parasite burdens were determined by limiting dilution (Figure 5F) and T cell cytokine secretion was analysed by flow cytometry after an overnight culture of total ear cells in the presence of *L. major* SLA, and additionally PMA, Ionomycin and Brefeldin A during the last 4 hours of culture. For each animal, including controls, parasite burden values were plotted against the frequencies of CD4+ T cells secreting IFN-γ, IL-10 or IL-4, in a way to assess their correlation (A). These frequencies are represented for VR-2001-SfSPChimera immunized and control animals, sub-grouped (Figure 5F) according to parasite burden values (B). IFN-γ/IL-10 and IFN-γ/IL-4 ratios were calculated and are represented using the group division criteria (C). Correlation between these ratio values and the parasite burdens were also performed, now only considering the animals immunized with VR-2001-SfSPChimera DNA vaccine (C). Results from two independent experiments are represented. Each dot represents one animal. Average and SD of the values within each group are shown. Statistical differences are properly identified (Mann-Whitney test: * $p \leq 0.05$ and ** $p \leq 0.01$) and refer to differences between sub-groups (black bars), or differences between VR-2001-SfSPChimera vaccinated and control groups (grey bars). Pearson correlation coefficients (r) and significance are also represented.

DISCUSSION

The quest for a vaccine against human leishmaniasis has still not been fruitful. Although many vaccine candidates were proposed and tested along the years, so far, the documented protection as a result of leishmanization campaigns was never again achieved in the clinical context[12]. There are a number of factors that together justify this fact, one of the most important being related to pre-clinical to clinical translation. The majority of the vaccine candidates are initially tested empirically, as proof-of concept studies in mouse models[12], recognized by the scientific community as invaluable tools for the advancement of science in general[57,58]. Still, there are well known differences between mouse and human immunology that may condition distinct vaccination outcomes, particularly when we consider T cell vaccines. T cell epitopes are usually HLA-restricted, frequently in a species specific fashion[59,60]. Additionally, together with the intrinsic physiological/biological/genetic limitations of murine models[57,59-61], again having in mind translation to humans, we also need

to consider the validity of the model chosen[62]. Most of the proposed anti-*Leishmania* vaccines were evaluated in models that disregarded either the contribution of the vector and vector derived components, or the transmission mechanism *per se*. On one hand, the parasites are deposited into the host skin through the bite of an infected sand fly. Therefore, the early immune response in the skin must be considered and not only from the pathophysiological perspective. This is often frequently overlooked, particularly when we think about visceral disease models that are often infected intravenously or intraperitoneally[63]. On the other hand, the parasites are inoculated at the bite site together not only with parasite-derived components (such as exosomes or PSG) but also with vector-derived ones (such as sand fly midgut microbiota and sand fly saliva), all demonstrated to impact parasite establishment[51,53,64-66]. Still, the majority of the inoculums used in experimental infections are composed exclusively of parasites (not rarely non-metacyclic promastigotes, the natural infectious form)[63,67]. Curiously, and in line with these raised issues, Peters *et al* have shown *in vivo* that a previously defined effective anti-*Leishmania* vaccine, lost its protective potential in the context of natural *Leishmania* transmission via sand fly bites[52], reproducing the disappointing results obtained in human clinical trials.

The abovementioned major issues of the anti-*Leishmania* vaccines' research were the premise of this study. The demonstration in different models that pre-exposure to vector saliva, at least from *P. papatasi*, *P. duboscqi*, *L. whitmani* or *L. longipalpis*, confers protection against vector-transmitted leishmaniasis[13,18,68,69], supports the study of defined sand fly salivary proteins as anti-*Leishmania* vaccines. Importantly, the fact that the protection is mediated by specific, but indirect non-parasite specific responses, can be considered an advantage if we think of a paracrine effect mediated by non-infected antigen-presenting cells[70] that are potentially less affected by the known *Leishmania* immunomodulatory potential[3]. However, using a sand fly saliva-based vaccine presents its own challenges. The natural evolutionary pressure that conditioned the speciation of the different Phlebotominae that transmit leishmaniasis[71], brought as a logical consequence the lower potential for cross-reactivity (due to the known – although not strict - relation between T cell epitopes' conservation and immunogenicity[72]). Therefore defined sand fly salivary proteins will have a limited anti-*Leishmania* vaccine spectrum: effectiveness will probably be limited to the geographical region where the vector species is prevalent, and consequently to the parasite species and disease manifestation associated to that specific vector[21,22]. In the hope of turning limitations into opportunities, we engineered a single antigen (vector-derived) composed of fractions of two distinct sand fly salivary vaccine candidates, through a reverse

vaccinology approach that potentially maximizes translation from the pre-clinical to the clinical context, which we propose as a proof of principle of a future vector-based pan-*Leishmania* vaccine.

Although our vaccine proposal is directed to humans, in our in silico approach, we predicted, both murine and human MHC-I and MHC-II restricted epitopes (Figures 1 and 2), to uncover the "immunogenic map" of the two antigens and the overlaps considering the two species. This approach was adopted to bridge the pre-clinical and clinical contexts. Peptide-based vaccines, particularly targeting T cell responses, have been exploited particularly in the cancer immunotherapy field, and have recognized advantages pertaining to simple and reproducible manufacturing processes, stability under simple storage conditions, and lower probability of generating allergic or autoimmune responses[73]. However, peptide vaccines are also recognized as poor immunogens, with a limited spectrum considering the multiplicity of MHC alleles of the human population, and a higher probability of *in vivo* degradation in comparison with folded proteins[73]. Therefore, instead of considering individual peptides in our convergent analysis, we opted to select highly immunogenic protein regions. One the one hand this enriches epitope diversity and ensures "natural" antigen processing and presentation and on the other allows for the potential targeting of a wider population. Indeed, the protein portions we have selected have the potential of pan-immunogenicity in humans, containing epitopes predicted to bind to 24/27 and 20/27 MHC-II and MHC-I human alleles, respectively in the case of PdSP15, and to 27/27 of both MHC-II and MHC-I human alleles in the case of LJL-143 (having in mind the MHC-II and MHC-I reference sets used provide >99% and >97% population coverage, respectively). Still, *in silico* can never replace *in vitro*/*in vivo* observations, and must always be performed rationally. We included, on purpose, the signal peptide sequences of both proteins in our predictions and analysis, and found these are indeed highly immunogenic (considering both murine and human MHC-I and MHC-II alleles), which goes in line with data published on cancer research and *M. tuberculosis* infection[74,75]. However, if in other contexts they can be considered as potential vaccine candidates (they are expected to be on the diverse pool of presented peptides in the context of bacterial infections or cancer)[74], this is virtually impossible when we think of sand fly-saliva based vaccines: in nature, the host immune system will only "see" the mature salivary proteins (that are deposited in the skin by the bite of the vector), and never the signal peptides that will remain within the insect salivary glands secretory cells.

One may argue that to simply fuse the sand fly salivary proteins in tandem, would be an equally valid approach, and perhaps easier or at least faster, considering the time the *in silico* predictions and rational analysis consumes. However, sand fly salivary proteins are recognized by their strong bioactive properties[23]. Indeed the two proteins used in this study have strong anti-haemostatic properties. LJL-143 is an anti-coagulant that acts through the inhibition of factor Xa of the coagulation cascade[56], and PdSP15 interferes with factor XII contact-system driven clotting[55]. Once the mere fusion of two proteins might not impact their bioactive properties (there are actually therapeutic approaches in the pre-clinical stage, as well as biotechnology strategies that depend on the maintenance of bioactive properties in the context of fusion proteins[76-78]) we opted to fuse the immunogenic portions of the two proteins in an interpolated manner (Figure 3), as a way to potentially destabilize protein structures and consequently eliminate the bioactive effects, thereby generating an innocuous antigen. Of note, to maximize the potential loss of protein bioactive potential we considered the protein residues thought to be important for their biological activities in the in silico convergent analysis, excluding them, at least partially from the selected protein portions, while assuring maintenance of potential broad-spectrum immunogenicity. The same rationale is valid if we consider a multivalent vaccine composed by different salivary antigens (for instance the two recombinant salivary proteins formulated together), with an additional drawback. Multivalent vaccines are associated with higher costs of production, a consequence of manufacturing costs increasing proportionally to the number of antigens contemplated, and the necessary application of exhaustive quality control measures[79], which is a limitation when we think of vaccine development for leishmaniasis, a neglected tropical disease (NTD). NTDs funding averages a total of 0.6% of total development assistance for health[80], although 1/6 of the human population suffer from at least one[81] – for the sake of comparison average funding for HIV averages a total of 36.3% of total development assistance for health (60 fold higher)[80], while the estimated prevalence among adults is around 0.8% (20 fold lower)[82].

To validate our in silico analysis we opted to perform recall experiments against sand fly saliva and not against the single recombinant proteins, to mimic better the natural infection context where a heterogeneous mixture of salivary proteins is inoculated into the host skin when the sand fly takes a blood meal. T cell responses, particularly activation, are known to be dependent, amongst other things, on antigen dose, relevant not only in the context of primary immune responses[83,84], but also in relation to memory responses[85,86]. Given the fact that around 500 ng representing more than 10 different salivary proteins described in distinct

species, dependent on species-specific differences in their relative abundance, are deposited in host skin during a sand fly blood meal[44], it´s not unreasonable to consider that some may not be relevant enough to reach the antigen threshold requirement for T cell proliferation, translating into non-immunogenicity. Remarkably, we demonstrate that T cells from mice immunized with VR-2001-SfSPChimera, expand in response to both *P. duboscqi* and *L. longipalpis* SGH (Figure 4), a result that validates our *in silico* predictions, and attests our hypothesis of vaccine-spectrum enlargement. These results, considering our convergent *in silico* analysis contemplating both murine and human MHC alleles, suggest that this fusion protein will potentially generate relevant responses both in individuals from South America and West Africa (*L. longipalpis* and *P. duboscqi* origins, respectively), hypothesis we are currently pursuing. Of note, T cells from the positive control groups immunized with *P. duboscqi* or *L. longipalpis SGH* did not respond to the heterologous SGH stimulus*, (L. longipalpis or P. duboscqi* SGH, respectively) indicating an absence of cross-reactivity and therefore pointing to the potential limited spectrum of sand fly salivary antigens.

Although we clearly demonstrate the vaccine spectrum enlargement with respect to immunogenicity, we were not able to conclude the same in regards to efficacy. When we assessed protection in the context of *L. major* transmission by *P. duboscqi* to C57BL/6 mice, a self-healing cutaneous leishmaniasis model that parallels best human disease, we did not observe any protection in animals that received the VR-2001-SfSPChimera DNA vaccine (Figure 5 B/C). In this particular infection context, we could mainly evaluate how the memory generated by the PdSP15 antigenic portions included in the fusion vaccine would influence parasite establishment (since the recall agent to consider is the native PdSP15 salivary protein from *P. duboscqi* sand flies). Importantly, this protein (either in the context of DNA vaccine, or as recombinant antigen) was never tested as an anti-*Leishmania* vaccine in murine models of cutaneous leishmaniasis, although it was demonstrated an effective vaccine in a Non-Human Primate model[18]. Still, a closely related homologue from *P. papatasi* (PpSP15; Supplementary Figure 3; 67% identity considering the full length protein), was demonstrated to protect C57BL/6 mice infected intradermally by needle with *L. major* metacyclic promastigotes together with *P. papatasi* SGH[17]. Curiously, our *in silico* analysis of the PdSP15 protein did not show any relevant MHC-II restricted epitopes in respect to the allele relevant for C57BL/6 background, making us wonder if the efficacy results would be different, for instance in the context of a NHP model, or even considering in humans where *in silico* predictions suggest a good immunogenic potential. On the other way, when we assessed the potential effect of the immune response elicited by the LJL-143 immunogenic

portions included in the engineered fusion antigen on parasites' establishment, we observed a modest, but significant effect six weeks post-infection (Figure 5 E/F). For this, we took advantage of *L. longipalpis* sand flies (main vector of VL and South America, containing the native LJL-143 in the saliva) and BALB/c mice (one of the relevant murine models of VL), but in the context of *L. major* transmission (dermotropic parasites). Curiously, this is the first time that protection mediated by LJL-143 is demonstrated in a murine model. Although this antigen was demonstrated to negatively impact *Leishmania* parasites in a relevant canine model, since dogs are natural reservoirs of VL[25], in a different murine model (C57BL/6 mice infected intradermally by needle with *L. major* parasites together with *L. longipalpis* SGH), LJL-143 failed to protect against disease[87], emphasizing the importance of host genetics. This different phenotype we report now, may be due to two different explanations. The first one relates directly to the model. Again, the *in silico* predictions indicate that this antigen is theoretically more immunogenic for the BALB/c background (Supplementary data 8 – various MHC-II restricted epitopes in at least 4 distinct protein regions), comparing with C57BL/6 mice (Supplementary data 9 – 6 MHC-II restricted epitopes in one single regions), which may suggest that the absence of efficacy in the C57BL/6 model may be consequence of poor immunogenicity (although a LJL-143 specific DTH response was reported in this background [87]). The second one comes as an (accidental) consequence of our strategy. *In silico*, we predicted MHC-I and MHC-II restricted epitopes. Still, this analysis does not allow to extrapolate to T cell functionality that has to be proven experimentally, strategy that is starting to be employed in T cell vaccinology[88]. It is therefore not impossible to imagine that, by removing 26% of the LJL-143 protein sequence (portions not included in our fusion antigen), we excluded important Th2 epitopes, overall improving the protective potential of the antigen. This is however a speculation that we think deserves further investigation.

The curious observation that the protective phenotype in BALB/c mice was observed starting only from week 4 post-infection (and not at the two weeks' time-point) led us to further explore the potential mechanism of protection. Since the protective mechanism in our context has to be dependent on the anti-vector saliva response, differences must be visible relatively early after vector transmission (the moment where the host gets in contact with vector saliva), which justifies our option to explore the immune context at two weeks post-infection (where no differences in terms of both pathology and parasite burdens were observed). Because the dispersion in terms of the quantified parasite burdens (at two weeks post-infection) might just be a consequence of the heterogeneity of sand fly transmission experiments, we divided both controls and VR-2001-SfSPChimera vaccinated animals in

two subgroups, and showed that indeed the phenotype observed at six weeks post-infection is vaccine specific (Figure 6). Importantly, we show here that a strong IFN-γ response by CD4+ T cells, accepted as the hallmark protective response[89], is not sufficient to warrant protection. In fact, the best correlates of protection in our model are the low *Leishmania*-specific secretion of the anti-inflammatory cytokines IL-4 and IL-10 by CD4+ T cells, and consequently the high anti/pro-inflammatory balance. The next step we will follow will be the evaluation of the vaccine candidate in the context of a visceral disease model (e.g. *L. infantum* transmission by *L. longipalpis*, the natural parasite-vector pairing from South America), with a certain degree of optimism, since also in visceral disease, a high IFN-γ response, together with a lower IL-10 response are essential for parasite elimination and disease resolution[90,91].

In summary, we show here that the enlargement of sand fly saliva-based vaccines is an achievable goal. This should however be done following a rational strategy, to ensure that the probability of translatability to humans, which are the target population, is the highest possible. We propose our approach as a proof of principle for a future vector-based pan-*Leishmania* vaccine encompassing immunogenic portions from more salivary proteins, selected based on a convergent analysis contemplating murine and human MHC alleles. For example, salivary proteins from *P. argentipes* or *P. sergenti*, relevant vectors of visceral and cutaneous disease, caused by *L. donovani* and *L. tropica*, in the Indian Sub-continent and Middle East, respectively[92,93] can be considered, to further enlarge the vaccine-spectrum of the present *P. duboscqi* and *L. longipalpis* fusion protein. There is still much room for improvement, starting by switching from DNA to a recombinant protein vaccine approach that can be administered together with a Th1 inducing adjuvant and/or combined with a parasite-derived highly conserved antigen, to maximize the negative impact on *Leishmania* parasites' establishment, through additive parasite-specific and vector saliva-specific immune responses.

METHODS

Rationale. As a proof-of principle that would help us understand if we could obtain a vector-derived large-spectrum anti-*Leishmania* vaccine candidate for humans (targeting distinct endemic areas, in both the New and Old Worlds; directed against different parasite species, and consequently distinct disease forms), we've selected two different sand fly salivary

proteins, already studied in the context of vector-based anti-*Leishmania* vaccines, and followed a reverse vaccinology approach to design a single antigen (fusion protein) that in theory would maintain the individual immunogenic properties of each of the single sand fly salivary proteins, but not their biological activities. The proteins this study focuses on are: i) PdSP15, a *P. duboscqi* salivary protein (Old World; studied in the context of Cutaneous Leishmaniasis) – GenBank acc. no. ABI15933[27]; and ii) LJL-143, a *L. longipalpis* salivary protein (New World; studied in the context of Visceral Leishmaniasis) – GenBank acc. no. AAS05319[28].

in silico analysis. Signal peptide sequences were predicted using the SignalP 3.0 server[29]. T cell epitope mapping was performed (for each individual protein) using the IEDB Analysis Resource (www.iedb.org). MHC-II binding predictions were made against 3 murine alleles (H2-IA^d and H2-IE^d - representative, among others, of BALB/c strain; and H2-IE^b - representative, among others, of C57BL/6 strain), and 27 human alleles (reference set that provides >99% population coverage[30,31]), considering 15-MER sized peptides, and using the consensus method[32,33], or whenever not possible the SMM-Align (ver. 1.1)[34] or the NetMHCIIpan (ver. 3.1)[35] tools. MHC-I binding predictions were made against 5 murine alleles (H2-D^d, H2-K^d and H2-L^d – representative, among others, of BALB/c strain; and H2-D^b and H2-K^b – representative, among others, of C57BL/6 strain), and 27 human alleles (reference set that provides >97% population coverage[30,36]), considering 8-14- or 9-10-MER sized peptides, respectively, and using the consensus tool[37], which combines predictions from ANN aka NetMHC (4.0)[38-40], SMM[41] and Comblib[42] tools, or whenever not possible the NetMHC (4.0) tool[38-40]. Additionally, human CD4+ T cell epitope mapping (MHC-II binding against the same 27 alleles that provide >99% population coverage), was performed with two other salivary proteins: PpSP15 - GenBank acc. no. AF335487 - a *Phlebotomus papatasi* salivary protein with known homology to PdSP15, studied in the context of Cutaneous Leishmaniasis[43,44]; and PpeSP06 - GenBank acc. no. *DQ153100* - a salivary protein from *Phlebotomus perniciosus*, the main vector of Visceral Leishmaniasis in the Mediterranean Basin, with known homology to LJL-143[43,45]. Selection of predicted binders was done based on the percentile ranks calculated, according to IEDB recommendations: top 10% or 1% hits for MHC-II or MHC-I, respectively[46]. A convergent analysis was performed to identify the protein portions to be part of the final fusion vaccine candidate based on: i) presence of murine and human CD4+ T cell epitopes (predicted); ii) presence

of murine and human CD8+ T cell epitopes (predicted); iii) conservation of protein (and epitopes) sequences between homologs; and iv) exclusion of signal peptides and other residues thought to be important for the proteins' biological activities.

Construction of the multiepitope vaccine sequence, cloning and other in-silico determinations. The sequences coding for the protein portions selected to be included in the fusion antigen were joined contiguously, respecting the open reading frames of interest. Sequences coding for a Flag-Tag and a His-Tag were added to the 3'end, followed by the introduction of a stop codon. Correct translation was confirmed using ExPASy translate tool, followed by codon optimization to a mammalian codon usage bias. The originated sequence was synthetized (GeneArt Strings - Thermo Fischer Scientific, MA, USA) and cloned into the VR2001-TOPO vector as previously described[47]. The VR-2001-SfSPChimera plasmid was then purified as previously reported[47], and used either as a DNA vaccine or as a vector in the attempt of protein expression using a mammalian system, described elsewhere[25].

Additional *in-silico* determinations were performed now using the multiepitope antigen protein sequence as template. The prediction of antigenicity probability (ANTIGENpro), solubility upon overexpression (SOLpro), formation of disulphide bonds (DIpro) and presence of transmembrane domains (ABTMpro) were performed using the SCRATCH protein predictor online tool (http://scratch.proteomics.ics.uci.edu/). The prediction of allergenic potential was performed using the AlgPred online tool[48].

***Ethics Statement*.** All animal experiments were carried out in accordance with i) the i3S Animal Ethics Committees and the Portuguese National Authorities for Animal Health guidelines, according to the statements on the Directive 2010/63/EU of the European Parliament and of the Council; ii) and with the National Institute of Allergy and Infectious Diseases (NIAID) Animal Care and Use Committee under the animal protocol LMVR4E (the NIAID DIR Animal Care and Use Program complies with the Guide for the Care and Use of Laboratory Animals and with the NIH Office of Animal Care and Use and Animal Research Advisory Committee guidelines).

Mice. Six- to eight-week-old female BALB/c and C57BL/6 mice were obtained from Charles River laboratories. Animals were maintained i) under specific pathogen-free conditions at the i3S facilities, in sterile IVC cabinets, with food and water available *ad libitum*; or ii) were housed under pathogen-free conditions at the NIAID Twinbrook animal facility, Rockville, MD. Animals were randomly assigned to groups.

Sand flies and Salivary Glands Homogenates (SGH). *P. duboscqi, L. longipalpis*, *P. papatasi and P. perniciosus* sand flies were mass reared at the Laboratory of Malaria and Vector Research insectary according to the protocols described elsewhere[49]. Adult females were maintained on a 30% sucrose diet (commercial sugar) and were starved for 12 hours before feeding. Three- to 4-day-old sand flies were used for transmission experiments. Five- to 7-day-old sand flies were used for preparation of SGH. Briefly, pools of 20 salivary glands were dissected in phosphate-buffered saline, subjected to ultra-sonication followed by centrifugation at 10,000g for 3 min at 4 °C. The supernatant (SGH) was then collected and stored at -80ºC until use.

Parasites and Soluble Leishmania Antigen (SLA). A cloned line of virulent *Leishmania major* (WR 2885) isolated from a soldier deployed to Iraq[16] was used in this study. Promastigotes were routinely maintained at 26ºC in Schneider's insect medium (Lonza, MD, USA) supplemented with 10% heat inactivated foetal bovine serum (Thermo Fischer Scientific, MA, USA), penicillin (100 U/ml) and streptomycin (100 mg/ml). SLA was prepared from 1×10^9 *L. major* promastigotes. Briefly, parasites in PBS were subjected to repeated freeze-thaw cycles, and centrifuged at 10000g for 20 min at 4 °C. The resultant supernatant (SLA) was collected, and its protein content was quantified by the BCA method. Aliquots (1 mg/ml) were prepared and frozen at −80°C until use.

Immunization scheme. Mice were immunized intradermally in the right ear three times at two weeks intervals with 5 µg of VR-2001-SfSPChimera plasmid in 10 µl PBS. Negative controls received either the same amount of empty vector (VR-2001 in 10 µl PBS; used as "true placebo" in efficacy trials, according to the WHO recommendations[50]) or the same volume of vehicle only (10 µl PBS). Additionally, for immunogenicity *ex-vivo* experiments, two additional control groups (positive controls) were immunized intradermally with either *P.*

duboscqi or *L. longipalpis* SGH - the equivalent of 1 sand fly salivary gland pairs in 10 µl PBS. One month after the last immunization, animals were either euthanized for vaccine immunogenic potential determination (and bioinformatics validation) or challenged via sand fly bites.

Transmission of L. major via sand fly bites. *P. duboscqi* or *L. longipalpis* sand flies were infected by artificial feeding through a chick membrane on defibrinated rabbit blood (Spring Valley Laboratories, MD, USA) containing *L. major* promastigotes, as previously described[16]. Fully blood-fed female sand flies were separated and maintained at 26°C with 75% humidity and were provided 30% sucrose until the development of a mature infection was confirmed (typically 11 to 15 days after blood feeding). Sugar pads were removed 12 h before transmission. Ten *L. major*-infected sand flies were applied to the left ear of each mouse (under anaesthesia, as previously described[51]), using vials with a meshed surface held in place by custom-made clamps, and allowed to feed for two hours in the dark (26°C with 75% humidity). The number of blood-fed flies was then determined by observing them under a stereomicroscope, as a potential measurement of transmission success.

Post-infection follow up and experimental endpoints. Animals were monitored to evaluate the evolution of the characteristic lesions caused by *L. major* infection. Ear thickness or the maximum lesion diameter were measured individually (results presented per mice) using a Digimatic calliper (Mitutoyo Corp., IL, USA) and recorded on a weekly basis. Animals were euthanized at two- or six-weeks post-infection for parasite burden determination, as well as for the dissection of potential correlates of protection (2-weeks' time-point only).

Euthanasia, organ collection and manipulation. Euthanasia was performed by cervical dislocation always under isoflurane anaesthesia (Piramal healthcare, UK). Spleens were aseptically collected from euthanized animals, weighed, and homogenized using Falcon®100 µm Cell Strainers (Corning Life Sciences, MA, USA). Ears were collected, immersed in 70% ethanol and then in PBS. Ear cell suspensions were obtained as previously described [52]. Briefly, the ventral and dorsal sheets were separated, deposited in RPMI containing 0.25 mg/ml Liberase CI purified enzyme blend and 10 µg/ml DNAse (both from Roche, Switzerland), and incubated for 1 hour at 37°C and 5% CO_2. Digested ear sheets were subsequently homogenized for 3 minutes using the Medicon/Medimachine

tissue homogenizer system (BD™, NJ, USA) and the resultant single cell suspensions were filtered using a 70 µm-pore size Falcon cell strainer (BD Biosciences, NJ, USA). Viable cell numbers were determined for all obtained cell suspensions (ear and spleen derived) under the microscope, using a Neubauer chamber and trypan blue solution as "viability marker".

ex-vivo splenic cell proliferation assays. Stimulations were performed with antigen-pulsed Bone Marrow-derived Dendritic Cells (BMDCs), differentiated from naïve strain-matched mice bone marrow precursors as previously described [53]. BMDCs ($5x10^4$/ well) were pulsed at day -1 with *P. duboscqi or L. longipalpis* or *P. papatasi or P. perniciosus* SGH (final concentration of 3 sand fly salivary gland pair/ml). Non-pulsed cells were cultured in parallel to be used in the experimental-control conditions. Splenic cell suspensions were aseptically pre-stained (day 0) as previously described[26]. Briefly, $1x10^7$ splenocytes per animal were pelleted, washed twice with PBS and stained for 10 minutes at 37ºC with CFSE (1 µM; in 1 ml of PBS; Thermo Fisher Scientific, MA, USA). Complete RPMI (Lonza, Switzerland) [10% heat-inactivated FBS (Lonza, Switzerland), 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin (BioWhittaker, MD, USA)] was added to the cell suspensions, that were then pelleted, re-suspended in complete RPMI and incubated at 4ºC for 5 minutes to stop the reaction. Afterwards, cells were once more centrifuged (5 min, 350 g), and resuspended in complete RPMI supplemented with 50 µM 2-mercaptoethanol (Sigma-Aldrich, MO, USA). Stained splenocytes were added to the pulsed/non-pulsed BMDCs at the final amount of 2.5×10^5 cells per well (1:5 - DC:Splenocyte ratio). Additionally, to the non-pulsed DCs + splenocytes co-cultures, concanavalin A (3 µg/ml) and complete RPMI medium were added as positive and negative controls, respectively. Co-cultures were incubated (37ºC and 5% CO_2) for three (positive control) or four days (remaining conditions), and proliferating T cell populations were determined by flow cytometry. Each determination was performed in duplicate.

ex-vivo ear cell stimulation for intracellular cytokine quantification. Ear cell suspensions (ideally at least $1x10^6$ cells in complete RPMI) were seeded in 24-well plates and cultured for 18 h in the presence of 50 µg/ml of SLA at 37ºC and 5% CO_2. PMA, Ionomycin and Brefeldin A (BD Biosciences, NJ, USA) were added during the last 4 hours of culture at the concentrations of 25 ng/mL, 500 ng/mL and 10 µg/ml, respectively. Cells were then harvested and T cell cytokine production was analysed by flow cytometry.

***Flow Cytometry*.** The anti-mouse monoclonal antibodies used to perform this study were either purchased to BD Biosciences (BD - NJ, USA) or BioLegend (BioL - CA, USA): FITC labelled anti-CD4 (GK1.5, BD); PE labelled anti-IFNγ (XMG1.2, BD); PerCP-Cy5.5 labelled anti-CD8α (53-6.7, BD); PE-Cy7 labelled anti-CD3 (HA2, BioL), and anti-IL-10 (JES5-16E3, BD); APC labelled anti-IL-4 (11B11, BD); BV421 labelled anti-TGFβ1 (TW7-16B4, BD); BV510 labelled anti-CD4 (RM4-5, BioL); and APC/Fire labelled anti-TCRβ (H57-597, BD). Cell viability was evaluated using either the LIVE/DEAD™ Fixable Yellow Dead Cell Stain Kit (Thermo Fischer Scientific, MA, USA) or the Zombie NIR™ Fixable Viability Kit (BioLegend, CA, USA). The viability stainings were performed first, according to the manufacturers' instructions. Afterwards, the cells were blocked with anti-CD16/CD32 (BD Fc block, 2.4G2; BD Pharmingen) for 15 minutes at 4°C. Surface stainings of splenic cells were performed in PBS + 0.5% BSA (30min, 4ºC) followed by 15 min fixation using 1% PFA. Surface stainings of ear cells were performed for 30min at 4ºC in Cell Staining Buffer (BioLegend, CA, USA), followed by 20 minutes fixation using a commercial Fixation Buffer (BioLegend, CA, USA). Intracellular staining of fixed ear cells was performed for 2 h at 4ºC in Perm/Wash Buffer (BioLegend, CA, USA). Cells were always washed between each step with the respective staining buffer used (PBS + 0.5% BSA, Cell Staining Buffer or Perm/Wash Buffer). Stained cells in either PBS + 0.5% BSA or Cell Staining Buffer were then acquired in a FACSCanto (BD^{TM}, NJ, USA) or a MACSQuant® Analyzer 16 (Miltenyi Biotech, Germany). Data analysis was performed with the FlowJo software v10 (TreeStar, OR, USA).

An initial gate plotting FSC-A versus SSC-A was performed to exclude cell debris. Afterwards, singlets were selected by plotting FSC-A versus FSC-H and the remaining cell populations were resolved within live cells. T lymphoid cell populations were defined as CD3+/CD4+ and CD3+/CD8+. Cytokine production by T cells was assessed within these two sub-populations. Proliferating T cells (total, and CD4+ or CD8+) were defined as CFSE$^{int/low/neg}$ (FITC channel), always comparing each condition with the respective negative control. A brief gating strategy is represented in Supplementary Figure 1.

***Parasite burdens' determination*.** Ear parasite burdens were assessed by the limit dilution method, as reported elsewhere[54]. Briefly, defined volumes of ear cell suspensions were serially diluted in 96-well plates containing 50 µl biphasic medium - NNN medium with 10% of defibrinated rabbit blood - overlaid with 200 µl of Schneider's insect medium (Lonza, MD,

USA) supplemented with 10% heat inactivated foetal bovine serum (Thermo Fischer Scientific, MA, USA), penicillin (100 U/ml) and streptomycin (100 mg/ml), and incubated for 14 days at 26ºC. The total number of parasites per ear was calculated as previously[54].

Statistical Analysis. Results are expressed per individual animals/samples, with a representation of the group mean value ± standard deviation. Statistical differences were analysed using GraphPad Prism software v6.01 (CA, USA). As parasite numbers in the ear of mice transmitted by infected sand flies bites do not follow a Gaussian distribution we used the Mann-Whitney test in the analysis of any transmission-related data. All of the remaining analysis were performed with parametric statistical tests: unpaired t-test or One Way ANOVA (with Tukey's post-hoc analysis) were used to compare two or more than two groups, respectively. Bivariate correlations were calculated and given by the Pearson correlation coefficient.

DATA AVAILABILITY

All relevant data are within the paper and its supplementary materials.

ACKNOWLEDGEMENTS

We are grateful to the i3S Translational Cytometry and Animal Facilities, and to the NIAID Animal Facility for all the technical assistance provided**.** We also thank Dr. Shaden Kamhawi (NIAID, NIH) for critical review of the manuscript.

FUNDING SOURCES

The research leading to these results has received funding from the project NORTE-01-0145-FEDER-000012, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). This research was also supported in part by the Intramural Research Program of the NIH, National Institute of Allergy and Infectious Diseases. PC was supported by Foundation for Science and Technology (FCT), Portugal, through the individual grant SFRH/BD/121252/2016. PC was also supported by Fulbright

Portugal. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

DECLARATION OF INTERESTS

The authors declare that there are no competing interests.

AUTHOR CONTRIBUTIONS

PC, ACdS and FO conceived and designed the experiments. PC, JO, TDS and FO performed the experiments. PC, JO, ACdS and FO analysed the data. PC, JO, JV, ACdS and FO critically discussed the results. JV and ACdS assured the funding. CM, JV and ACdS contributed with the reagents, materials, and analysis tools. PC wrote the original draft, under the supervision of ACdS and FO. PC, JO, CM, TDS, JGV, ACdS and FO critically revised and edited the paper. All the authors approved the current version of this manuscript.

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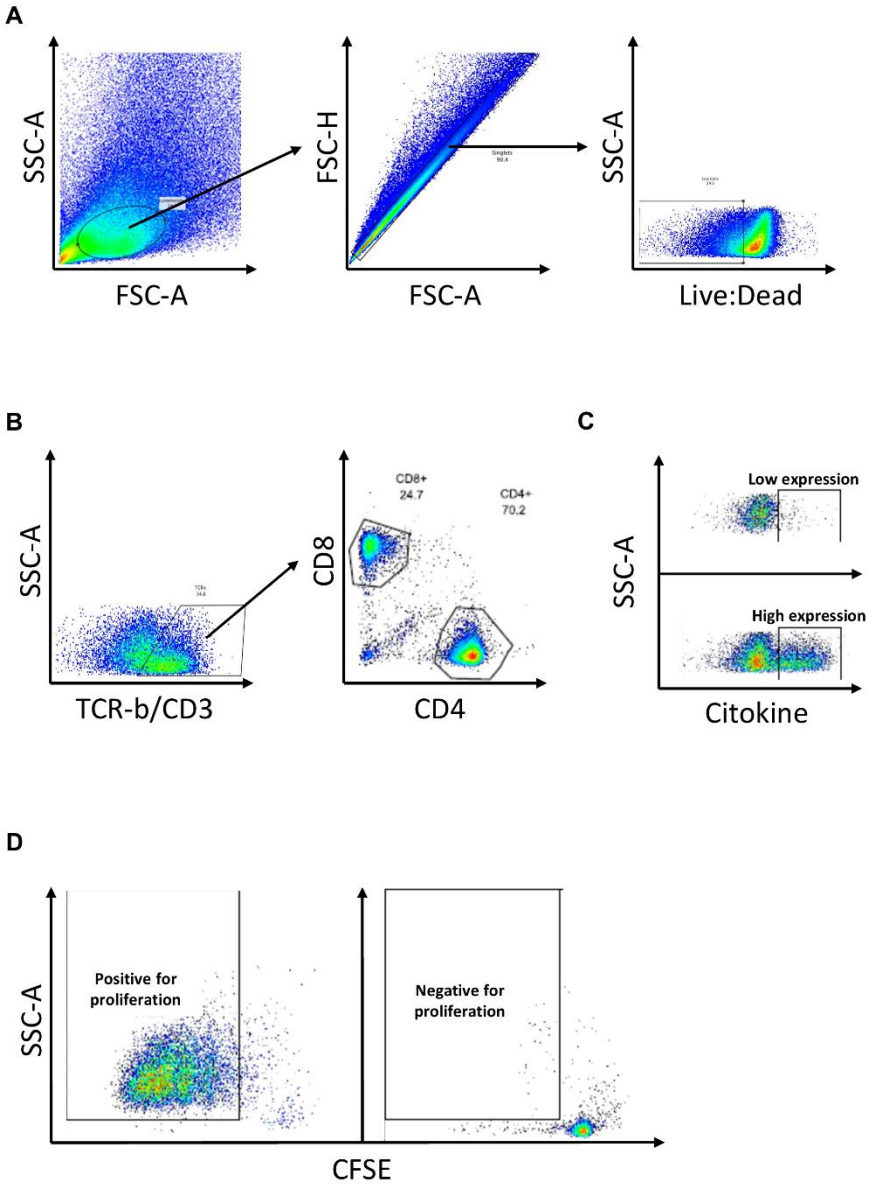
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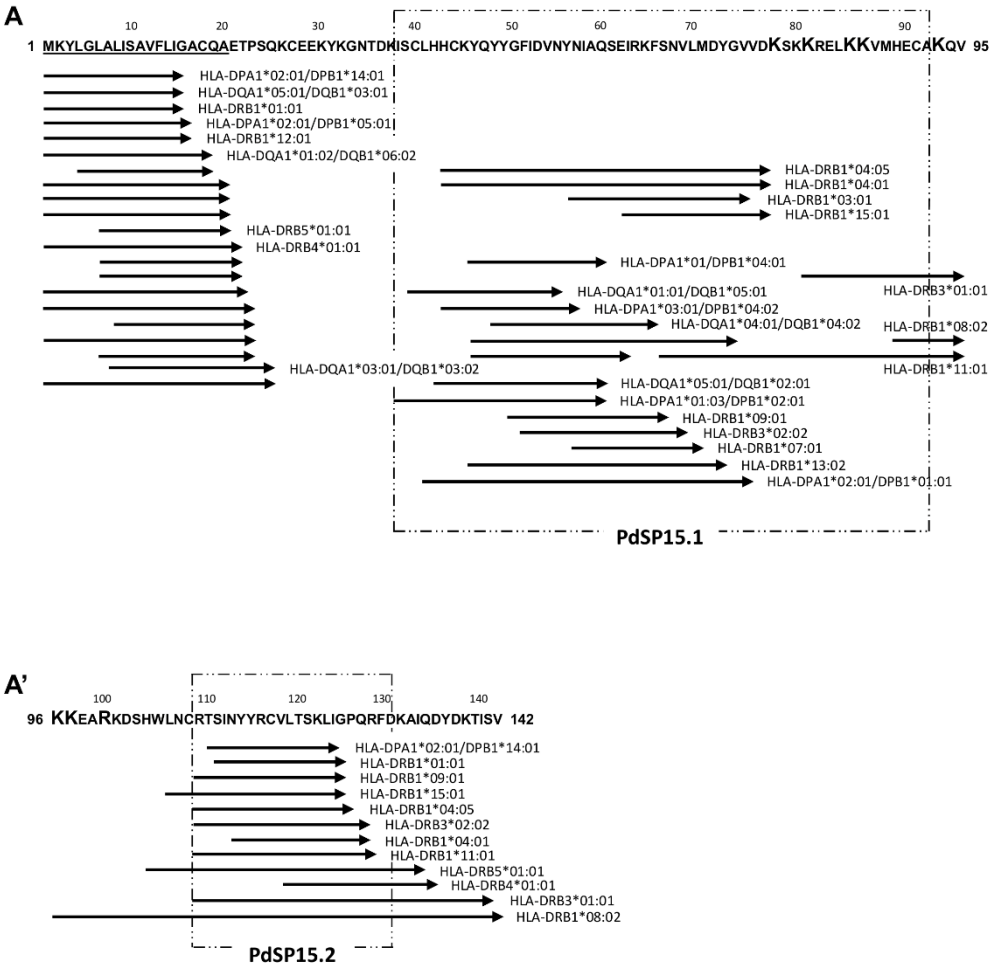
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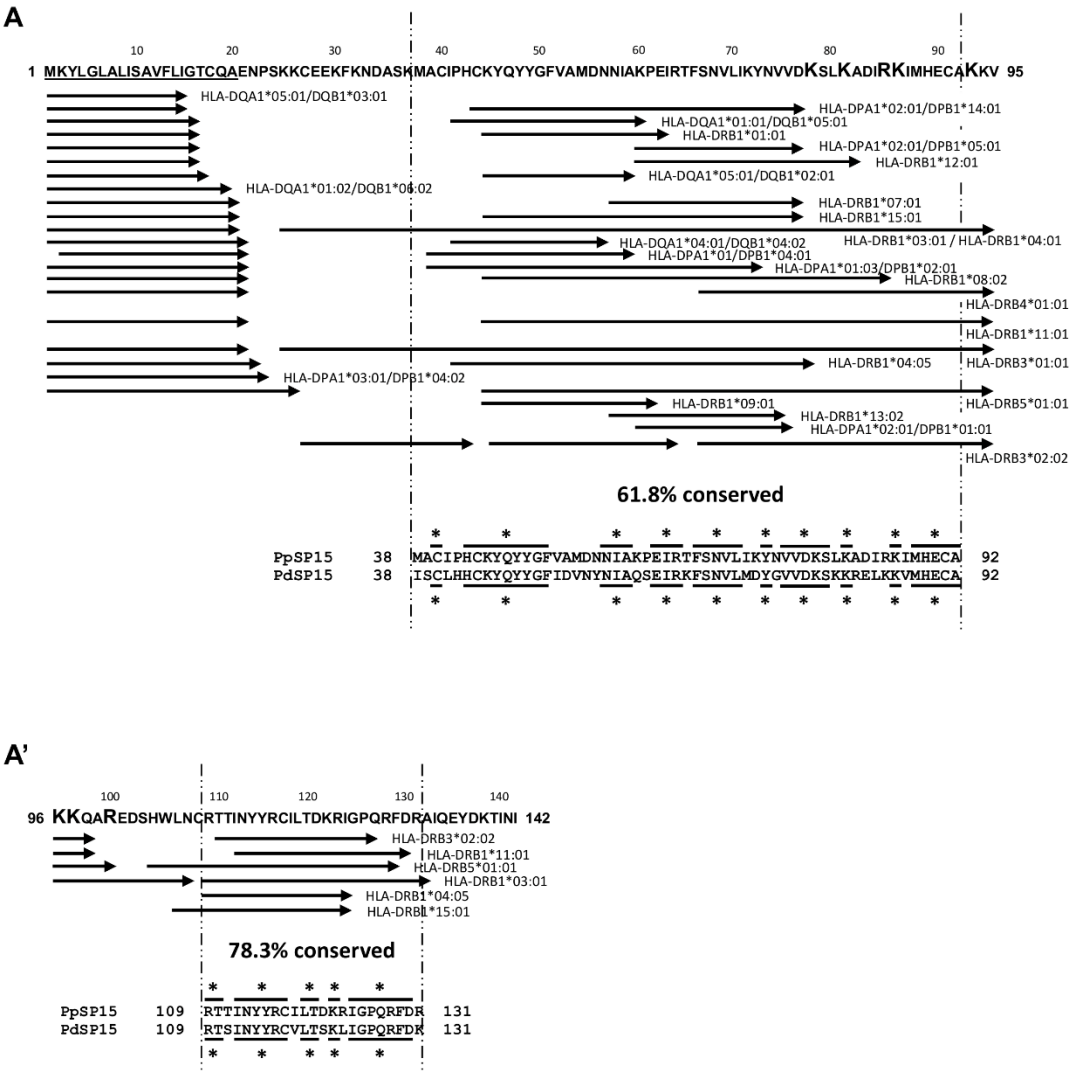
93 Poche, D. M., Grant, W. E. & Wang, H. H. Visceral Leishmaniasis on the Indian Subcontinent: Modelling the Dynamic Relationship between Vector Control Schemes and Vector Life Cycles. *PLoS Negl Trop Dis* **10**, e0004868, doi:10.1371/journal.pntd.0004868 (2016).



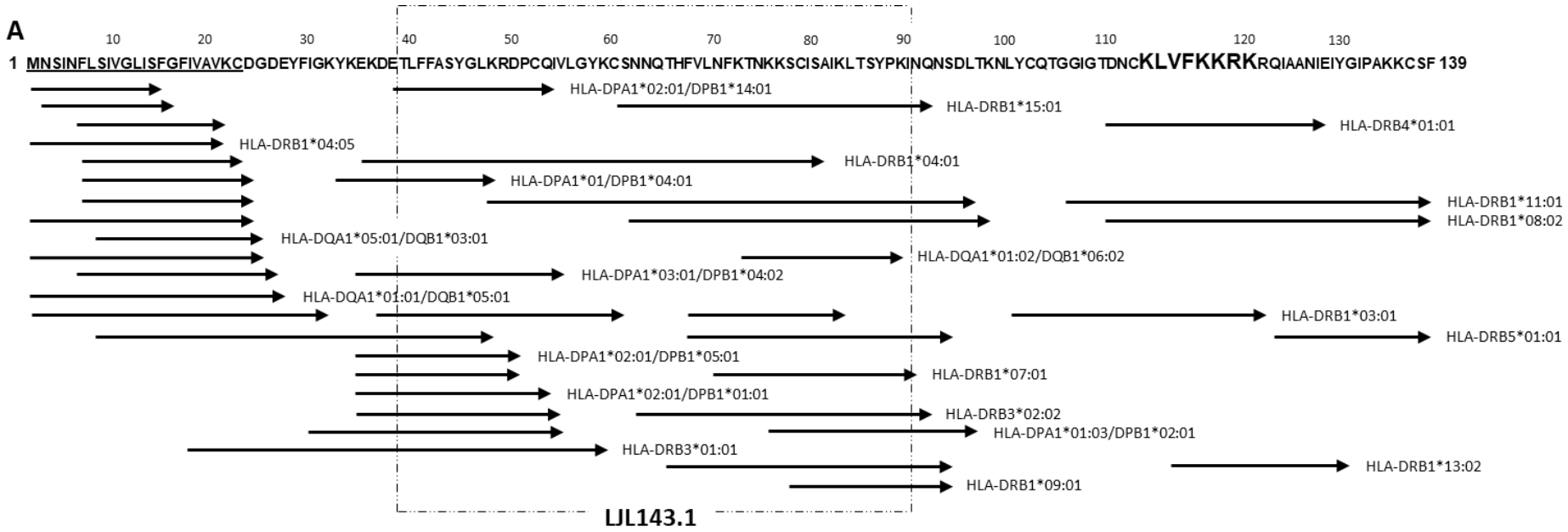
Supplementary Figure 1. Brief representation of the flow cytometry gating strategy applied in this work. (A) An initial gate plotting FSC-A versus SSC-A was performed to exclude cell debris and select for lymphocytes. Afterwards, singlets were selected by plotting FSC-A versus FSC-H and the remaining cell populations were resolved within live cells. (B) T lymphoid cell populations were defined as CD3+(or TCRβ+)/CD4+ and CD3+(or TCRβ+)/CD8+. (C) Cytokine production by T cells was assessed within these two sub-populations. (D) Proliferating T cells (total, and CD4+ or CD8+) were defined as $CFSE^{int/low/neg}$ (FITC channel), always comparing each condition with the respective negative control.

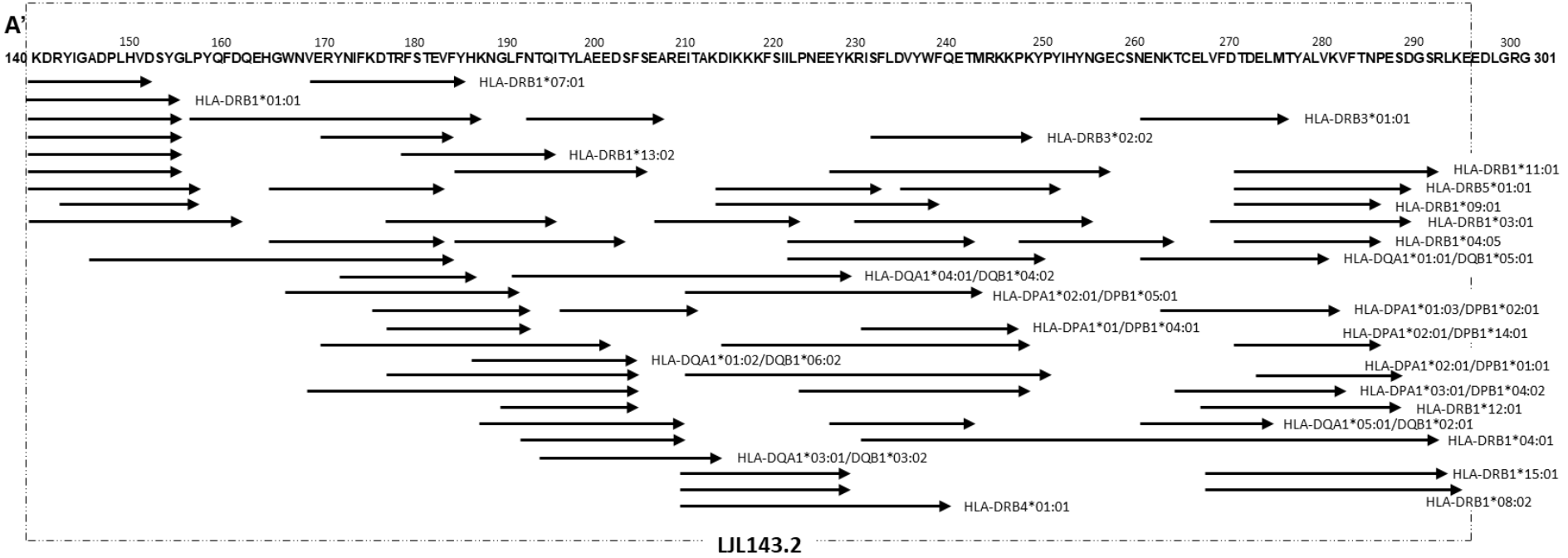


Supplementary Figure 2. PdSP15 CD4+ T cell epitope mapping: extended representation of human MHC-II restricted epitopes. PdSP15 (*GenBank acc. no. ABI15933*) CD4+ T cell epitope mapping was performed using the IEDB Analysis Resource considering 27 human MHC-II alleles. A/A' represent the extended translation of the data set obtained (Supplementary data 4) and show predicted epitopes with percentile rank values between 2.5 and 7.5. Results are presented by allele. Each arrow represents one or more (contiguous) predicted epitopes. Underlined protein residues represent the signal peptide sequence. Magnified protein residues are potentially important for protein biological activity. Dashed boxes represent the two protein portions selected to be part of the final chimeric sand fly salivary antigen.

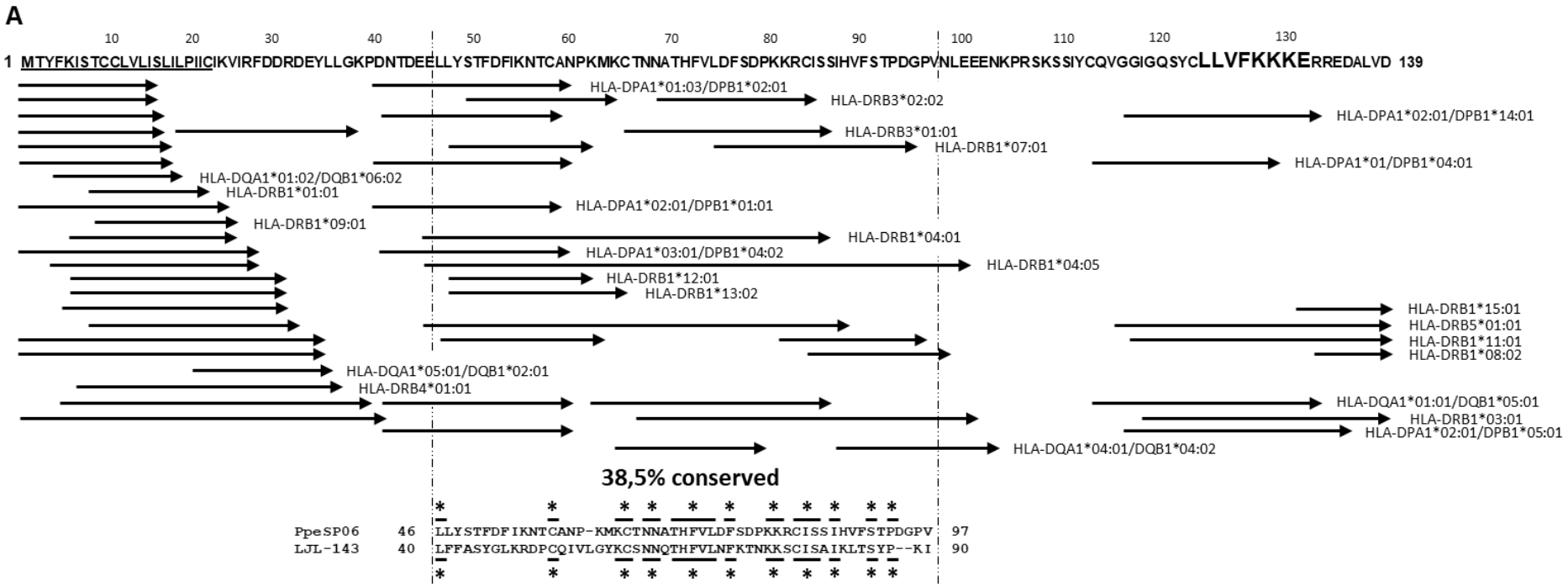


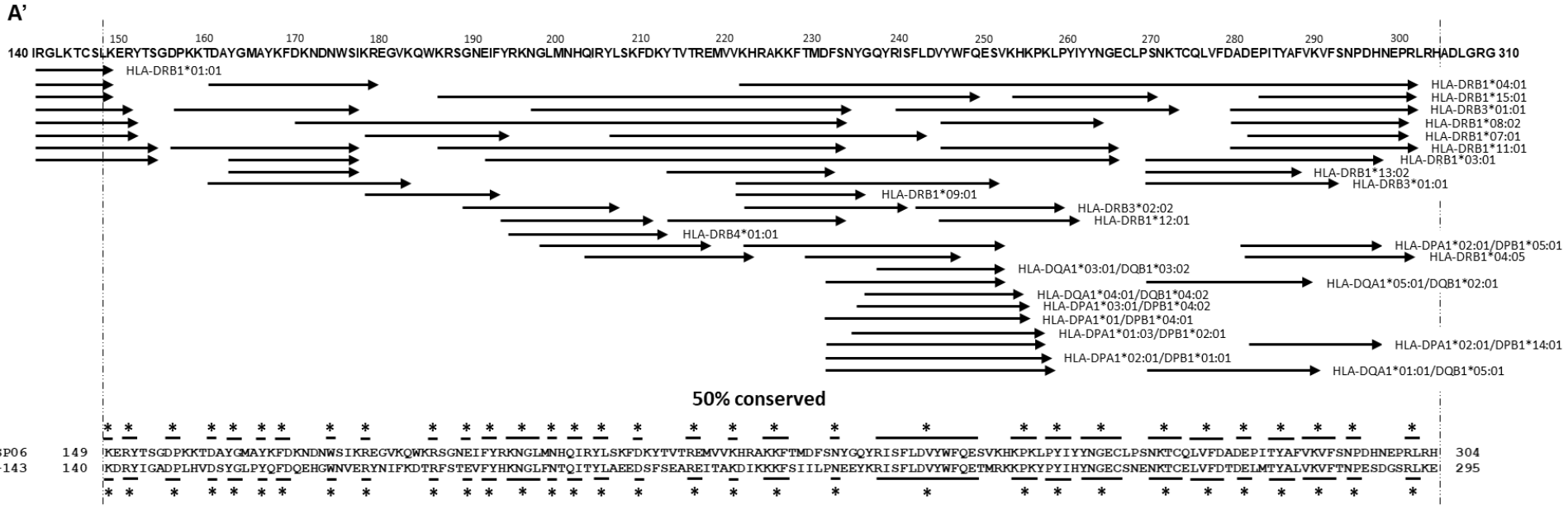
Supplementary Figure 3. *in* silico determination of the human MHC-II restricted epitopes of PpSP15, a known PdSP15 homologue. PpSP15 (*GenBank acc. no. AF335487*) CD4+ T cell epitope mapping was performed using the IEDB Analysis Resource considering 27 human MHC-II alleles. A/A' represent the translation of the data set obtained (Supplementary data 5) and show the top 7.5% hits. Results are presented by allele. Each arrow represents one or more (contiguous) predicted epitopes. Underlined protein residues represent the signal peptide sequence. Magnified protein residues are potentially important for protein biological activity. Dashed vertical lines limit the homologous regions to PdSP15 portions selected to be part of the final chimeric sand fly salivary antigen. The alignment of PpSP15 and PdSP15 portions of interest is represented, with the conserved residues highlighted. Percentage of amino acid sequence conservation within each portion was calculated and is also shown.


A
10 20 30 40 50 60 70 80 90 100 110 120 130
1 MNSINFLSIVGLISFGFIVAVKCDGDEYFIGKYKEKDETLFFASYGLKRDPCQIVLGYKCSNNQTHFVLNFKTNKKSCISAIKLTSYPKINQNSDLTKNLYCQTGGIGTDNCKLVFKKRKRQIAANIEIYGIPAKKCSF 139
HLA-DPA1*02:01/DPB1*14:01
HLA-DRB1*15:01
HLA-DRB4*01:01
HLA-DRB1*04:05
HLA-DRB1*04:01
HLA-DPA1*01/DPB1*04:01
HLA-DRB1*11:01
HLA-DRB1*08:02
HLA-DQA1*05:01/DQB1*03:01
HLA-DQA1*01:02/DQB1*06:02
HLA-DPA1*03:01/DPB1*04:02
HLA-DQA1*01:01/DQB1*05:01
HLA-DRB1*03:01
HLA-DRB5*01:01
HLA-DPA1*02:01/DPB1*05:01
HLA-DRB1*07:01
HLA-DPA1*02:01/DPB1*01:01
HLA-DRB3*02:02
HLA-DPA1*01:03/DPB1*02:01
HLA-DRB3*01:01
HLA-DRB1*13:02
HLA-DRB1*09:01
LJL143.1

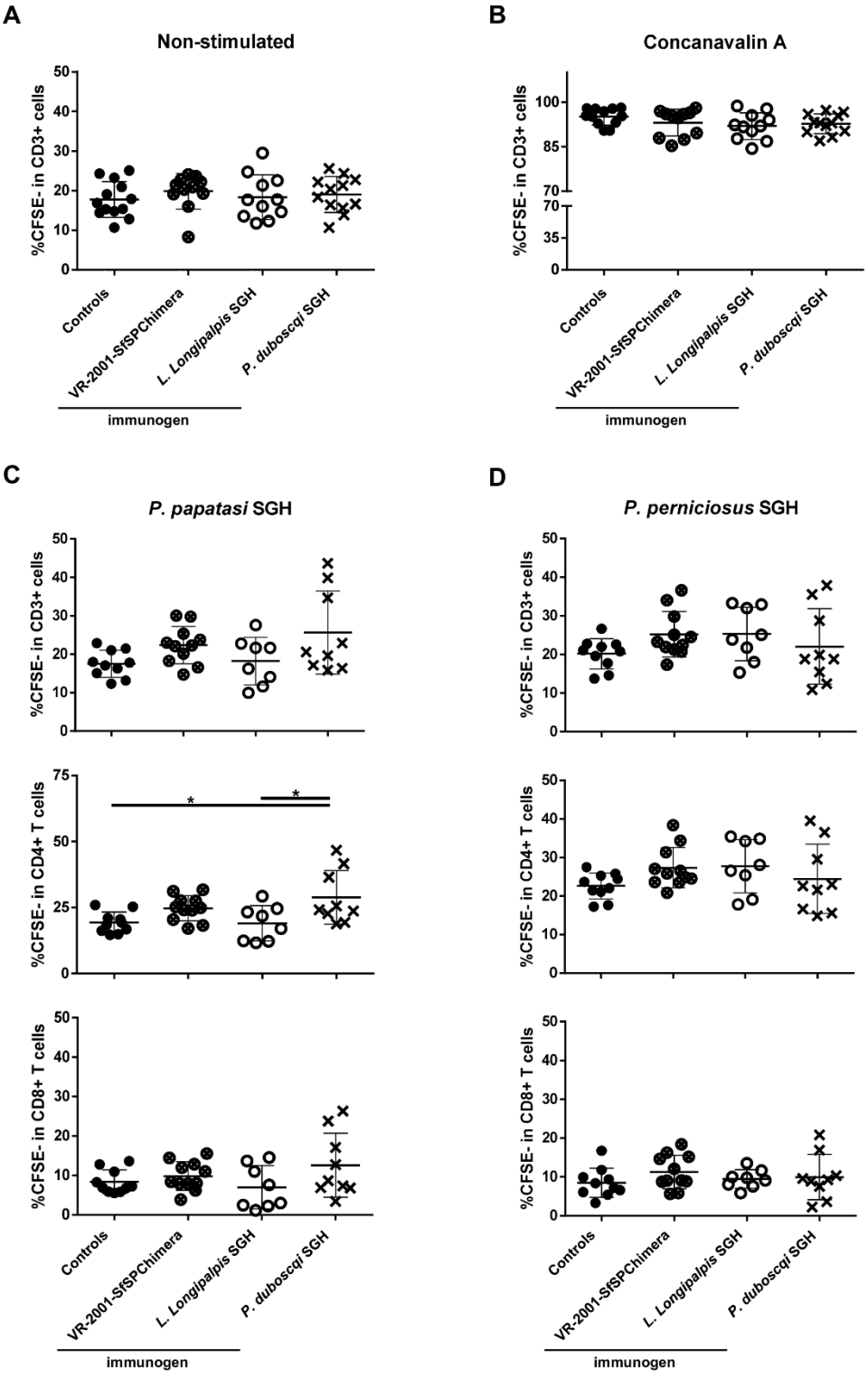


Supplementary Figure 4. LJL-143 CD4+ T cell epitope mapping: extended representation of human MHC-II restricted epitopes. LJL-143 (GenBank acc. no. AAS05319) CD4+ T cell epitope mapping was performed using the IEDB Analysis Resource considering 27 human MHC-II alleles. A/A' represent the extended translation of the data set obtained (Supplementary data 11) and show predicted epitopes with percentile rank values between 2.5 and 7.5. Results are presented by allele. Each arrow represents one or more (contiguous) predicted epitopes. Underlined protein residues represent the signal peptide sequence. Magnified protein residues are potentially important for protein biological activity. Dashed boxes represent the two protein portions selected to be part of the final chimeric sand fly salivary antigen.


A
1 MTYFKISTCCLVLISLILPIICIKVIRFDDRDEYLLGKPDNTDEELLYSTFDFIKNTCANPKMKCTNNATHFVLDFSDPKKRCISSIHVFSTPDGPVNLEEENKPRSKSSIYCQVGGIGQSYCLLVFKKKERREDALVD 139
HLA-DPA1*01:03/DPB1*02:01
HLA-DRB3*02:02
HLA-DPA1*02:01/DPB1*14:01
HLA-DRB3*01:01
HLA-DRB1*07:01
HLA-DPA1*01/DPB1*04:01
HLA-DQA1*01:02/DQB1*06:02
HLA-DRB1*01:01
HLA-DPA1*02:01/DPB1*01:01
HLA-DRB1*09:01
HLA-DRB1*04:01
HLA-DPA1*03:01/DPB1*04:02
HLA-DRB1*04:05
HLA-DRB1*12:01
HLA-DRB1*13:02
HLA-DRB1*15:01
HLA-DRB5*01:01
HLA-DRB1*11:01
HLA-DRB1*08:02
HLA-DQA1*05:01/DQB1*02:01
HLA-DRB4*01:01
HLA-DQA1*01:01/DQB1*05:01
HLA-DRB1*03:01
HLA-DPA1*02:01/DPB1*05:01
HLA-DQA1*04:01/DQB1*04:02
38,5% conserved
PpeSP06 46 LLYSTFDFIKNTCANP-KMKCTNNATHFVLDFSDPKKRCISSIHVFSTPDGPV 97
LJL-143 40 LFFASYGLKRDPCQIVLGYKCSNNQTHFVLNFKTNKKSCISAIKLTSYP--KI 90



Supplementary Figure 5. *in* silico determination of the human MHC-II restricted epitopes of PpeSP06, a LJL-143 homologue present in the saliva of the most relevant vector of Visceral Leishmaniasis in the Mediterranean Basin. PpeSP06 (*GenBank acc. no.* DQ153100) CD4+ T cell epitope mapping was performed using the IEDB Analysis Resource considering 27 human MHC-II alleles. A/A' represent the translation of the data set obtained (Supplementary data 12) and show the top 7.5% hits. Results are presented by allele. Each arrow represents one or more (contiguous) predicted epitopes. Underlined protein residues represent the signal peptide sequence. Magnified protein residues are potentially important for protein biological activity. Dashed vertical lines limit the homologous regions to LJL-143 portions selected to be part of the final chimeric sand fly salivary antigen. The alignment of PpeSP06 and LJL-143 portions of interest is represented, with the conserved residues highlighted. Percentage of amino acid sequence conservation within each portion was calculated and is also shown.


A
Non-stimulated
%CFSE- in CD3+ cells
B
Concanavalin A
%CFSE- in CD3+ cells
C
P. papatasi SGH
%CFSE- in CD3+ cells
%CFSE- in CD4+ T cells
%CFSE- in CD8+ T cells
D
P. perniciosus SGH
%CFSE- in CD3+ cells
%CFSE- in CD4+ T cells
%CFSE- in CD8+ T cells
Controls
VR-2001-SfSPChimera
L. Longipalpis SGH
P. duboscqi SGH
immunogen

Supplementary Figure 6. *Ex-vivo* cell proliferation experiments: controls and evaluation of the sand fly-derived DNA chimeric vaccine cross-reactive potential. BALB/C mice were immunized intradermally in the right ear three times at two weeks intervals with *5 µg* of VR-2001-SfSPChimera plasmid, or with either *L. longipalpis* or *P. duboscqi* SGH - the equivalent of 1 sand fly salivary gland pairs. Control animals received the same volume of the vehicle solution (PBS). One month after the last immunization, animals were euthanized, their spleens collected and processed to obtain CFSE-stained splenocytes suspensions. Frequencies of proliferating splenic T cells (total CD3+ and CD3+/CD4+ or CD3+/CD8+) were determined by flow cytometry after four days of culture *in the presence of BMDCs (*5:1 ratio), non-pulsed in the absence (A) or presence of 3 µg/ml concanavalin A (B), or *pulsed with P. papatasi (C) or P. perniciosus (D)* SGH (final concentration of 3 sand fly salivary gland pair/ml). Results from three independent experiments are shown. Each dot represents one animal. Average and SD of the values within each group are shown. Statistical differences are properly identified (One-Way ANOVA with Tukey´s *post hoc* analysis: * p≤0.05).

Supplementary data 1. PdSP15 in silico analysis: murine MHC-II restricted-epitopes prediction - BALB/c (H2-IAd and H2-IEd)

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align core	SMM align IC50 (nM)	SMM align rank	NN align core	NN align IC50 (nM)	NN align rank
H2-IAd	57	71	NIAQSEIRKFSNVLM	Consensus	4.33	AQSEIRKFS	3044	2.45	IAQSEIRKF	792.10	6.22
H2-IAd	1	15	MKYLGLALISAVFLI	Consensus	4.53	YLGLALISA	2704	1.89	MKYLGLALI	903.90	7.16
H2-IAd	58	72	IAQSEIRKFSNVLMD	Consensus	4.76	IRKFSNVLM	3047	2.46	IAQSEIRKF	892.80	7.06
H2-IAd	113	127	NYYRCVLTSKLIGPQ	Consensus	6.41	YRCVLTSKL	2076	1.01	YRCVLTSKL	1469.40	11.80
H2-IAd	114	128	YYRCVLTSKLIGPQR	Consensus	6.82	RCVLTSKLI	3213	2.74	YRCVLTSKL	1355.80	10.89
H2-IAd	115	129	YRCVLTSKLIGPQRF	Consensus	6.92	YRCVLTSKL	4544	5.42	YRCVLTSKL	1055	8.43
H2-IAd	112	126	INYYRCVLTSKLIGP	Consensus	7.27	YRCVLTSKL	1944	0.86	YRCVLTSKL	1712	13.68
H2-IAd	3	17	YLGLALISAVFLIGA	Consensus	7.61	LALISAVFL	3644	3.56	LGLALISAV	1450.90	11.65
H2-IAd	79	93	SKKRELKKVMHECAK	Consensus	8.20	KRELKKVMH	4092	4.46	SKKRELKKV	1486.90	11.94
H2-IAd	2	16	KYLGLALISAVFLIG	Consensus	8.39	LALISAVFL	3310	2.91	LGLALISAV	1735.20	13.87
H2-IAd	111	125	SINYYRCVLTSKLIG	Consensus	8.50	YRCVLTSKL	1806	0.71	YRCVLTSKL	2060.30	16.28
H2-IAd	125	139	GPQRFDKAIQDYDKT	Consensus	9.58	QRFDKAIQD	3813	3.89	RFDKAIQDY	1925.20	15.27
H2-IAd	4	18	LGLALISAVFLIGAC	Consensus	9.83	LALISAVFL	4262	4.81	LGLALISAV	1867.30	14.85
H2-IAd	78	92	KSKKRELKKVMHECA	Consensus	9.88	KRELKKVMH	3866	4	SKKRELKKV	1988.70	15.75

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align core	SMM align IC50 (nM)	SMM align rank	NN align core	NN align IC50 (nM)	NN align rank
H2-IEd	NO PREDICTED BINDERS										

Note: The MHCII binding predictions were made on 11/18/2016 using the IEDB analysis resources Consensus tool (H2-IAd), or SMM-Align (ver. 1.1) tool (H2-IEd). Results are presented by allele, and from lowest to highest percentile rank. Peptides predicted as good binders are all of those with a percentile ranking lower or equal to 10.

Supplementary data 2. PdSP15 in silico analysis: murine MHC-I restricted-epitopes prediction - BALB/c (H-2-Dd, H-2-Kd and H-2-Ld)

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Dd	123	133	11	LIGPQRFDKAI	ann	0.1	282.19	0.1	-	-	-	-
H-2-Dd	124	134	11	IGPQRFDKAIQ	ann	0.1	406.53	0.1	-	-	-	-
H-2-Dd	120	133	14	TSKLIGPQRFDKAI	ann	0.1	234.91	0.1	-	-	-	-
H-2-Dd	121	134	14	SKLIGPQRFDKAIQ	ann	0.1	266.26	0.1	-	-	-	-
H-2-Dd	122	135	14	KLIGPQRFDKAIQD	ann	0.1	293.01	0.1	-	-	-	-
H-2-Dd	123	136	14	LIGPQRFDKAIQDY	ann	0.1	326.41	0.1	-	-	-	-
H-2-Dd	124	137	14	IGPQRFDKAIQDYD	ann	0.1	431.30	0.1	-	-	-	-
H-2-Dd	121	133	13	SKLIGPQRFDKAI	ann	0.1	242.53	0.1	-	-	-	-
H-2-Dd	122	134	13	KLIGPQRFDKAIQ	ann	0.1	280.27	0.1	-	-	-	-
H-2-Dd	123	135	13	LIGPQRFDKAIQD	ann	0.1	322.62	0.1	-	-	-	-
H-2-Dd	124	136	13	IGPQRFDKAIQDY	ann	0.1	428.54	0.1	-	-	-	-
H-2-Dd	124	131	8	IGPQRFDK	ann	0.1	2088.83	0.1	-	-	-	-
H-2-Dd	122	133	12	KLIGPQRFDKAI	ann	0.1	255.59	0.1	-	-	-	-
H-2-Dd	123	134	12	LIGPQRFDKAIQ	ann	0.1	308.81	0.1	-	-	-	-
H-2-Dd	124	135	12	IGPQRFDKAIQD	ann	0.1	423.95	0.1	-	-	-	-
H-2-Dd	124	133	10	IGPQRFDKAI	Consensus	0.15	119.97	0.1	741.99	0.2	-	-
H-2-Dd	122	132	11	KLIGPQRFDKA	ann	0.2	6851.02	0.2	-	-	-	-
H-2-Dd	120	132	13	TSKLIGPQRFDKA	ann	0.2	6025.88	0.2	-	-	-	-
H-2-Dd	125	132	8	GPQRFDKA	ann	0.2	3493.86	0.2	-	-	-	-
H-2-Dd	124	132	9	IGPQRFDKA	Consensus	0.3	892.45	0.1	20336.68	27	1.02e-05	0.3
H-2-Dd	121	132	12	SKLIGPQRFDKA	ann	0.3	6409.29	0.3	-	-	-	-
H-2-Dd	119	132	14	LTSKLIGPQRFDKA	ann	0.5	5779.08	0.5	-	-	-	-

Supplementary data 2 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Kd	113	124	12	NYYRCVLTSKLI	ann	0.1	89.43	0.1	-	-	-	-
H-2-Kd	1	13	13	MKYLGLALISAVF	ann	0.1	232.22	0.1	-	-	-	-
H-2-Kd	2	14	13	KYLGLALISAVFL	ann	0.1	256.32	0.1	-	-	-	-
H-2-Kd	51	63	13	FIDVNYNIAQSEI	ann	0.1	285.52	0.1	-	-	-	-
H-2-Kd	100	112	13	RKDSHWLNCRTSI	ann	0.1	385.20	0.1	-	-	-	-
H-2-Kd	111	123	13	SINYYRCVLTSKL	ann	0.1	238	0.1	-	-	-	-
H-2-Kd	112	124	13	INYYRCVLTSKLI	ann	0.1	95.59	0.1	-	-	-	-
H-2-Kd	113	125	13	NYYRCVLTSKLIG	ann	0.1	182.45	0.1	-	-	-	-
H-2-Kd	114	126	13	YYRCVLTSKLIGP	ann	0.1	210.01	0.1	-	-	-	-
H-2-Kd	1	14	14	MKYLGLALISAVFL	ann	0.1	306.95	0.1	-	-	-	-
H-2-Kd	2	15	14	KYLGLALISAVFLI	ann	0.1	220.23	0.1	-	-	-	-
H-2-Kd	45	58	14	KYQYYGFIDVNYNI	ann	0.1	289.75	0.1	-	-	-	-
H-2-Kd	50	63	14	GFIDVNYNIAQSEI	ann	0.1	345.33	0.1	-	-	-	-
H-2-Kd	99	112	14	ARKDSHWLNCRTSI	ann	0.1	391.07	0.1	-	-	-	-
H-2-Kd	110	123	14	TSINYYRCVLTSKL	ann	0.1	242.38	0.1	-	-	-	-
H-2-Kd	111	124	14	SINYYRCVLTSKLI	ann	0.1	98.06	0.1	-	-	-	-
H-2-Kd	112	125	14	INYYRCVLTSKLIG	ann	0.1	196.03	0.1	-	-	-	-
H-2-Kd	113	126	14	NYYRCVLTSKLIGP	ann	0.1	240.23	0.1	-	-	-	-
H-2-Kd	114	127	14	YYRCVLTSKLIGPQ	ann	0.1	236.88	0.1	-	-	-	-
H-2-Kd	113	122	10	NYYRCVLTSK	Consensus	0.15	1337.49	0.1	511.05	0.2	-	-
H-2-Kd	55	63	9	NYNIAQSEI	Consensus	0.2	54.91	0.1	35.42	0.2	1.51e-05	0.5
H-2-Kd	1	12	12	MKYLGLALISAV	ann	0.2	110.62	0.2	-	-	-	-
H-2-Kd	114	125	12	YYRCVLTSKLIG	ann	0.2	160.12	0.2	-	-	-	-
H-2-Kd	46	58	13	YQYYGFIDVNYNI	ann	0.2	744.29	0.2	-	-	-	-
H-2-Kd	101	113	13	KDSHWLNCRTSIN	ann	0.2	769.28	0.2	-	-	-	-

Supplementary data 2 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Kd	103	115	13	SHWLNCRTSINYY	ann	0.2	876.06	0.2	-	-	-	-
H-2-Kd	51	64	14	FIDVNYNIAQSEIR	ann	0.2	649.49	0.2	-	-	-	-
H-2-Kd	2	13	12	KYLGLALISAVF	ann	0.3	194.21	0.3	-	-	-	-
H-2-Kd	101	112	12	KDSHWLNCRTSI	ann	0.3	375.59	0.3	-	-	-	-
H-2-Kd	112	123	12	INYYRCVLTSKL	ann	0.3	229.63	0.3	-	-	-	-
H-2-Kd	102	114	13	DSHWLNCRTSINY	ann	0.3	917.75	0.3	-	-	-	-
H-2-Kd	103	112	10	SHWLNCRTSI	Consensus	0.3	307.56	0.1	684.64	0.5	-	-
H-2-Kd	100	113	14	RKDSHWLNCRTSIN	ann	0.3	787.84	0.3	-	-	-	-
H-2-Kd	101	114	14	KDSHWLNCRTSINY	ann	0.3	978.24	0.3	-	-	-	-
H-2-Kd	102	115	14	DSHWLNCRTSINYY	ann	0.3	1020.52	0.3	-	-	-	-
H-2-Kd	103	116	14	SHWLNCRTSINYYR	ann	0.3	934.03	0.3	-	-	-	-
H-2-Kd	2	9	8	KYLGLALI	Consensus	0.4	542.62	0.1	976.02	0.7	-	-
H-2-Kd	47	58	12	QYYGFIDVNYNI	ann	0.4	671.59	0.4	-	-	-	-
H-2-Kd	52	63	12	IDVNYNIAQSEI	ann	0.4	734.99	0.4	-	-	-	-
H-2-Kd	102	113	12	DSHWLNCRTSIN	ann	0.4	720.30	0.4	-	-	-	-
H-2-Kd	103	114	12	SHWLNCRTSINY	ann	0.4	786.31	0.4	-	-	-	-
H-2-Kd	2	11	10	KYLGLALISA	Consensus	0.45	4864.12	0.7	498.26	0.2	-	-
H-2-Kd	104	112	9	HWLNCRTSI	Consensus	0.5	387.22	0.5	1242.40	3.5	4.4e-06	0.2
H-2-Kd	114	122	9	YYRCVLTSK	Consensus	0.5	227.09	0.5	122.53	0.4	3.15e-05	1.2
H-2-Kd	30	37	8	KYKGNTDK	Consensus	0.65	3915.14	0.6	945.06	0.7	-	-
H-2-Kd	110	122	13	TSINYYRCVLTSK	Ann	0.7	1404.25	0.7	-	-	-	-
H-2-Kd	45	54	10	KYQYYGFIDV	Consensus	0.75	5559.15	0.7	894.25	0.8	-	-
H-2-Kd	114	121	8	YYRCVLTS	Consensus	0.8	2643.13	0.5	1200.77	1.1	-	-
H-2-Kd	4	12	9	LGLALISAV	Consensus	0.8	6164.30	0.8	1186.48	3.3	4.16e-06	0.2

Supplementary data 2 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Kd	47	59	13	QYYGFIDVNYNIA	Ann	0.8	1451.32	0.8	-	-	-	-
H-2-Kd	109	122	14	RTSINYYRCVLTSK	Ann	0.8	1443.85	0.8	-	-	-	-
H-2-Kd	46	59	14	YQYYGFIDVNYNIA	Ann	0.9	1598.54	0.9	-	-	-	-
H-2-Kd	30	38	9	KYKGNTDKI	Consensus	1	831.34	0.5	349.35	1	0.000354	9.9
H-2-Kd	47	60	14	QYYGFIDVNYNIAQ	ann	1	1907.50	1	-	-	-	-
H-2-Kd	48	61	14	YYGFIDVNYNIAQS	ann	1	2030.07	1	-	-	-	-
H-2-Kd	52	65	14	IDVNYNIAQSEIRK	ann	1	2175.30	1	-	-	-	-

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Ld	NO PREDICTED BINDERS											

Note: The MHCI binding predictions were made on 11/20/2016 using the IEDB analysis resource Consensus tool which combines predictions from ANN aka NetMHC (4.0), SMM and Comblib. Results are presented by allele, and from lowest to highest percentile rank. Peptides predicted as good binders are all of those with a percentile ranking lower or equal to 1.

Supplementary data 3. PdSP15 in silico analysis: murine MHC-I restricted-epitopes prediction - C57BL/6 (H-2-Db and H-2-Kd)

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Db	3	14	12	YLGLALISAVFL	ann	0.1	923.04	0.1	-	-	-	-
H-2-Db	129	140	12	FDKAIQDYDKTI	ann	0.1	840.58	0.1	-	-	-	-
H-2-Db	127	140	14	QRFDKAIQDYDKTI	ann	0.2	891.33	0.2	-	-	-	-
H-2-Db	131	140	10	KAIQDYDKTI	Consensus	0.25	110.73	0.1	2245.17	0.4	-	-
H-2-Db	128	140	13	RFDKAIQDYDKTI	ann	0.3	871.94	0.3	-	-	-	-
H-2-Db	1	14	14	MKYLGLALISAVFL	ann	0.4	1322.31	0.4	-	-	-	-
H-2-Db	103	112	10	SHWLNCRTSI	Consensus	0.45	3604.13	0.7	1072.14	0.2	-	-
H-2-Db	2	14	13	KYLGLALISAVFL	ann	0.5	1175.59	0.5	-	-	-	-
H-2-Db	64	75	12	RKFSNVLMDYGV	ann	0.6	3359.51	0.6	-	-	-	-
H-2-Db	65	76	12	KFSNVLMDYGVV	ann	0.6	2639.56	0.6	-	-	-	-
H-2-Db	130	141	12	DKAIQDYDKTIS	ann	0.6	2765.78	0.6	-	-	-	-
H-2-Db	131	142	12	KAIQDYDKTISV	ann	0.6	3080.48	0.6	-	-	-	-
H-2-Db	46	59	14	YQYYGFIDVNYNIA	ann	0.6	1763.24	0.6	-	-	-	-
H-2-Db	6	15	10	LALISAVFLI	Consensus	0.65	2776.45	0.6	3007.81	0.7	-	-
H-2-Db	48	59	12	YYGFIDVNYNIA	ann	0.8	6673.96	0.8	-	-	-	-
H-2-Db	66	77	12	FSNVLMDYGVVD	ann	0.8	5882.10	0.8	-	-	-	-
H-2-Db	63	76	14	IRKFSNVLMDYGVV	ann	0.8	3008.25	0.8	-	-	-	-
H-2-Db	128	141	14	RFDKAIQDYDKTIS	ann	0.8	3158.21	0.8	-	-	-	-
H-2-Db	6	14	9	LALISAVFL	Consensus	0.8	437.16	0.1	700.84	0.8	9.57e-06	0.8
H-2-Db	43	52	10	HCKYQYYGFI	Consensus	0.85	7696.66	1	2993.99	0.7	-	-
H-2-Db	47	58	12	QYYGFIDVNYNI	ann	0.9	8137.08	0.9	-	-	-	-
H-2-Db	2	15	14	KYLGLALISAVFLI	ann	0.9	4126.08	0.9	-	-	-	-

Supplementary data 3 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Db	3	16	14	YLGLALISAVFLIG	ann	0.9	5101.95	0.9	-	-	-	-
H-2-Db	45	58	14	KYQYYGFIDVNYNI	ann	0.9	5337.88	0.9	-	-	-	-
H-2-Db	62	75	14	EIRKFSNVLMDYGV	ann	0.9	3525.34	0.9	-	-	-	-
H-2-Db	129	142	14	FDKAIQDYDKTISV	ann	0.9	4336.97	0.9	-	-	-	-
H-2-Db	66	74	9	FSNVLMDYG	Consensus	0.9	3582.24	0.9	1103.11	1.3	6.58e-06	0.5

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Kb	106	119	14	LNCRTSINYYRCVL	ann	0.2	412.32	0.2	-	-	-	-
H-2-Kb	110	123	14	TSINYYRCVLTSKL	ann	0.2	60.85	0.2	-	-	-	-
H-2-Kb	58	69	12	IAQSEIRKFSNV	ann	0.3	263.25	0.3	-	-	-	-
H-2-Kb	108	119	12	CRTSINYYRCVL	ann	0.3	361.44	0.3	-	-	-	-
H-2-Kb	109	120	12	RTSINYYRCVLT	ann	0.3	611.23	0.3	-	-	-	-
H-2-Kb	110	121	12	TSINYYRCVLTS	ann	0.3	535.02	0.3	-	-	-	-
H-2-Kb	112	123	12	INYYRCVLTSKL	ann	0.3	158.77	0.3	-	-	-	-
H-2-Kb	111	123	13	SINYYRCVLTSKL	ann	0.3	244.99	0.3	-	-	-	-
H-2-Kb	56	69	14	YNIAQSEIRKFSNV	ann	0.3	512.02	0.3	-	-	-	-
H-2-Kb	57	70	14	NIAQSEIRKFSNVL	ann	0.3	939.27	0.3	-	-	-	-
H-2-Kb	58	71	14	IAQSEIRKFSNVLM	ann	0.3	803.12	0.3	-	-	-	-
H-2-Kb	107	120	14	NCRTSINYYRCVLT	ann	0.3	751.16	0.3	-	-	-	-
H-2-Kb	108	121	14	CRTSINYYRCVLTS	ann	0.3	983.07	0.3	-	-	-	-
H-2-Kb	109	122	14	RTSINYYRCVLTSK	ann	0.3	1012.05	0.3	-	-	-	-
H-2-Kb	111	124	14	SINYYRCVLTSKLI	ann	0.3	485.56	0.3	-	-	-	-
H-2-Kb	112	125	14	INYYRCVLTSKLIG	ann	0.3	434.35	0.3	-	-	-	-
H-2-Kb	57	69	13	NIAQSEIRKFSNV	ann	0.4	436.39	0.4	-	-	-	-

Supplementary data 3 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Kb	58	70	13	IAQSEIRKFSNVL	ann	0.4	576.12	0.4	-	-	-	-
H-2-Kb	106	118	13	LNCRTSINYYRCV	ann	0.4	924.11	0.4	-	-	-	-
H-2-Kb	107	119	13	NCRTSINYYRCVL	ann	0.4	392.49	0.4	-	-	-	-
H-2-Kb	108	120	13	CRTSINYYRCVLT	ann	0.4	697.40	0.4	-	-	-	-
H-2-Kb	109	121	13	RTSINYYRCVLTS	ann	0.4	861.88	0.4	-	-	-	-
H-2-Kb	110	122	13	TSINYYRCVLTSK	ann	0.4	625.57	0.4	-	-	-	-
H-2-Kb	112	124	13	INYYRCVLTSKLI	ann	0.4	309.03	0.4	-	-	-	-
H-2-Kb	110	118	9	TSINYYRCV	Consensus	0.45	16.96	0.2	214.84	0.7	-	-
H-2-Kb	112	119	8	INYYRCVL	Consensus	0.45	31.34	0.3	123.52	0.6	-	-
H-2-Kb	105	118	14	WLNCRTSINYYRCV	ann	0.5	1081.92	0.5	-	-	-	-
H-2-Kb	112	120	9	INYYRCVLT	Consensus	0.5	184.28	0.6	109.93	0.4	-	-
H-2-Kb	107	118	12	NCRTSINYYRCV	ann	0.6	1092.87	0.6	-	-	-	-
H-2-Kb	44	52	9	CKYQYYGFI	Consensus	0.8	258.21	0.6	269.84	1	-	-
H-2-Kb	110	117	8	TSINYYRC	Consensus	0.8	275.38	0.8	159.49	0.8	-	-
H-2-Kb	40	52	13	CLHHCKYQYYGFI	ann	0.9	3148.48	0.9	-	-	-	-
H-2-Kb	41	53	13	LHHCKYQYYGFID	ann	0.9	3985.41	0.9	-	-	-	-
H-2-Kb	46	58	13	YQYYGFIDVNYNI	ann	0.9	3850.91	0.9	-	-	-	-
H-2-Kb	59	71	13	AQSEIRKFSNVLM	ann	0.9	3323.18	0.9	-	-	-	-
H-2-Kb	60	72	13	QSEIRKFSNVLMD	ann	0.9	2400.74	0.9	-	-	-	-
H-2-Kb	38	51	14	ISCLHHCKYQYYGF	ann	1	1865.29	1	-	-	-	-
H-2-Kb	39	52	14	SCLHHCKYQYYGFI	ann	1	1950.74	1	-	-	-	-

Note: The MHCI binding predictions were made on 11/20/2016 using the IEDB analysis resource Consensus tool which combines predictions from ANN aka NetMHC (4.0), SMM and Comblib. Results are presented by allele, and from lowest to highest percentile rank. Peptides predicted as good binders are all of those with a percentile ranking lower or equal to 1.

Supplementary data 4. PdSP15 in silico analysis: MHC-II restricted-epitopes prediction - Human (99% population coverage)

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*01/DPB1*04:01	42	56	HHCKYQYYGFIDVNY	Consensus	0.48	2.18	14.91	112	0.06	9.10	0.48
HLA-DPA1*01/DPB1*04:01	43	57	HCKYQYYGFIDVNYN	Consensus	0.52	2.18	14.91	113	0.06	9.60	0.52
HLA-DPA1*01/DPB1*04:01	41	55	LHHCKYQYYGFIDVN	Consensus	0.53	2.18	14.91	113	0.06	9.70	0.53
HLA-DPA1*01/DPB1*04:01	40	54	CLHHCKYQYYGFIDV	Consensus	0.60	100.21	33.49	112	0.06	10.60	0.60
HLA-DPA1*01/DPB1*04:01	44	58	CKYQYYGFIDVNYNI	Consensus	0.85	2.18	14.91	112	0.06	14	0.85
HLA-DPA1*01/DPB1*04:01	3	17	YLGLALISAVFLIGA	Consensus	0.90	0.01	0.01	370	0.90	608.20	16.64
HLA-DPA1*01/DPB1*04:01	4	18	LGLALISAVFLIGAC	Consensus	0.94	0.01	0.01	378	0.94	799.60	19.33
HLA-DPA1*01/DPB1*04:01	2	16	KYLGLALISAVFLIG	Consensus	0.98	0.01	0.01	385	0.98	507.40	15.02
HLA-DPA1*01/DPB1*04:01	5	19	GLALISAVFLIGACQ	Consensus	0.99	0.01	0.01	387	0.99	867.50	20.20
HLA-DPA1*01/DPB1*04:01	6	20	LALISAVFLIGACQA	Consensus	1.12	0.01	0.01	412	1.12	735.80	18.45
HLA-DPA1*01/DPB1*04:01	45	59	KYQYYGFIDVNYNIA	Consensus	2.33	2.18	14.91	291	0.54	36.20	2.33
HLA-DPA1*01/DPB1*04:01	7	21	ALISAVFLIGACQAE	Consensus	5.50	0.01	0.01	987	5.50	1816.10	29.74
HLA-DPA1*01/DPB1*04:01	46	60	YQYYGFIDVNYNIAQ	Consensus	5.79	2.18	14.91	311	0.62	113.10	5.79

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*01:02/DQB1*06:02	3	17	YLGLALISAVFLIGA	Consensus	2.37	0.02	2.37	38	0.21	284	18.55
HLA-DQA1*01:02/DQB1*06:02	4	18	LGLALISAVFLIGAC	Consensus	3.28	0.03	3.28	38	0.21	289.30	18.80
HLA-DQA1*01:02/DQB1*06:02	5	19	GLALISAVFLIGACQ	Consensus	3.28	0.03	3.28	38	0.21	321.80	20.32
HLA-DQA1*01:02/DQB1*06:02	1	15	MKYLGLALISAVFLI	Consensus	5.01	0.02	2.37	244	5.01	274.70	18.07

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*01:03/DPB1*02:01	3	17	YLGLALISAVFLIGA	Consensus	0.08	0.01	0.01	97	0.08	127	9.73
HLA-DPA1*01:03/DPB1*02:01	2	16	KYLGLALISAVFLIG	Consensus	0.09	0.01	0.01	99	0.09	133.20	10.03
HLA-DPA1*01:03/DPB1*02:01	4	18	LGLALISAVFLIGAC	Consensus	0.09	0.01	0.01	99	0.09	161.70	11.33
HLA-DPA1*01:03/DPB1*02:01	5	19	GLALISAVFLIGACQ	Consensus	0.10	0.01	0.01	107	0.10	210.80	13.30
HLA-DPA1*01:03/DPB1*02:01	6	20	LALISAVFLIGACQA	Consensus	0.11	0.01	0.01	111	0.11	209.50	13.25
HLA-DPA1*01:03/DPB1*02:01	7	21	ALISAVFLIGACQAE	Consensus	0.60	0.01	0.01	302	0.60	372.40	18.36
HLA-DPA1*01:03/DPB1*02:01	42	56	HHCKYQYYGFIDVNY	Consensus	0.66	2.33	12	43	0.02	6.70	0.66
HLA-DPA1*01:03/DPB1*02:01	41	55	LHHCKYQYYGFIDVN	Consensus	0.71	2.33	12	43	0.02	7	0.71
HLA-DPA1*01:03/DPB1*02:01	1	15	MKYLGLALISAVFLI	Consensus	0.72	0.01	0.01	337	0.72	171.70	11.72
HLA-DPA1*01:03/DPB1*02:01	40	54	CLHHCKYQYYGFIDV	Consensus	0.73	2.33	12	43	0.02	7.10	0.73
HLA-DPA1*01:03/DPB1*02:01	43	57	HCKYQYYGFIDVNYN	Consensus	0.79	2.33	12	43	0.02	7.50	0.79
HLA-DPA1*01:03/DPB1*02:01	44	58	CKYQYYGFIDVNYNI	Consensus	1.03	2.33	12	43	0.02	9.20	1.03
HLA-DPA1*01:03/DPB1*02:01	45	59	KYQYYGFIDVNYNIA	Consensus	2.01	2.33	12	112	0.11	16.70	2.01
HLA-DPA1*01:03/DPB1*02:01	46	60	YQYYGFIDVNYNIAQ	Consensus	4.15	78.86	27.84	119	0.13	37.90	4.15
HLA-DPA1*01:03/DPB1*02:01	39	53	SCLHHCKYQYYGFID	Consensus	4.64	2.33	12	676	2.63	43.80	4.64
HLA-DPA1*01:03/DPB1*02:01	38	52	ISCLHHCKYQYYGFI	Consensus	6.13	16.53	19.82	713	2.87	63.80	6.13

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*02:01/DPB1*05:01	1	15	MKYLGLALISAVFLI	Consensus	4.34	0.59	2.39	721	4.34	852	15.82
HLA-DPA1*02:01/DPB1*05:01	2	16	KYLGLALISAVFLIG	Consensus	4.64	0.59	2.39	755	4.64	1591.40	24.43

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*02:01/DPB1*01:01	43	57	HCKYQYYGFIDVNYN	Consensus	4.85	187.19	26.85	47	0.03	46.40	4.85
HLA-DPA1*02:01/DPB1*01:01	42	56	HHCKYQYYGFIDVNY	Consensus	4.96	187.19	26.85	47	0.03	47.30	4.96
HLA-DPA1*02:01/DPB1*01:01	44	58	CKYQYYGFIDVNYNI	Consensus	5.27	187.19	26.85	45	0.02	49.90	5.27
HLA-DPA1*02:01/DPB1*01:01	61	75	SEIRKFSNVLMDYGV	Consensus	5.66	145.17	24.60	328	1.10	53.20	5.66
HLA-DPA1*02:01/DPB1*01:01	60	74	QSEIRKFSNVLMDYG	Consensus	5.71	145.17	24.60	337	1.16	53.70	5.71
HLA-DPA1*02:01/DPB1*01:01	59	73	AQSEIRKFSNVLMDY	Consensus	5.95	145.17	24.60	319	1.04	55.80	5.95
HLA-DPA1*02:01/DPB1*01:01	45	59	KYQYYGFIDVNYNIA	Consensus	6.14	8.39	7.57	118	0.17	57.50	6.14
HLA-DPA1*02:01/DPB1*01:01	41	55	LHHCKYQYYGFIDVN	Consensus	6.16	187.19	26.85	47	0.03	57.70	6.16
HLA-DPA1*02:01/DPB1*01:01	62	76	EIRKFSNVLMDYGVV	Consensus	7.09	145.17	24.60	368	1.37	66.20	7.09

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*03:01/DPB1*04:02	3	17	YLGLALISAVFLIGA	Consensus	2.97	0.08	2.97	116	0.10	115.40	10.62
HLA-DPA1*03:01/DPB1*04:02	4	18	LGLALISAVFLIGAC	Consensus	2.97	0.08	2.97	117	0.10	146.70	12.33
HLA-DPA1*03:01/DPB1*04:02	5	19	GLALISAVFLIGACQ	Consensus	2.97	0.08	2.97	122	0.11	154.20	12.71
HLA-DPA1*03:01/DPB1*04:02	6	20	LALISAVFLIGACQA	Consensus	2.97	0.08	2.97	126	0.12	171.70	13.55
HLA-DPA1*03:01/DPB1*04:02	7	21	ALISAVFLIGACQAE	Consensus	2.97	0.08	2.97	285	0.51	285	17.91
HLA-DPA1*03:01/DPB1*04:02	8	22	LISAVFLIGACQAET	Consensus	2.97	0.08	2.97	373	0.84	451.30	22.52
HLA-DPA1*03:01/DPB1*04:02	1	15	MKYLGLALISAVFLI	Consensus	3.03	0.09	3.03	229	0.35	117.30	10.73
HLA-DPA1*03:01/DPB1*04:02	2	16	KYLGLALISAVFLIG	Consensus	3.03	0.09	3.03	124	0.11	134.40	11.69
HLA-DPA1*03:01/DPB1*04:02	9	23	ISAVFLIGACQAETP	Consensus	4.70	0.08	2.97	1023	4.70	705.30	27.52
HLA-DPA1*03:01/DPB1*04:02	43	57	HCKYQYYGFIDVNYN	Consensus	6.89	1723.96	50.72	136	0.14	62.50	6.89

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*01:01/DQB1*05:01	44	58	CKYQYYGFIDVNYNI	Consensus	0.56	0.29	3.16	323	0.03	33.10	0.56
HLA-DQA1*01:01/DQB1*05:01	45	59	KYQYYGFIDVNYNIA	Consensus	0.61	0.29	3.16	361	0.04	35.20	0.61
HLA-DQA1*01:01/DQB1*05:01	46	60	YQYYGFIDVNYNIAQ	Consensus	0.74	0.29	3.16	400	0.05	40.70	0.74
HLA-DQA1*01:01/DQB1*05:01	43	57	HCKYQYYGFIDVNYN	Consensus	0.81	0.29	3.16	330	0.03	43.50	0.81
HLA-DQA1*01:01/DQB1*05:01	42	56	HHCKYQYYGFIDVNY	Consensus	1.26	0.29	3.16	329	0.03	60.90	1.26
HLA-DQA1*01:01/DQB1*05:01	47	61	QYYGFIDVNYNIAQS	Consensus	1.31	0.95	5.14	938	0.34	63	1.31
HLA-DQA1*01:01/DQB1*05:01	48	62	YYGFIDVNYNIAQSE	Consensus	2.44	0.95	5.14	978	0.38	110.50	2.44
HLA-DQA1*01:01/DQB1*05:01	7	21	ALISAVFLIGACQAE	Consensus	2.92	0.21	2.77	2728	2.92	1771.80	21.45
HLA-DQA1*01:01/DQB1*05:01	40	54	CLHHCKYQYYGFIDV	Consensus	3.16	0.29	3.16	2201	1.95	1120.40	16.01
HLA-DQA1*01:01/DQB1*05:01	41	55	LHHCKYQYYGFIDVN	Consensus	3.16	0.29	3.16	1809	1.32	266.70	5.54
HLA-DQA1*01:01/DQB1*05:01	4	18	LGLALISAVFLIGAC	Consensus	3.50	0.21	2.77	3015	3.50	1847.70	21.99
HLA-DQA1*01:01/DQB1*05:01	6	20	LALISAVFLIGACQA	Consensus	3.69	0.21	2.77	3105	3.69	1837.10	21.92
HLA-DQA1*01:01/DQB1*05:01	5	19	GLALISAVFLIGACQ	Consensus	3.78	0.21	2.77	3147	3.78	1936.20	22.64
HLA-DQA1*01:01/DQB1*05:01	8	22	LISAVFLIGACQAET	Consensus	4.27	0.21	2.77	3366	4.27	1842.30	21.95
HLA-DQA1*01:01/DQB1*05:01	3	17	YLGLALISAVFLIGA	Consensus	4.78	0.21	2.77	3590	4.78	2727	27.83
HLA-DQA1*01:01/DQB1*05:01	1	15	MKYLGLALISAVFLI	Consensus	7.05	2.18	7.05	2508	2.50	1277.10	17.45
HLA-DQA1*01:01/DQB1*05:01	2	16	KYLGLALISAVFLIG	Consensus	7.05	2.18	7.05	3948	5.63	2530.80	26.63

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*05:01/DQB1*03:01	1	15	MKYLGLALISAVFLI	Consensus	7.29	0.14	3.39	43	14.09	39.10	7.29

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*04:01/DQB1*04:02	1	15	MKYLGLALISAVFLI	Consensus	0.01	0.01	0.01	294	0.01	692.50	11.04
HLA-DQA1*04:01/DQB1*04:02	2	16	KYLGLALISAVFLIG	Consensus	0.01	0.01	0.01	299	0.01	905.70	14.40
HLA-DQA1*04:01/DQB1*04:02	3	17	YLGLALISAVFLIGA	Consensus	0.07	0.01	0.01	634	0.07	1039.10	16.39
HLA-DQA1*04:01/DQB1*04:02	4	18	LGLALISAVFLIGAC	Consensus	0.08	0.01	0.01	664	0.08	1507.70	22.79
HLA-DQA1*04:01/DQB1*04:02	7	21	ALISAVFLIGACQAE	Consensus	0.25	0.01	0.01	990	0.25	359.80	5.35
HLA-DQA1*04:01/DQB1*04:02	5	19	GLALISAVFLIGACQ	Consensus	0.67	0.01	0.01	1468	0.67	2441.30	33.33
HLA-DQA1*04:01/DQB1*04:02	49	63	YGFIDVNYNIAQSEI	Consensus	1.39	645.32	40.78	578	0.06	134.20	1.39
HLA-DQA1*04:01/DQB1*04:02	6	20	LALISAVFLIGACQA	Consensus	1.86	0.01	0.01	2297	1.86	1225.70	19.06
HLA-DQA1*04:01/DQB1*04:02	50	64	GFIDVNYNIAQSEIR	Consensus	2.15	645.32	40.78	570	0.05	178.90	2.15
HLA-DQA1*04:01/DQB1*04:02	48	62	YYGFIDVNYNIAQSE	Consensus	3.32	645.32	40.78	1231	0.43	243.70	3.32
HLA-DQA1*04:01/DQB1*04:02	51	65	FIDVNYNIAQSEIRK	Consensus	3.55	3033.04	48.34	578	0.06	256.80	3.55
HLA-DQA1*04:01/DQB1*04:02	8	22	LISAVFLIGACQAET	Consensus	5.78	22124.90	56.75	576	0.06	383.50	5.78
HLA-DQA1*04:01/DQB1*04:02	52	66	IDVNYNIAQSEIRKF	Consensus	5.83	3033.04	48.34	592	0.06	386.20	5.83
HLA-DQA1*04:01/DQB1*04:02	9	23	ISAVFLIGACQAETP	Consensus	6.68	22124.90	56.75	579	0.06	435	6.68

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*01:01	1	15	MKYLGLALISAVFLI	Consensus	4.30	0.38	15.04	7	0.77	8.90	4.30
HLA-DRB1*01:01	2	16	KYLGLALISAVFLIG	Consensus	5.73	0.38	15.04	8	0.96	10.70	5.73
HLA-DRB1*01:01	111	125	SINYYRCVLTSKLIG	Consensus	6.67	3.13	24.08	25	4.77	12	6.67

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*03:01	63	77	IRKFSNVLMDYGVVD	Consensus	1.55	410	0.33	401.70	12.39	3.60	1.55
HLA-DRB1*03:01	64	78	RKFSNVLMDYGVVDK	Consensus	1.55	402	0.31	236.80	8.92	3.60	1.55
HLA-DRB1*03:01	65	79	KFSNVLMDYGVVDKS	Consensus	1.55	402	0.31	144.90	6.51	3.60	1.55
HLA-DRB1*03:01	66	80	FSNVLMDYGVVDKSK	Consensus	1.55	394	0.30	98.70	4.96	3.60	1.55
HLA-DRB1*03:01	67	81	SNVLMDYGVVDKSKK	Consensus	1.55	404	0.31	161	6.97	3.60	1.55
HLA-DRB1*03:01	68	82	NVLMDYGVVDKSKKR	Consensus	1.55	531	0.56	230	8.75	3.60	1.55
HLA-DRB1*03:01	69	83	VLMDYGVVDKSKKRE	Consensus	1.55	529	0.55	457.60	13.33	3.60	1.55
HLA-DRB1*03:01	1	15	MKYLGLALISAVFLI	Consensus	3.67	2000	3.27	4118.50	41.50	2.70	3.67
HLA-DRB1*03:01	2	16	KYLGLALISAVFLIG	Consensus	4.29	1930	3.14	4483.20	43.17	2.57	4.29
HLA-DRB1*03:01	3	17	YLGLALISAVFLIGA	Consensus	4.29	2208	3.73	4742.30	44.26	2.57	4.29
HLA-DRB1*03:01	4	18	LGLALISAVFLIGAC	Consensus	4.29	2206	3.73	4265.50	42.18	2.57	4.29
HLA-DRB1*03:01	6	20	LALISAVFLIGACQA	Consensus	7.12	3824	7.12	7452.50	53.99	2.30	5.24
HLA-DRB1*03:01	57	71	NIAQSEIRKFSNVLM	Consensus	7.21	1813	2.91	1590.40	26.25	1.90	7.21
HLA-DRB1*03:01	58	72	IAQSEIRKFSNVLMD	Consensus	7.21	1968	3.21	2551.60	32.95	1.90	7.21
HLA-DRB1*03:01	59	73	AQSEIRKFSNVLMDY	Consensus	7.21	2638	4.60	3152.30	36.46	1.90	7.21
HLA-DRB1*03:01	60	74	QSEIRKFSNVLMDYG	Consensus	7.21	2793	4.92	3480.90	38.29	1.90	7.21
HLA-DRB1*03:01	61	75	SEIRKFSNVLMDYGV	Consensus	7.21	2917	5.20	3963	40.74	1.90	7.21
HLA-DRB1*03:01	53	67	DVNYNIAQSEIRKFS	Consensus	7.50	999	1.36	179.70	7.50	1.50	9.61

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*12:01	1	15	MKYLGLALISAVFLI	smm	4.39	-	-	451	4.39	-	-
HLA-DRB1*12:01	3	17	YLGLALISAVFLIGA	smm	5.65	-	-	543	5.65	-	-
HLA-DRB1*12:01	2	16	KYLGLALISAVFLIG	smm	6.06	-	-	573	6.06	-	-

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:01	45	59	KYQYYGFIDVNYNIA	Consensus	1.79	391	4.22	24.90	1.42	2.20	1.79
HLA-DRB1*04:01	46	60	YQYYGFIDVNYNIAQ	Consensus	2.09	374	4.03	31.90	2.09	2.20	1.79
HLA-DRB1*04:01	44	58	CKYQYYGFIDVNYNI	Consensus	2.16	437	4.76	32.70	2.16	2.20	1.79
HLA-DRB1*04:01	110	124	TSINYYRCVLTSKLI	Consensus	2.25	221	2.25	106.50	8.62	2	2.16
HLA-DRB1*04:01	111	125	SINYYRCVLTSKLIG	Consensus	2.31	226	2.31	82.30	6.66	2	2.16
HLA-DRB1*04:01	109	123	RTSINYYRCVLTSKL	Consensus	2.35	230	2.35	199.10	14.93	2	2.16
HLA-DRB1*04:01	112	126	INYYRCVLTSKLIGP	Consensus	2.37	231	2.37	103.80	8.41	2	2.16
HLA-DRB1*04:01	47	61	QYYGFIDVNYNIAQS	Consensus	2.69	580	6.34	38.30	2.69	2.20	1.79
HLA-DRB1*04:01	113	127	NYYRCVLTSKLIGPQ	Consensus	2.84	272	2.84	146.50	11.55	2	2.16
HLA-DRB1*04:01	60	74	QSEIRKFSNVLMDYG	Consensus	2.85	56	0.39	39.90	2.85	1.28	4.11
HLA-DRB1*04:01	59	73	AQSEIRKFSNVLMDY	Consensus	3.11	58	0.41	42.70	3.11	1.28	4.11
HLA-DRB1*04:01	43	57	HCKYQYYGFIDVNYN	Consensus	3.50	492	5.39	46.80	3.50	2.20	1.79
HLA-DRB1*04:01	58	72	IAQSEIRKFSNVLMD	Consensus	3.68	57	0.40	48.60	3.68	1.28	4.11
HLA-DRB1*04:01	57	71	NIAQSEIRKFSNVLM	Consensus	3.77	57	0.40	49.60	3.77	1.28	4.11
HLA-DRB1*04:01	61	75	SEIRKFSNVLMDYGV	Consensus	4.11	57	0.40	55.80	4.35	1.28	4.11
HLA-DRB1*04:01	62	76	EIRKFSNVLMDYGVV	Consensus	4.11	174	1.73	74.90	6.05	1.28	4.11
HLA-DRB1*04:01	63	77	IRKFSNVLMDYGVVD	Consensus	4.11	177	1.77	102.20	8.29	1.28	4.11
HLA-DRB1*04:01	1	15	MKYLGLALISAVFLI	Consensus	5.14	171	1.70	130.50	10.43	0.98	5.14
HLA-DRB1*04:01	2	16	KYLGLALISAVFLIG	Consensus	5.14	199	2.01	221.60	16.26	0.98	5.14
HLA-DRB1*04:01	3	17	YLGLALISAVFLIGA	Consensus	5.14	209	2.12	494.20	28.62	0.98	5.14
HLA-DRB1*04:01	48	62	YYGFIDVNYNIAQSE	Consensus	6.86	651	7.08	84.60	6.86	2.20	1.79
HLA-DRB1*04:01	6	20	LALISAVFLIGACQA	Consensus	6.98	641	6.98	1665.10	55.09	1.60	3.05
HLA-DRB1*04:01	114	128	YYRCVLTSKLIGPQR	Consensus	7.28	670	7.28	238.80	17.20	2	2.16

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:05	46	60	YQYYGFIDVNYNIAQ	Consensus	1.98	386	5.69	25.80	1.98	4.60	0.21
HLA-DRB1*04:05	45	59	KYQYYGFIDVNYNIA	Consensus	2	420	6.18	26	2	4.60	0.21
HLA-DRB1*04:05	44	58	CKYQYYGFIDVNYNI	Consensus	2.20	434	6.39	27.70	2.20	4.60	0.21
HLA-DRB1*04:05	61	75	SEIRKFSNVLMDYGV	Consensus	3	246	3.62	34.10	3	4.10	0.38
HLA-DRB1*04:05	47	61	QYYGFIDVNYNIAQS	Consensus	3.01	620	8.88	34.20	3.01	4.60	0.21
HLA-DRB1*04:05	43	57	HCKYQYYGFIDVNYN	Consensus	3.06	454	6.66	34.60	3.06	4.60	0.21
HLA-DRB1*04:05	110	124	TSINYYRCVLTSKLI	Consensus	3.13	214	3.13	116.80	10.98	5.10	0.11
HLA-DRB1*04:05	62	76	EIRKFSNVLMDYGVV	Consensus	3.17	422	6.20	35.50	3.17	4.10	0.38
HLA-DRB1*04:05	109	123	RTSINYYRCVLTSKL	Consensus	3.28	223	3.28	156.30	13.81	5.10	0.11
HLA-DRB1*04:05	111	125	SINYYRCVLTSKLIG	Consensus	3.37	229	3.37	136.20	12.43	5.10	0.11
HLA-DRB1*04:05	112	126	INYYRCVLTSKLIGP	Consensus	3.51	238	3.51	201.20	16.63	5.10	0.11
HLA-DRB1*04:05	60	74	QSEIRKFSNVLMDYG	Consensus	3.62	248	3.65	39.20	3.62	4.10	0.38
HLA-DRB1*04:05	63	77	IRKFSNVLMDYGVVD	Consensus	3.70	422	6.20	39.90	3.70	4.10	0.38
HLA-DRB1*04:05	113	127	NYYRCVLTSKLIGPQ	Consensus	3.98	272	3.98	282.10	20.90	5.10	0.11
HLA-DRB1*04:05	58	72	IAQSEIRKFSNVLMD	Consensus	4.87	331	4.87	106	10.12	4.10	0.38
HLA-DRB1*04:05	59	73	AQSEIRKFSNVLMDY	Consensus	4.87	331	4.87	100.60	9.69	4.10	0.38
HLA-DRB1*04:05	57	71	NIAQSEIRKFSNVLM	Consensus	4.95	337	4.95	194.20	16.23	4.10	0.38
HLA-DRB1*04:05	48	62	YYGFIDVNYNIAQSE	Consensus	5.64	487	7.14	57.30	5.64	4.60	0.21
HLA-DRB1*04:05	1	15	MKYLGLALISAVFLI	Consensus	5.88	192	2.77	131.80	12.12	1.18	5.88
HLA-DRB1*04:05	2	16	KYLGLALISAVFLIG	Consensus	5.88	231	3.41	183.60	15.59	1.18	5.88
HLA-DRB1*04:05	3	17	YLGLALISAVFLIGA	Consensus	5.88	374	5.51	305.50	21.99	1.18	5.88
HLA-DRB1*04:05	4	18	LGLALISAVFLIGAC	Consensus	6.32	426	6.26	536.90	30.43	1	6.32
HLA-DRB1*04:05	50	64	GFIDVNYNIAQSEIR	Consensus	7.19	491	7.19	317.10	22.51	1.60	4.45
HLA-DRB1*04:05	51	65	FIDVNYNIAQSEIRK	Consensus	7.36	504	7.36	263.40	19.98	1.60	4.45

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*07:01	1	15	MKYLGLALISAVFLI	Consensus	1.31	0.01	1.31	32	0.21	131.30	16.81
HLA-DRB1*07:01	2	16	KYLGLALISAVFLIG	Consensus	1.31	0.01	1.31	34	0.23	330.20	27.49
HLA-DRB1*07:01	3	17	YLGLALISAVFLIGA	Consensus	1.31	0.01	1.31	34	0.23	593.50	35.84
HLA-DRB1*07:01	4	18	LGLALISAVFLIGAC	Consensus	1.31	0.01	1.31	34	0.23	925.50	42.98
HLA-DRB1*07:01	5	19	GLALISAVFLIGACQ	Consensus	1.31	0.01	1.31	105	0.96	847.50	41.55
HLA-DRB1*07:01	6	20	LALISAVFLIGACQA	Consensus	1.31	0.01	1.31	110	1.01	819.70	40.98
HLA-DRB1*07:01	110	124	TSINYYRCVLTSKLI	Consensus	2.05	1.61	11.31	44	0.33	11.70	2.05
HLA-DRB1*07:01	111	125	SINYYRCVLTSKLIG	Consensus	2.70	1.61	11.31	47	0.35	14.50	2.70
HLA-DRB1*07:01	112	126	INYYRCVLTSKLIGP	Consensus	3.23	1.61	11.31	48	0.36	17.10	3.23
HLA-DRB1*07:01	57	71	NIAQSEIRKFSNVLM	Consensus	3.33	71.16	31.67	232	2.56	17.60	3.33
HLA-DRB1*07:01	109	123	RTSINYYRCVLTSKL	Consensus	4.41	1.61	11.31	51	0.39	22.80	4.41
HLA-DRB1*07:01	58	72	IAQSEIRKFSNVLMD	Consensus	4.79	71.16	31.67	236	2.61	24.80	4.79
HLA-DRB1*07:01	113	127	NYYRCVLTSKLIGPQ	Consensus	5.02	1.61	11.31	51	0.39	26	5.02
HLA-DRB1*07:01	114	128	YYRCVLTSKLIGPQR	Consensus	6.60	1.61	11.31	129	1.24	35.80	6.60

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*03:01/DQB1*03:02	8	22	LISAVFLIGACQAET	Consensus	3.63	2.42	22.72	705	1.47	245.50	3.63
HLA-DQA1*03:01/DQB1*03:02	7	21	ALISAVFLIGACQAE	Consensus	3.66	0.09	10.62	694	1.43	247	3.66
HLA-DQA1*03:01/DQB1*03:02	9	23	ISAVFLIGACQAETP	Consensus	3.96	2.42	22.72	751	1.67	262.40	3.96
HLA-DQA1*03:01/DQB1*03:02	10	24	SAVFLIGACQAETPS	Consensus	4.54	2.42	22.72	815	1.95	291	4.54
HLA-DQA1*03:01/DQB1*03:02	11	25	AVFLIGACQAETPSQ	Consensus	6.25	275.10	47.08	1072	3.20	376.20	6.25

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*08:02	1	15	MKYLGLALISAVFLI	Consensus	2.59	871	1.88	1859.60	34.59	1.50	2.29
HLA-DRB1*08:02	3	17	YLGLALISAVFLIGA	Consensus	2.73	993	2.31	1665	32.25	1.30	2.73
HLA-DRB1*08:02	2	16	KYLGLALISAVFLIG	Consensus	3.06	1221	3.06	2379.90	40.21	1.30	2.73
HLA-DRB1*08:02	6	20	LALISAVFLIGACQA	Consensus	4.37	1596	4.37	1943.50	35.59	1.10	3.25
HLA-DRB1*08:02	109	123	RTSINYYRCVLTSKL	Consensus	4.60	1665	4.60	560.10	13.60	1	3.80
HLA-DRB1*08:02	128	142	RFDKAIQDYDKTISV	Consensus	4.65	1679	4.65	1200.30	25.67	0.70	4.45
HLA-DRB1*08:02	110	124	TSINYYRCVLTSKLI	Consensus	4.68	1689	4.68	623.60	15.01	1	3.80
HLA-DRB1*08:02	127	141	QRFDKAIQDYDKTIS	Consensus	4.76	1713	4.76	2016.70	36.42	0.70	4.45
HLA-DRB1*08:02	7	21	ALISAVFLIGACQAE	Consensus	5.23	1844	5.23	1869	34.71	1.10	3.25
HLA-DRB1*08:02	108	122	CRTSINYYRCVLTSK	Consensus	6.16	2102	6.16	577.30	14	0.50	5.13
HLA-DRB1*08:02	111	125	SINYYRCVLTSKLIG	Consensus	6.22	2115	6.22	665.40	15.88	1	3.80
HLA-DRB1*08:02	112	126	INYYRCVLTSKLIGP	Consensus	6.47	2184	6.47	968.50	21.91	1	3.80
HLA-DRB1*08:02	9	23	ISAVFLIGACQAETP	Consensus	7.01	2327	7.01	732.60	17.33	1.10	3.25
HLA-DRB1*08:02	47	61	QYYGFIDVNYNIAQS	Consensus	7.13	1444	3.83	2257.30	38.95	0.10	7.13
HLA-DRB1*08:02	48	62	YYGFIDVNYNIAQSE	Consensus	7.13	1412	3.72	2291.20	39.31	0.10	7.13
HLA-DRB1*08:02	49	63	YGFIDVNYNIAQSEI	Consensus	7.13	1388	3.64	2185.70	38.25	0.10	7.13
HLA-DRB1*08:02	46	60	YQYYGFIDVNYNIAQ	Consensus	7.15	2367	7.15	2738.50	43.65	1.30	2.73
HLA-DRB1*08:02	106	120	LNCRTSINYYRCVLT	Consensus	7.21	2385	7.21	1174.30	25.25	0.50	5.13
HLA-DRB1*08:02	89	103	HECAKQVKKEARKDS	Consensus	7.32	1483	3.98	5127.60	59.50	0	7.32
HLA-DRB1*08:02	90	104	ECAKQVKKEARKDSH	Consensus	7.32	1508	4.07	4342.20	55.13	0	7.32
HLA-DRB1*08:02	91	105	CAKQVKKEARKDSHW	Consensus	7.32	1552	4.22	4287.40	54.82	0	7.32
HLA-DRB1*08:02	92	106	AKQVKKEARKDSHWL	Consensus	7.32	1558	4.24	5018.30	58.96	0	7.32
HLA-DRB1*08:02	93	107	KQVKKEARKDSHWLN	Consensus	7.32	1611	4.42	5081.80	59.28	0	7.32
HLA-DRB1*08:02	60	74	QSEIRKFSNVLMDYG	Consensus	7.39	203	0.17	311.30	7.39	-0.40	9.17
HLA-DRB1*08:02	8	22	LISAVFLIGACQAET	Consensus	7.48	2459	7.48	1030.60	22.91	1.10	3.25

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*11:01	1	15	MKYLGLALISAVFLI	Consensus	0.49	274	0.49	194.90	20.04	3.30	0.43
HLA-DRB1*11:01	3	17	YLGLALISAVFLIGA	Consensus	0.69	336	0.69	1126.70	42.57	3.30	0.43
HLA-DRB1*11:01	2	16	KYLGLALISAVFLIG	Consensus	0.71	340	0.71	345.60	26.41	3.30	0.43
HLA-DRB1*11:01	81	95	KRELKKVMHECAKQV	Consensus	2.44	626	1.82	14.80	2.44	-0.90	13.67
HLA-DRB1*11:01	6	20	LALISAVFLIGACQA	Consensus	2.51	775	2.51	1837.60	50.46	1.70	2.41
HLA-DRB1*11:01	78	92	KSKKRELKKVMHECA	Consensus	3.04	453	1.14	17.50	3.04	-0.90	13.67
HLA-DRB1*11:01	80	94	KKRELKKVMHECAKQ	Consensus	3.17	710	2.22	18.10	3.17	-0.90	13.67
HLA-DRB1*11:01	79	93	SKKRELKKVMHECAK	Consensus	3.75	502	1.33	20.80	3.75	-0.90	13.67
HLA-DRB1*11:01	67	81	SNVLMDYGVVDKSKK	Consensus	4.02	377	0.84	907.40	39.31	1.10	4.02
HLA-DRB1*11:01	68	82	NVLMDYGVVDKSKKR	Consensus	4.02	360	0.78	493.70	30.89	1.10	4.02
HLA-DRB1*11:01	69	83	VLMDYGVVDKSKKRE	Consensus	4.02	357	0.77	376.60	27.46	1.10	4.02
HLA-DRB1*11:01	70	84	LMDYGVVDKSKKREL	Consensus	4.02	97	0.07	226.40	21.61	1.10	4.02
HLA-DRB1*11:01	71	85	MDYGVVDKSKKRELK	Consensus	4.02	95	0.07	239.80	22.23	1.10	4.02
HLA-DRB1*11:01	72	86	DYGVVDKSKKRELKK	Consensus	4.02	107	0.09	223.10	21.44	1.10	4.02
HLA-DRB1*11:01	73	87	YGVVDKSKKRELKKV	Consensus	4.02	104	0.09	239.40	22.21	1.10	4.02
HLA-DRB1*11:01	109	123	RTSINYYRCVLTSKL	Consensus	4.02	134	0.15	65.10	10.35	1.10	4.02
HLA-DRB1*11:01	110	124	TSINYYRCVLTSKLI	Consensus	4.02	128	0.13	37.30	6.70	1.10	4.02
HLA-DRB1*11:01	111	125	SINYYRCVLTSKLIG	Consensus	4.02	115	0.11	26.10	4.81	1.10	4.02
HLA-DRB1*11:01	112	126	INYYRCVLTSKLIGP	Consensus	4.02	117	0.12	23.40	4.28	1.10	4.02
HLA-DRB1*11:01	113	127	NYYRCVLTSKLIGPQ	Consensus	4.02	135	0.15	30.80	5.66	1.10	4.02
HLA-DRB1*11:01	114	128	YYRCVLTSKLIGPQR	Consensus	4.02	262	0.46	44.70	7.81	1.10	4.02
HLA-DRB1*11:01	115	129	YRCVLTSKLIGPQRF	Consensus	4.02	292	0.55	87.60	12.65	1.10	4.02
HLA-DRB1*11:01	82	96	RELKKVMHECAKQVK	Consensus	4.39	586	1.66	24	4.39	-0.90	13.67
HLA-DRB1*11:01	47	61	QYYGFIDVNYNIAQS	Consensus	4.67	1231	4.67	360.40	26.94	1	4.33
HLA-DRB1*11:01	46	60	YQYYGFIDVNYNIAQ	Consensus	4.73	1243	4.73	262.80	23.25	1	4.33
HLA-DRB1*11:01	49	63	YGFIDVNYNIAQSEI	Consensus	4.80	1258	4.80	425.70	28.99	1	4.33
HLA-DRB1*11:01	48	62	YYGFIDVNYNIAQSE	Consensus	5.03	1303	5.03	393.90	28.03	1	4.33

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*11:01	77	91	DKSKKRELKKVMHEC	Consensus	5.17	677	2.07	28.10	5.17	-12.50	34.99
HLA-DRB1*11:01	7	21	ALISAVFLIGACQAE	Consensus	5.37	1369	5.37	1492.40	47.01	1.70	2.41
HLA-DRB1*11:01	8	22	LISAVFLIGACQAET	Consensus	6.03	1498	6.03	1047.20	41.46	1.70	2.41
HLA-DRB1*11:01	83	97	ELKKVMHECAKQVKK	Consensus	6.38	935	3.23	35.20	6.38	-0.90	13.67
HLA-DRB1*11:01	9	23	ISAVFLIGACQAETP	Consensus	6.78	1643	6.78	981	40.48	1.70	2.41
HLA-DRB1*11:01	76	90	VDKSKKRELKKVMHE	Consensus	6.86	266	0.47	38.30	6.86	-3.10	26.77

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*05:01/DQB1*02:01	9	23	ISAVFLIGACQAETP	Consensus	4.82	0.01	0.01	794	4.82	305.80	6.88
HLA-DQA1*05:01/DQB1*02:01	10	24	SAVFLIGACQAETPS	Consensus	5.11	0.01	0.01	820	5.11	409	9.23
HLA-DQA1*05:01/DQB1*02:01	45	59	KYQYYGFIDVNYNIA	Consensus	5.17	0.01	0.01	825	5.17	456.40	10.26
HLA-DQA1*05:01/DQB1*02:01	44	58	CKYQYYGFIDVNYNI	Consensus	5.39	0.01	0.01	845	5.39	422.80	9.53
HLA-DQA1*05:01/DQB1*02:01	1	15	MKYLGLALISAVFLI	Consensus	5.52	0.01	0.01	856	5.52	1260.20	24.41
HLA-DQA1*05:01/DQB1*02:01	46	60	YQYYGFIDVNYNIAQ	Consensus	5.52	0.01	0.01	856	5.52	542.50	12.06
HLA-DQA1*05:01/DQB1*02:01	43	57	HCKYQYYGFIDVNYN	Consensus	5.99	0.01	0.01	897	5.99	404	9.12
HLA-DQA1*05:01/DQB1*02:01	11	25	AVFLIGACQAETPSQ	Consensus	6	0.01	0.01	898	6	561.40	12.45
HLA-DQA1*05:01/DQB1*02:01	3	17	YLGLALISAVFLIGA	Consensus	6.21	0.01	0.01	915	6.21	1219	23.81
HLA-DQA1*05:01/DQB1*02:01	2	16	KYLGLALISAVFLIG	Consensus	6.39	0.01	0.01	931	6.39	1451.40	27.06
HLA-DQA1*05:01/DQB1*02:01	42	56	HHCKYQYYGFIDVNY	Consensus	7.06	0.01	0.01	987	7.06	678.90	14.79
HLA-DQA1*05:01/DQB1*02:01	4	18	LGLALISAVFLIGAC	Consensus	7.27	0.01	0.01	1004	7.27	1454.20	27.10

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*15:01	59	73	AQSEIRKFSNVLMDY	Consensus	0.97	115	1.55	15.40	0.97	5.68	0.15
HLA-DRB1*15:01	60	74	QSEIRKFSNVLMDYG	Consensus	1.25	118	1.61	17.80	1.25	5.68	0.15
HLA-DRB1*15:01	58	72	IAQSEIRKFSNVLMD	Consensus	1.43	117	1.59	19.30	1.43	5.68	0.15
HLA-DRB1*15:01	57	71	NIAQSEIRKFSNVLM	Consensus	1.55	115	1.55	20.20	1.55	5.68	0.15
HLA-DRB1*15:01	61	75	SEIRKFSNVLMDYGV	Consensus	1.84	142	2.07	22.60	1.84	5.68	0.15
HLA-DRB1*15:01	1	15	MKYLGLALISAVFLI	Consensus	2.61	82	0.94	193.10	16.57	3.30	2.61
HLA-DRB1*15:01	110	124	TSINYYRCVLTSKLI	Consensus	3.18	193	3.18	104.60	10.32	4.20	1.11
HLA-DRB1*15:01	62	76	EIRKFSNVLMDYGVV	Consensus	3.35	326	6.18	35.10	3.35	5.68	0.15
HLA-DRB1*15:01	109	123	RTSINYYRCVLTSKL	Consensus	3.93	227	3.93	161.20	14.54	4.20	1.11
HLA-DRB1*15:01	106	120	LNCRTSINYYRCVLT	Consensus	4.86	267	4.86	151.90	13.92	4.20	1.11
HLA-DRB1*15:01	108	122	CRTSINYYRCVLTSK	Consensus	5.20	282	5.20	276	20.84	4.20	1.11
HLA-DRB1*15:01	107	121	NCRTSINYYRCVLTS	Consensus	5.35	289	5.35	200.90	17.01	4.20	1.11
HLA-DRB1*15:01	2	16	KYLGLALISAVFLIG	Consensus	5.61	87	1.02	272	20.66	2.40	5.61
HLA-DRB1*15:01	3	17	YLGLALISAVFLIGA	Consensus	5.61	87	1.02	344.90	23.79	2.40	5.61
HLA-DRB1*15:01	4	18	LGLALISAVFLIGAC	Consensus	5.61	90	1.08	373.10	24.88	2.40	5.61
HLA-DRB1*15:01	5	19	GLALISAVFLIGACQ	Consensus	5.61	255	4.57	466.90	28.06	2.40	5.61
HLA-DRB1*15:01	6	20	LALISAVFLIGACQA	Consensus	5.61	274	5.03	1721.50	49.65	2.40	5.61
HLA-DRB1*15:01	111	125	SINYYRCVLTSKLIG	Consensus	6.74	352	6.74	133.40	12.61	4.20	1.11
HLA-DRB1*15:01	112	126	INYYRCVLTSKLIGP	Consensus	6.78	354	6.78	202	17.07	4.20	1.11
HLA-DRB1*15:01	63	77	IRKFSNVLMDYGVVD	Consensus	7.05	367	7.05	344.40	23.77	5.68	0.15

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB3*01:01	44	58	CKYQYYGFIDVNYNI	Consensus	0.12	0.01	0.01	929	0.12	495	11.68
HLA-DRB3*01:01	45	59	KYQYYGFIDVNYNIA	Consensus	0.14	0.01	0.01	984	0.14	511.80	11.92
HLA-DRB3*01:01	46	60	YQYYGFIDVNYNIAQ	Consensus	0.14	0.01	0.01	992	0.14	562.60	12.59
HLA-DRB3*01:01	47	61	QYYGFIDVNYNIAQS	Consensus	0.14	0.01	0.01	999	0.14	650.90	13.74
HLA-DRB3*01:01	48	62	YYGFIDVNYNIAQSE	Consensus	0.14	0.01	0.01	1008	0.14	1206.40	20.01
HLA-DRB3*01:01	1	15	MKYLGLALISAVFLI	Consensus	0.80	0.01	0.01	2333	0.80	2415.70	30.99
HLA-DRB3*01:01	2	16	KYLGLALISAVFLIG	Consensus	0.83	0.01	0.01	2389	0.83	2415.40	30.99
HLA-DRB3*01:01	3	17	YLGLALISAVFLIGA	Consensus	0.84	0.01	0.01	2399	0.84	2290.50	29.96
HLA-DRB3*01:01	4	18	LGLALISAVFLIGAC	Consensus	0.86	0.01	0.01	2417	0.86	2206	29.25
HLA-DRB3*01:01	50	64	GFIDVNYNIAQSEIR	Consensus	0.92	0.01	0.01	2507	0.92	2307.90	30.11
HLA-DRB3*01:01	49	63	YGFIDVNYNIAQSEI	Consensus	1.02	0.01	0.01	2686	1.02	1836.20	26.05
HLA-DRB3*01:01	5	19	GLALISAVFLIGACQ	Consensus	1.09	0.01	0.01	2781	1.09	2670.70	32.99
HLA-DRB3*01:01	6	20	LALISAVFLIGACQA	Consensus	4.69	0.01	0.01	7320	4.69	3420.60	38.49
HLA-DRB3*01:01	121	135	SKLIGPQRFDKAIQD	Consensus	5.07	0.04	5.07	1749	0.48	775.20	15.25
HLA-DRB3*01:01	122	136	KLIGPQRFDKAIQDY	Consensus	5.07	0.04	5.07	1740	0.47	630.90	13.48
HLA-DRB3*01:01	123	137	LIGPQRFDKAIQDYD	Consensus	5.07	0.04	5.07	1692	0.45	490.80	11.62
HLA-DRB3*01:01	124	138	IGPQRFDKAIQDYDK	Consensus	5.07	0.04	5.07	1713	0.46	485.30	11.55
HLA-DRB3*01:01	125	139	GPQRFDKAIQDYDKT	Consensus	5.07	0.04	5.07	1763	0.48	930.40	17.07
HLA-DRB3*01:01	126	140	PQRFDKAIQDYDKTI	Consensus	5.07	0.04	5.07	1389	0.29	902.90	16.76
HLA-DRB3*01:01	127	141	QRFDKAIQDYDKTIS	Consensus	5.07	0.04	5.07	1401	0.30	1205.70	20
HLA-DRB3*01:01	7	21	ALISAVFLIGACQAE	Consensus	5.13	0.01	0.01	7837	5.13	3747.90	40.70
HLA-DRB3*01:01	84	98	LKKVMHECAKQVKKE	Consensus	6.22	6150.66	47.19	561	0.03	169.90	6.22
HLA-DRB3*01:01	83	97	ELKKVMHECAKQVKK	Consensus	6.54	6150.66	47.19	561	0.03	184.30	6.54
HLA-DRB3*01:01	110	124	TSINYYRCVLTSKLI	Consensus	6.71	34825.06	57.13	3574	1.65	192.50	6.71
HLA-DRB3*01:01	82	96	RELKKVMHECAKQVK	Consensus	6.80	6150.66	47.19	554	0.03	196.90	6.80
HLA-DRB3*01:01	109	123	RTSINYYRCVLTSKL	Consensus	7.30	34825.06	57.13	3620	1.69	221.80	7.30
HLA-DRB3*01:01	81	95	KRELKKVMHECAKQV	Consensus	7.31	6150.66	47.19	547	0.03	222.20	7.31

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB5*01:01	110	124	TSINYYRCVLTSKLI	Consensus	1.10	146	1.10	11.10	2.68	3.60	0.95
HLA-DRB5*01:01	111	125	SINYYRCVLTSKLIG	Consensus	1.13	151	1.13	10.20	2.42	3.60	0.95
HLA-DRB5*01:01	50	64	GFIDVNYNIAQSEIR	Consensus	1.16	32	0.14	46.50	9.50	3.40	1.16
HLA-DRB5*01:01	51	65	FIDVNYNIAQSEIRK	Consensus	1.16	30	0.13	27.40	6.40	3.40	1.16
HLA-DRB5*01:01	52	66	IDVNYNIAQSEIRKF	Consensus	1.16	29	0.12	20.10	4.91	3.40	1.16
HLA-DRB5*01:01	53	67	DVNYNIAQSEIRKFS	Consensus	1.16	29	0.12	18.40	4.51	3.40	1.16
HLA-DRB5*01:01	54	68	VNYNIAQSEIRKFSN	Consensus	1.16	29	0.12	22.70	5.46	3.40	1.16
HLA-DRB5*01:01	55	69	NYNIAQSEIRKFSNV	Consensus	1.16	88	0.57	35.30	7.80	3.40	1.16
HLA-DRB5*01:01	56	70	YNIAQSEIRKFSNVL	Consensus	1.16	88	0.57	59.30	11.17	3.40	1.16
HLA-DRB5*01:01	109	123	RTSINYYRCVLTSKL	Consensus	1.19	158	1.19	16.80	4.14	3.60	0.95
HLA-DRB5*01:01	112	126	INYYRCVLTSKLIGP	Consensus	1.19	158	1.19	11.20	2.71	3.60	0.95
HLA-DRB5*01:01	113	127	NYYRCVLTSKLIGPQ	Consensus	1.35	172	1.35	14.80	3.66	3.60	0.95
HLA-DRB5*01:01	67	81	SNVLMDYGVVDKSKK	Consensus	1.54	145	1.08	135.50	18.05	3.10	1.54
HLA-DRB5*01:01	68	82	NVLMDYGVVDKSKKR	Consensus	1.54	96	0.64	94.90	14.85	3.10	1.54
HLA-DRB5*01:01	69	83	VLMDYGVVDKSKKRE	Consensus	1.54	96	0.64	99.90	15.29	3.10	1.54
HLA-DRB5*01:01	70	84	LMDYGVVDKSKKREL	Consensus	1.54	89	0.58	84.60	13.89	3.10	1.54
HLA-DRB5*01:01	71	85	MDYGVVDKSKKRELK	Consensus	1.54	90	0.59	107.60	15.94	3.10	1.54
HLA-DRB5*01:01	72	86	DYGVVDKSKKRELKK	Consensus	1.54	148	1.11	144.40	18.67	3.10	1.54
HLA-DRB5*01:01	73	87	YGVVDKSKKRELKKV	Consensus	2.11	261	2.11	180.20	20.90	3.10	1.54
HLA-DRB5*01:01	115	129	YRCVLTSKLIGPQRF	Consensus	2.84	341	2.84	32.10	7.24	3.60	1.05
HLA-DRB5*01:01	1	15	MKYLGLALISAVFLI	Consensus	3.19	145	1.08	815	40.07	2.30	3.19
HLA-DRB5*01:01	2	16	KYLGLALISAVFLIG	Consensus	3.19	149	1.11	1243.70	46.75	2.30	3.19
HLA-DRB5*01:01	3	17	YLGLALISAVFLIGA	Consensus	3.19	148	1.11	1466.10	49.47	2.30	3.19
HLA-DRB5*01:01	4	18	LGLALISAVFLIGAC	Consensus	3.19	150	1.12	2002.80	54.92	2.30	3.19
HLA-DRB5*01:01	114	128	YYRCVLTSKLIGPQR	Consensus	3.34	398	3.34	22	5.32	3.60	0.95
HLA-DRB5*01:01	5	19	GLALISAVFLIGACQ	Consensus	3.52	418	3.52	2988.80	62.19	2.30	3.19
HLA-DRB5*01:01	6	20	LALISAVFLIGACQA	Consensus	3.74	443	3.74	3499.50	65.11	2.30	3.19
HLA-DRB5*01:01	105	119	WLNCRTSINYYRCVL	Consensus	4.10	345	2.87	16.60	4.10	0.50	11.11

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB5*01:01	117	131	CVLTSKLIGPQRFDK	Consensus	5.05	596	5.02	195.50	21.74	1.70	5.05
HLA-DRB5*01:01	118	132	VLTSKLIGPQRFDKA	Consensus	5.29	627	5.29	231.50	23.60	1.70	5.05
HLA-DRB5*01:01	106	120	LNCRTSINYYRCVLT	Consensus	5.84	332	2.76	24.50	5.84	0.10	12.86
HLA-DRB5*01:01	119	133	LTSKLIGPQRFDKAI	Consensus	6.08	722	6.08	371.20	29.16	1.70	5.05
HLA-DRB5*01:01	108	122	CRTSINYYRCVLTSK	Consensus	6.41	758	6.41	44.30	9.20	3.60	0.95
HLA-DRB5*01:01	104	118	HWLNCRTSINYYRCV	Consensus	6.56	375	3.15	28.30	6.56	0.50	11.11

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB4*01:01	121	135	SKLIGPQRFDKAIQD	Consensus	3.89	63.54	27.31	337	2.44	52	3.89
HLA-DRB4*01:01	120	134	TSKLIGPQRFDKAIQ	Consensus	4.52	0.82	9.66	337	2.44	59.30	4.52
HLA-DRB4*01:01	1	15	MKYLGLALISAVFLI	Consensus	5.57	0.32	7.40	168	0.95	71.70	5.57
HLA-DRB4*01:01	119	133	LTSKLIGPQRFDKAI	Consensus	6.57	0.82	9.66	186	1.10	84.30	6.57
HLA-DRB4*01:01	2	16	KYLGLALISAVFLIG	Consensus	7.40	0.32	7.40	274	1.87	695.30	33.36
HLA-DRB4*01:01	3	17	YLGLALISAVFLIGA	Consensus	7.40	0.32	7.40	347	2.53	1312.70	46.34
HLA-DRB4*01:01	4	18	LGLALISAVFLIGAC	Consensus	7.40	0.32	7.40	353	2.60	1201.80	44.45
HLA-DRB4*01:01	5	19	GLALISAVFLIGACQ	Consensus	7.40	0.32	7.40	555	4.56	2243	58.42
HLA-DRB4*01:01	6	20	LALISAVFLIGACQA	Consensus	7.40	0.32	7.40	519	4.21	1687.10	51.89
HLA-DRB4*01:01	7	21	ALISAVFLIGACQAE	Consensus	7.40	0.32	7.40	694	5.90	1820.80	53.63

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*09:01	109	123	RTSINYYRCVLTSKL	Consensus	3.79	0.01	0.01	456	3.79	133	9.06
HLA-DRB1*09:01	53	67	DVNYNIAQSEIRKFS	Consensus	4.30	0.01	0.01	502	4.30	380.40	21.16
HLA-DRB1*09:01	52	66	IDVNYNIAQSEIRKF	Consensus	4.33	0.01	0.01	505	4.33	482.80	24.91
HLA-DRB1*09:01	50	64	GFIDVNYNIAQSEIR	Consensus	5.95	0.01	0.01	645	5.95	1158.20	42.16
HLA-DRB1*09:01	51	65	FIDVNYNIAQSEIRK	Consensus	6	0.01	0.01	649	6	853.70	35.57
HLA-DRB1*09:01	111	125	SINYYRCVLTSKLIG	Consensus	6.27	1000000	61.16	401	3.16	90.30	6.27
HLA-DRB1*09:01	110	124	TSINYYRCVLTSKLI	Consensus	6.35	1000000	61.16	381	2.93	91.50	6.35
HLA-DRB1*09:01	112	126	INYYRCVLTSKLIGP	Consensus	6.96	539046.91	59.78	410	3.25	100.50	6.96
HLA-DRB1*09:01	113	127	NYYRCVLTSKLIGPQ	Consensus	8.95	539046.91	59.78	429	3.46	131.40	8.95

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*13:02	50	64	GFIDVNYNIAQSEIR	Consensus	4.87	251	7.13	58.40	3.99	1.70	4.87
HLA-DRB1*13:02	47	61	QYYGFIDVNYNIAQS	Consensus	5.77	304	8.51	83.40	5.33	1.40	5.77
HLA-DRB1*13:02	48	62	YYGFIDVNYNIAQSE	Consensus	5.77	242	6.89	70.70	4.66	1.40	5.77
HLA-DRB1*13:02	49	63	YGFIDVNYNIAQSEI	Consensus	5.77	246	6.99	55.10	3.80	1.40	5.77
HLA-DRB1*13:02	51	65	FIDVNYNIAQSEIRK	Consensus	6.08	425	11.27	100.30	6.08	1.70	4.87
HLA-DRB1*13:02	46	60	YQYYGFIDVNYNIAQ	Consensus	6.41	305	8.53	107.80	6.41	1.40	5.77
HLA-DRB1*13:02	52	66	IDVNYNIAQSEIRKF	Consensus	6.63	414	11.02	113	6.63	1.70	4.87
HLA-DRB1*13:02	59	73	AQSEIRKFSNVLMDY	Consensus	6.87	317	8.82	118.80	6.87	2.40	2.29

Supplementary data 4 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NetMHCIIpan IC50 (nM)	NetMHCIIpan rank
HLA-DRB3*02:02	111	125	SINYYRCVLTSKLIG	NetMHCIIpan	1.64	-	-	90.56	1.64
HLA-DRB3*02:02	112	126	INYYRCVLTSKLIGP	NetMHCIIpan	1.74	-	-	94.29	1.74
HLA-DRB3*02:02	110	124	TSINYYRCVLTSKLI	NetMHCIIpan	2	-	-	103.23	2
HLA-DRB3*02:02	53	67	DVNYNIAQSEIRKFS	NetMHCIIpan	2.49	-	-	116.38	2.49
HLA-DRB3*02:02	54	68	VNYNIAQSEIRKFSN	NetMHCIIpan	2.59	-	-	118.98	2.59
HLA-DRB3*02:02	52	66	IDVNYNIAQSEIRKF	NetMHCIIpan	3.25	-	-	135.78	3.25
HLA-DRB3*02:02	113	127	NYYRCVLTSKLIGPQ	NetMHCIIpan	3.39	-	-	139.72	3.39
HLA-DRB3*02:02	55	69	NYNIAQSEIRKFSNV	NetMHCIIpan	4.14	-	-	159.43	4.14
HLA-DRB3*02:02	109	123	RTSINYYRCVLTSKL	NetMHCIIpan	4.47	-	-	165.58	4.47
HLA-DRB3*02:02	114	128	YYRCVLTSKLIGPQR	NetMHCIIpan	5.60	-	-	198.57	5.60
HLA-DRB3*02:02	51	65	FIDVNYNIAQSEIRK	NetMHCIIpan	7.21	-	-	233.24	7.21

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NetMHCIIpan IC50 (nM)	NetMHCIIpan rank
HLA-DPA1*02:01/DPB1*14:01	111	125	SINYYRCVLTSKLIG	NetMHCIIpan	5.54	-	-	32.89	5.54
HLA-DPA1*02:01/DPB1*14:01	110	124	TSINYYRCVLTSKLI	NetMHCIIpan	5.80	-	-	33.88	5.80
HLA-DPA1*02:01/DPB1*14:01	1	15	MKYLGLALISAVFLI	NetMHCIIpan	5.96	-	-	34.48	5.96
HLA-DPA1*02:01/DPB1*14:01	112	126	INYYRCVLTSKLIGP	NetMHCIIpan	5.96	-	-	34.52	5.96

Notes: The MHCII binding predictions were made on 11/18/2016 using the IEDB analysis resource Consensus tool. In terms of population coverage, the reference sets for class II should provide >99%. Results are presented by allele, and from lowest to highest percentile rank. Peptides predicted as good binders are all of those with a percentile ranking lower or equal to 10.

Supplementary data 5. PpSP15 in silico analysis: MHC-II restricted-epitopes prediction - Human (99% population coverage)

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*01/DPB1*04:01	3	17	YLGLALISAVFLIGT	Consensus	0.86	0.01	0.01	362	0.86	497.60	14.86
HLA-DPA1*01/DPB1*04:01	4	18	LGLALISAVFLIGTC	Consensus	0.90	0.01	0.01	370	0.90	654.10	17.30
HLA-DPA1*01/DPB1*04:01	5	19	GLALISAVFLIGTCQ	Consensus	0.94	0.01	0.01	377	0.94	722.60	18.27
HLA-DPA1*01/DPB1*04:01	2	16	KYLGLALISAVFLIG	Consensus	0.98	0.01	0.01	385	0.98	507.40	15.02
HLA-DPA1*01/DPB1*04:01	6	20	LALISAVFLIGTCQA	Consensus	1.04	0.01	0.01	397	1.04	559.10	15.86
HLA-DPA1*01/DPB1*04:01	41	55	IPHCKYQYYGFVAMD	Consensus	1.40	0.03	4.39	173	0.17	21.60	1.40
HLA-DPA1*01/DPB1*04:01	40	54	CIPHCKYQYYGFVAM	Consensus	1.43	0.03	4.39	173	0.17	22	1.43
HLA-DPA1*01/DPB1*04:01	43	57	HCKYQYYGFVAMDNN	Consensus	1.43	0.03	4.39	176	0.17	22	1.43
HLA-DPA1*01/DPB1*04:01	42	56	PHCKYQYYGFVAMDN	Consensus	1.46	0.03	4.39	174	0.17	22.40	1.46
HLA-DPA1*01/DPB1*04:01	44	58	CKYQYYGFVAMDNNI	Consensus	2.39	0.03	4.39	190	0.21	37.20	2.39
HLA-DPA1*01/DPB1*04:01	39	53	ACIPHCKYQYYGFVA	Consensus	4.39	0.03	4.39	2554	21.19	72.80	4.16
HLA-DPA1*01/DPB1*04:01	45	59	KYQYYGFVAMDNNIA	Consensus	4.39	0.03	4.39	496	1.60	101.80	5.37
HLA-DPA1*01/DPB1*04:01	7	21	ALISAVFLIGTCQAE	Consensus	5	0.01	0.01	932	5	1057	22.47

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*02:01/DPB1*01:01	61	75	PEIRTFSNVLIKYNV	Consensus	6.33	39.94	15.05	160	0.29	59.30	6.33
HLA-DPA1*02:01/DPB1*01:01	62	76	EIRTFSNVLIKYNVV	Consensus	6.41	39.94	15.05	185	0.38	60.10	6.41
HLA-DPA1*02:01/DPB1*01:01	60	74	KPEIRTFSNVLIKYN	Consensus	6.73	39.94	15.05	164	0.31	62.90	6.73

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*01:03/DPB1*02:01	3	17	YLGLALISAVFLIGT	Consensus	0.08	0.01	0.01	98	0.08	124.30	9.60
HLA-DPA1*01:03/DPB1*02:01	2	16	KYLGLALISAVFLIG	Consensus	0.09	0.01	0.01	99	0.09	133.20	10.03
HLA-DPA1*01:03/DPB1*02:01	4	18	LGLALISAVFLIGTC	Consensus	0.09	0.01	0.01	100	0.09	158.20	11.18
HLA-DPA1*01:03/DPB1*02:01	5	19	GLALISAVFLIGTCQ	Consensus	0.11	0.01	0.01	109	0.11	204.80	13.06
HLA-DPA1*01:03/DPB1*02:01	6	20	LALISAVFLIGTCQA	Consensus	0.12	0.01	0.01	115	0.12	183.70	12.23
HLA-DPA1*01:03/DPB1*02:01	7	21	ALISAVFLIGTCQAE	Consensus	0.68	0.01	0.01	324	0.68	161.50	11.32
HLA-DPA1*01:03/DPB1*02:01	1	15	MKYLGLALISAVFLI	Consensus	0.72	0.01	0.01	337	0.72	171.70	11.72
HLA-DPA1*01:03/DPB1*02:01	40	54	CIPHCKYQYYGFVAM	Consensus	2.53	16.53	19.82	120	0.13	21.30	2.53
HLA-DPA1*01:03/DPB1*02:01	41	55	IPHCKYQYYGFVAMD	Consensus	2.70	16.53	19.82	120	0.13	22.80	2.70
HLA-DPA1*01:03/DPB1*02:01	42	56	PHCKYQYYGFVAMDN	Consensus	2.77	16.53	19.82	120	0.13	23.50	2.77
HLA-DPA1*01:03/DPB1*02:01	43	57	HCKYQYYGFVAMDNN	Consensus	2.95	16.53	19.82	124	0.14	25.30	2.95
HLA-DPA1*01:03/DPB1*02:01	44	58	CKYQYYGFVAMDNNI	Consensus	4.11	35.48	23.42	126	0.14	37.40	4.11
HLA-DPA1*01:03/DPB1*02:01	59	73	AKPEIRTFSNVLIKY	Consensus	5.01	0.03	2.99	1061	5.01	412.60	19.40
HLA-DPA1*01:03/DPB1*02:01	58	72	IAKPEIRTFSNVLIK	Consensus	5.80	0.03	2.99	1198	5.80	1588.90	37.96
HLA-DPA1*01:03/DPB1*02:01	57	71	NIAKPEIRTFSNVLI	Consensus	6.07	0.03	2.99	1246	6.07	1779.90	39.94
HLA-DPA1*01:03/DPB1*02:01	39	53	ACIPHCKYQYYGFVA	Consensus	6.99	16.53	19.82	925	4.21	76.80	6.99

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*03:01/DQB1*03:02	NO PREDICTED BINDERS										

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*03:01	77	91	DKSLKADIRKIMHEC	Consensus	0.12	87	0.02	5.60	0.12	4.60	0.41
HLA-DRB1*03:01	76	90	VDKSLKADIRKIMHE	Consensus	0.19	87	0.02	6.70	0.19	4.60	0.41
HLA-DRB1*03:01	78	92	KSLKADIRKIMHECA	Consensus	0.32	87	0.02	8.60	0.32	4.60	0.41
HLA-DRB1*03:01	75	89	VVDKSLKADIRKIMH	Consensus	0.34	87	0.02	8.80	0.34	4.60	0.41
HLA-DRB1*03:01	74	88	NVVDKSLKADIRKIM	Consensus	0.41	87	0.02	13.80	0.69	4.60	0.41
HLA-DRB1*03:01	79	93	SLKADIRKIMHECAK	Consensus	0.41	260	0.14	17.10	0.95	4.60	0.41
HLA-DRB1*03:01	80	94	LKADIRKIMHECAKK	Consensus	0.41	262	0.14	46.40	2.70	4.60	0.41
HLA-DRB1*03:01	112	126	INYYRCILTDKRIGP	Consensus	0.53	326	0.20	99.90	5.02	4.40	0.53
HLA-DRB1*03:01	113	127	NYYRCILTDKRIGPQ	Consensus	0.53	324	0.20	65	3.56	4.40	0.53
HLA-DRB1*03:01	114	128	YYRCILTDKRIGPQR	Consensus	0.53	318	0.19	43.50	2.56	4.40	0.53
HLA-DRB1*03:01	115	129	YRCILTDKRIGPQRF	Consensus	0.53	310	0.18	29.30	1.71	4.40	0.53
HLA-DRB1*03:01	116	130	RCILTDKRIGPQRFD	Consensus	0.53	311	0.18	55.20	3.11	4.40	0.53
HLA-DRB1*03:01	68	82	NVLIKYNVVDKSLKA	Consensus	1.14	793	0.99	31.60	1.85	3.80	1.14
HLA-DRB1*03:01	69	83	VLIKYNVVDKSLKAD	Consensus	1.14	802	1	50	2.86	3.80	1.14
HLA-DRB1*03:01	70	84	LIKYNVVDKSLKADI	Consensus	1.14	800	1	61.20	3.37	3.80	1.14
HLA-DRB1*03:01	71	85	IKYNVVDKSLKADIR	Consensus	1.14	789	0.98	89.50	4.59	3.80	1.14
HLA-DRB1*03:01	118	132	ILTDKRIGPQRFDRA	Consensus	1.22	922	1.22	311.40	10.58	4.40	0.53
HLA-DRB1*03:01	117	131	CILTDKRIGPQRFDR	Consensus	1.25	941	1.25	122.80	5.78	4.40	0.53
HLA-DRB1*03:01	67	81	SNVLIKYNVVDKSLK	Consensus	1.35	995	1.35	44.90	2.63	3.80	1.14
HLA-DRB1*03:01	66	80	FSNVLIKYNVVDKSL	Consensus	1.38	1014	1.38	82.20	4.29	3.80	1.14
HLA-DRB1*03:01	65	79	TFSNVLIKYNVVDKS	Consensus	1.48	1071	1.48	203.30	8.11	3.80	1.14
HLA-DRB1*03:01	46	60	YQYYGFVAMDNNIAK	Consensus	2.18	365	0.26	236.50	8.92	3.30	2.18
HLA-DRB1*03:01	47	61	QYYGFVAMDNNIAKP	Consensus	2.18	383	0.29	158.90	6.93	3.30	2.18
HLA-DRB1*03:01	48	62	YYGFVAMDNNIAKPE	Consensus	2.18	381	0.28	98.70	4.96	3.30	2.18
HLA-DRB1*03:01	49	63	YGFVAMDNNIAKPEI	Consensus	2.18	364	0.26	62.30	3.43	3.30	2.18

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*03:01	50	64	GFVAMDNNIAKPEIR	Consensus	2.18	371	0.27	128.70	5.97	3.30	2.18
HLA-DRB1*03:01	51	65	FVAMDNNIAKPEIRT	Consensus	2.18	1065	1.47	280.10	9.90	3.30	2.18
HLA-DRB1*03:01	52	66	VAMDNNIAKPEIRTF	Consensus	2.18	1103	1.54	710.90	17.11	3.30	2.18
HLA-DRB1*03:01	1	15	MKYLGLALISAVFLI	Consensus	3.67	2000	3.27	4118.50	41.50	2.70	3.67
HLA-DRB1*03:01	2	16	KYLGLALISAVFLIG	Consensus	4.29	1930	3.14	4483.20	43.17	2.57	4.29
HLA-DRB1*03:01	3	17	YLGLALISAVFLIGT	Consensus	4.29	2191	3.70	4627.30	43.74	2.57	4.29
HLA-DRB1*03:01	4	18	LGLALISAVFLIGTC	Consensus	4.29	2188	3.69	4153.70	41.67	2.57	4.29
HLA-DRB1*03:01	81	95	KADIRKIMHECAKKV	Consensus	4.77	1629	2.56	3800	39.92	2.40	4.77
HLA-DRB1*03:01	82	96	ADIRKIMHECAKKVK	Consensus	4.77	1583	2.47	3213.90	36.79	2.40	4.77
HLA-DRB1*03:01	83	97	DIRKIMHECAKKVKK	Consensus	4.77	1553	2.41	2323.80	31.46	2.40	4.77
HLA-DRB1*03:01	84	98	IRKIMHECAKKVKKQ	Consensus	4.77	1541	2.39	1568.80	26.08	2.40	4.77
HLA-DRB1*03:01	109	123	RTTINYYRCILTDKR	Consensus	6	3295	6	1267.90	23.41	2.40	4.77
HLA-DRB1*03:01	110	124	TTINYYRCILTDKRI	Consensus	6.31	3438	6.31	2054.40	29.68	2.40	4.77
HLA-DRB1*03:01	6	20	LALISAVFLIGTCQA	Consensus	7.12	3822	7.12	7119.10	52.92	3.10	2.66
HLA-DRB1*03:01	25	39	KKCEEKFKNDASKMA	Consensus	7.21	678	0.77	888.40	19.35	1.90	7.21
HLA-DRB1*03:01	26	40	KCEEKFKNDASKMAC	Consensus	7.21	680	0.78	412	12.57	1.90	7.21
HLA-DRB1*03:01	27	41	CEEKFKNDASKMACI	Consensus	7.21	649	0.72	189.50	7.77	1.90	7.21
HLA-DRB1*03:01	28	42	EEKFKNDASKMACIP	Consensus	7.21	680	0.78	173.30	7.34	1.90	7.21
HLA-DRB1*03:01	29	43	EKFKNDASKMACIPH	Consensus	7.21	689	0.79	351.40	11.39	1.90	7.21
HLA-DRB1*03:01	30	44	KFKNDASKMACIPHC	Consensus	7.21	2058	3.40	760.60	17.76	1.90	7.21
HLA-DRB1*03:01	31	45	FKNDASKMACIPHCK	Consensus	7.21	2096	3.49	1955.40	28.98	1.90	7.21
HLA-DRB1*03:01	73	87	YNVVDKSLKADIRKI	Consensus	7.47	972	1.31	178.20	7.47	1.47	10.09
HLA-DRB1*03:01	93	107	KKVKKQAREDSHWLN	Consensus	7.48	4001	7.48	12419.40	66.42	3	2.71

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*02:01/DPB1*05:01	1	15	MKYLGLALISAVFLI	Consensus	4.34	0.59	2.39	721	4.34	852	15.82
HLA-DPA1*02:01/DPB1*05:01	2	16	KYLGLALISAVFLIG	Consensus	4.64	0.59	2.39	755	4.64	1591.40	24.43
HLA-DPA1*02:01/DPB1*05:01	61	75	PEIRTFSNVLIKYNV	Consensus	5.93	180.27	23.87	900	5.93	101.10	2.03
HLA-DPA1*02:01/DPB1*05:01	60	74	KPEIRTFSNVLIKYN	Consensus	6.14	180.27	23.87	923	6.14	134	2.85
HLA-DPA1*02:01/DPB1*05:01	62	76	EIRTFSNVLIKYNVV	Consensus	6.39	180.27	23.87	950	6.39	84.50	1.61
HLA-DPA1*02:01/DPB1*05:01	63	77	IRTFSNVLIKYNVVD	Consensus	6.66	180.27	23.87	979	6.66	121.60	2.54

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	NetMHCIIpan IC50 (nM)	NetMHCIIpan rank
HLA-DPA1*02:01/DPB1*14:01	1	15	MKYLGLALISAVFLI	NetMHCIIpan	4.67	-	-	-	-	29.21	4.67
HLA-DPA1*02:01/DPB1*14:01	60	74	KPEIRTFSNVLIKYN	NetMHCIIpan	6.12	-	-	-	-	35.06	6.12
HLA-DPA1*02:01/DPB1*14:01	61	75	PEIRTFSNVLIKYNV	NetMHCIIpan	6.12	-	-	-	-	35.10	6.12
HLA-DPA1*02:01/DPB1*14:01	62	76	EIRTFSNVLIKYNVV	NetMHCIIpan	6.32	-	-	-	-	36.09	6.32
HLA-DPA1*02:01/DPB1*14:01	45	59	KYQYYGFVAMDNNIA	NetMHCIIpan	6.45	-	-	-	-	36.53	6.45
HLA-DPA1*02:01/DPB1*14:01	59	73	AKPEIRTFSNVLIKY	NetMHCIIpan	7.02	-	-	-	-	39.74	7.02
HLA-DPA1*02:01/DPB1*14:01	46	60	YQYYGFVAMDNNIAK	NetMHCIIpan	7.27	-	-	-	-	40.82	7.27
HLA-DPA1*02:01/DPB1*14:01	44	58	CKYQYYGFVAMDNNI	NetMHCIIpan	7.32	-	-	-	-	40.93	7.32

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*05:01/DQB1*03:01	1	15	MKYLGLALISAVFLI	Consensus	7.29	0.14	3.39	43	14.09	39.10	7.29

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:01	47	61	QYYGFVAMDNNIAKP	Consensus	0.07	49	0.33	6.70	0.06	4.90	0.07
HLA-DRB1*04:01	45	59	KYQYYGFVAMDNNIA	Consensus	0.07	49	0.33	7.10	0.07	4.90	0.07
HLA-DRB1*04:01	46	60	YQYYGFVAMDNNIAK	Consensus	0.07	47	0.31	7	0.07	4.90	0.07
HLA-DRB1*04:01	48	62	YYGFVAMDNNIAKPE	Consensus	0.08	48	0.32	7.40	0.08	4.90	0.07
HLA-DRB1*04:01	49	63	YGFVAMDNNIAKPEI	Consensus	0.18	49	0.33	9.60	0.18	4.90	0.07
HLA-DRB1*04:01	50	64	GFVAMDNNIAKPEIR	Consensus	0.41	132	1.25	13.20	0.41	4.90	0.07
HLA-DRB1*04:01	27	41	CEEKFKNDASKMACI	Consensus	0.73	87	0.73	55.20	4.30	3.70	0.33
HLA-DRB1*04:01	25	39	KKCEEKFKNDASKMA	Consensus	0.74	88	0.74	152.30	11.95	3.70	0.33
HLA-DRB1*04:01	28	42	EEKFKNDASKMACIP	Consensus	0.74	88	0.74	43.80	3.21	3.70	0.33
HLA-DRB1*04:01	26	40	KCEEKFKNDASKMAC	Consensus	0.76	90	0.76	91.40	7.42	3.70	0.33
HLA-DRB1*04:01	29	43	EKFKNDASKMACIPH	Consensus	0.79	93	0.79	51.50	3.96	3.70	0.33
HLA-DRB1*04:01	51	65	FVAMDNNIAKPEIRT	Consensus	1.44	150	1.44	31.50	2.05	4.90	0.07
HLA-DRB1*04:01	30	44	KFKNDASKMACIPHC	Consensus	2.80	269	2.80	69.80	5.61	3.70	0.33
HLA-DRB1*04:01	31	45	FKNDASKMACIPHCK	Consensus	3.14	299	3.14	219.10	16.11	3.70	0.33
HLA-DRB1*04:01	57	71	NIAKPEIRTFSNVLI	Consensus	3.80	110	0.98	245.10	17.55	1.38	3.80
HLA-DRB1*04:01	58	72	IAKPEIRTFSNVLIK	Consensus	3.80	106	0.94	177.50	13.59	1.38	3.80
HLA-DRB1*04:01	59	73	AKPEIRTFSNVLIKY	Consensus	3.80	107	0.95	126.80	10.15	1.38	3.80
HLA-DRB1*04:01	60	74	KPEIRTFSNVLIKYN	Consensus	3.80	106	0.94	99.60	8.08	1.38	3.80
HLA-DRB1*04:01	61	75	PEIRTFSNVLIKYNV	Consensus	3.80	111	0.99	120.80	9.71	1.38	3.80
HLA-DRB1*04:01	62	76	EIRTFSNVLIKYNVV	Consensus	3.80	311	3.27	124.90	10.01	1.38	3.80
HLA-DRB1*04:01	63	77	IRTFSNVLIKYNVVD	Consensus	3.80	316	3.33	180.20	13.77	1.38	3.80
HLA-DRB1*04:01	1	15	MKYLGLALISAVFLI	Consensus	5.14	171	1.70	130.50	10.43	0.98	5.14
HLA-DRB1*04:01	2	16	KYLGLALISAVFLIG	Consensus	5.14	199	2.01	221.60	16.26	0.98	5.14
HLA-DRB1*04:01	3	17	YLGLALISAVFLIGT	Consensus	5.14	211	2.14	506.50	29.06	0.98	5.14
HLA-DRB1*04:01	44	58	CKYQYYGFVAMDNNI	Consensus	5.41	494	5.41	21.90	1.15	-0.40	11.01

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:01	43	57	HCKYQYYGFVAMDNN	Consensus	5.55	507	5.55	39.60	2.82	-0.40	11.01
HLA-DRB1*04:01	42	56	PHCKYQYYGFVAMDN	Consensus	5.71	521	5.71	57.90	4.55	-0.40	11.01
HLA-DRB1*04:01	68	82	NVLIKYNVVDKSLKA	Consensus	7.02	645	7.02	529.80	29.90	3.10	0.70
HLA-DRB1*04:01	82	96	ADIRKIMHECAKKVK	Consensus	7.13	656	7.13	543	30.35	0.80	5.48
HLA-DRB1*04:01	6	20	LALISAVFLIGTCQA	Consensus	7.20	662	7.20	1872.60	58.02	3.50	0.42
HLA-DRB1*04:01	84	98	IRKIMHECAKKVKKQ	Consensus	7.22	664	7.22	326.70	21.69	0.80	5.48
HLA-DRB1*04:01	81	95	KADIRKIMHECAKKV	Consensus	7.28	670	7.28	667.40	34.30	0.80	5.48
HLA-DRB1*04:01	83	97	DIRKIMHECAKKVKK	Consensus	7.33	674	7.33	384.30	24.27	0.80	5.48

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*03:01/DPB1*04:02	1	15	MKYLGLALISAVFLI	Consensus	3.03	0.09	3.03	229	0.35	117.30	10.73
HLA-DPA1*03:01/DPB1*04:02	2	16	KYLGLALISAVFLIG	Consensus	3.03	0.09	3.03	124	0.11	134.40	11.69
HLA-DPA1*03:01/DPB1*04:02	3	17	YLGLALISAVFLIGT	Consensus	3.03	0.09	3.03	113	0.09	99.20	9.61
HLA-DPA1*03:01/DPB1*04:02	4	18	LGLALISAVFLIGTC	Consensus	4.11	0.20	4.11	114	0.09	127.30	11.30
HLA-DPA1*03:01/DPB1*04:02	5	19	GLALISAVFLIGTCQ	Consensus	4.11	0.20	4.11	118	0.10	136.70	11.81
HLA-DPA1*03:01/DPB1*04:02	6	20	LALISAVFLIGTCQA	Consensus	4.11	0.20	4.11	120	0.11	149.80	12.49
HLA-DPA1*03:01/DPB1*04:02	7	21	ALISAVFLIGTCQAE	Consensus	4.11	0.20	4.11	256	0.42	236	16.19
HLA-DPA1*03:01/DPB1*04:02	8	22	LISAVFLIGTCQAEN	Consensus	4.11	0.20	4.11	343	0.71	342.60	19.67
HLA-DPA1*03:01/DPB1*04:02	9	23	ISAVFLIGTCQAENP	Consensus	4.11	0.20	4.11	804	3.30	444.30	22.35

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:05	45	59	KYQYYGFVAMDNNIA	Consensus	0.54	45	0.54	19	1.17	5.90	0.04
HLA-DRB1*04:05	46	60	YQYYGFVAMDNNIAK	Consensus	0.54	45	0.54	17.60	1	5.90	0.04
HLA-DRB1*04:05	47	61	QYYGFVAMDNNIAKP	Consensus	0.93	73	0.93	20.20	1.30	5.90	0.04
HLA-DRB1*04:05	48	62	YYGFVAMDNNIAKPE	Consensus	0.93	73	0.93	21.60	1.47	5.90	0.04
HLA-DRB1*04:05	49	63	YGFVAMDNNIAKPEI	Consensus	1.30	100	1.30	35	3.11	5.90	0.04
HLA-DRB1*04:05	44	58	CKYQYYGFVAMDNNI	Consensus	1.32	101	1.32	39.50	3.65	3.40	0.82
HLA-DRB1*04:05	43	57	HCKYQYYGFVAMDNN	Consensus	1.40	106	1.40	52.60	5.14	3.40	0.82
HLA-DRB1*04:05	42	56	PHCKYQYYGFVAMDN	Consensus	1.41	107	1.41	64.10	6.32	3.40	0.82
HLA-DRB1*04:05	60	74	KPEIRTFSNVLIKYN	Consensus	2.30	162	2.30	61.80	6.09	3.30	0.94
HLA-DRB1*04:05	61	75	PEIRTFSNVLIKYNV	Consensus	2.41	168	2.41	61.50	6.06	3.30	0.94
HLA-DRB1*04:05	63	77	IRTFSNVLIKYNVVD	Consensus	3.08	211	3.08	74.20	7.30	3.30	0.94
HLA-DRB1*04:05	62	76	EIRTFSNVLIKYNVV	Consensus	3.11	213	3.11	64.70	6.37	3.30	0.94
HLA-DRB1*04:05	64	78	RTFSNVLIKYNVVDK	Consensus	3.23	220	3.23	88.60	8.65	2.20	2.62
HLA-DRB1*04:05	58	72	IAKPEIRTFSNVLIK	Consensus	4.22	289	4.22	121.70	11.36	3.30	0.94
HLA-DRB1*04:05	59	73	AKPEIRTFSNVLIKY	Consensus	4.22	289	4.22	134.30	12.30	3.30	0.94
HLA-DRB1*04:05	57	71	NIAKPEIRTFSNVLI	Consensus	4.25	291	4.25	116.20	10.93	3.30	0.94
HLA-DRB1*04:05	50	64	GFVAMDNNIAKPEIR	Consensus	4.30	295	4.30	109.50	10.40	5.90	0.04
HLA-DRB1*04:05	51	65	FVAMDNNIAKPEIRT	Consensus	4.39	301	4.39	255.70	19.59	5.90	0.04
HLA-DRB1*04:05	1	15	MKYLGLALISAVFLI	Consensus	5.88	192	2.77	131.80	12.12	1.18	5.88
HLA-DRB1*04:05	2	16	KYLGLALISAVFLIG	Consensus	5.88	231	3.41	183.60	15.59	1.18	5.88
HLA-DRB1*04:05	3	17	YLGLALISAVFLIGT	Consensus	5.88	359	5.29	305.60	21.99	1.18	5.88
HLA-DRB1*04:05	4	18	LGLALISAVFLIGTC	Consensus	6.32	406	5.97	529.10	30.19	1	6.32
HLA-DRB1*04:05	110	124	TTINYYRCILTDKRI	Consensus	6.74	459	6.74	85.50	8.38	3	1.26

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:05	109	123	RTTINYYRCILTDKR	Consensus	6.85	467	6.85	114.50	10.79	3	1.26
HLA-DRB1*04:05	8	22	LISAVFLIGTCQAEN	Consensus	7.45	510	7.45	307.90	22.09	3.80	0.54

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*01:01/DQB1*05:01	44	58	CKYQYYGFVAMDNNI	Consensus	5.40	35.31	17.40	2279	2.08	258.90	5.40
HLA-DQA1*01:01/DQB1*05:01	45	59	KYQYYGFVAMDNNIA	Consensus	5.97	35.31	17.40	3026	3.52	292.10	5.97
HLA-DQA1*01:01/DQB1*05:01	43	57	HCKYQYYGFVAMDNN	Consensus	6.52	35.31	17.40	2298	2.12	325.50	6.52
HLA-DQA1*01:01/DQB1*05:01	46	60	YQYYGFVAMDNNIAK	Consensus	6.62	35.31	17.40	3307	4.14	332.50	6.62
HLA-DQA1*01:01/DQB1*05:01	1	15	MKYLGLALISAVFLI	Consensus	7.05	2.18	7.05	2508	2.50	1277.10	17.45
HLA-DQA1*01:01/DQB1*05:01	2	16	KYLGLALISAVFLIG	Consensus	7.05	2.18	7.05	3948	5.63	2530.80	26.63
HLA-DQA1*01:01/DQB1*05:01	42	56	PHCKYQYYGFVAMDN	Consensus	7.17	35.31	17.40	2318	2.16	368.10	7.17

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*01:02/DQB1*06:02	3	17	YLGLALISAVFLIGT	Consensus	2.37	0.02	2.37	122	1.77	310.20	19.79
HLA-DQA1*01:02/DQB1*06:02	4	18	LGLALISAVFLIGTC	Consensus	3.28	0.03	3.28	124	1.82	352	21.65
HLA-DQA1*01:02/DQB1*06:02	5	19	GLALISAVFLIGTCQ	Consensus	3.28	0.03	3.28	132	2.01	452.40	25.63
HLA-DQA1*01:02/DQB1*06:02	1	15	MKYLGLALISAVFLI	Consensus	5.01	0.02	2.37	244	5.01	274.70	18.07

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*08:02	67	81	SNVLIKYNVVDKSLK	Consensus	1.41	719	1.41	210.90	4.67	2.10	1.30
HLA-DRB1*08:02	68	82	NVLIKYNVVDKSLKA	Consensus	1.43	724	1.43	146.20	2.89	2.10	1.30
HLA-DRB1*08:02	65	79	TFSNVLIKYNVVDKS	Consensus	1.56	769	1.56	251.80	5.79	2.10	1.30
HLA-DRB1*08:02	66	80	FSNVLIKYNVVDKSL	Consensus	1.56	768	1.56	277.30	6.44	2.10	1.30
HLA-DRB1*08:02	64	78	RTFSNVLIKYNVVDK	Consensus	1.96	895	1.96	410.40	9.94	2.10	1.30
HLA-DRB1*08:02	1	15	MKYLGLALISAVFLI	Consensus	2.29	871	1.88	1859.60	34.59	1.50	2.29
HLA-DRB1*08:02	46	60	YQYYGFVAMDNNIAK	Consensus	2.29	775	1.58	1256.70	26.56	1.60	2.29
HLA-DRB1*08:02	47	61	QYYGFVAMDNNIAKP	Consensus	2.29	779	1.59	881.10	20.30	1.60	2.29
HLA-DRB1*08:02	48	62	YYGFVAMDNNIAKPE	Consensus	2.29	775	1.58	720.10	17.06	1.60	2.29
HLA-DRB1*08:02	49	63	YGFVAMDNNIAKPEI	Consensus	2.29	764	1.55	741.50	17.51	1.60	2.29
HLA-DRB1*08:02	3	17	YLGLALISAVFLIGT	Consensus	2.73	1099	2.65	2418.60	40.61	1.30	2.73
HLA-DRB1*08:02	69	83	VLIKYNVVDKSLKAD	Consensus	2.74	1126	2.74	180.30	3.84	2.10	1.30
HLA-DRB1*08:02	45	59	KYQYYGFVAMDNNIA	Consensus	2.89	1170	2.89	2334.30	39.75	1.60	2.29
HLA-DRB1*08:02	2	16	KYLGLALISAVFLIG	Consensus	3.06	1221	3.06	2379.90	40.21	1.30	2.73
HLA-DRB1*08:02	50	64	GFVAMDNNIAKPEIR	Consensus	3.82	1440	3.82	885.10	20.36	1.60	2.29
HLA-DRB1*08:02	70	84	LIKYNVVDKSLKADI	Consensus	3.86	1449	3.86	185.40	3.96	2.10	1.30
HLA-DRB1*08:02	71	85	IKYNVVDKSLKADIR	Consensus	4.95	1767	4.95	252.20	5.80	1.30	2.73
HLA-DRB1*08:02	6	20	LALISAVFLIGTCQA	Consensus	5.55	1931	5.55	2690.90	43.22	1.90	1.57
HLA-DRB1*08:02	63	77	IRTFSNVLIKYNVVD	Consensus	5.86	604	1.08	392.30	9.49	0.40	5.86
HLA-DRB1*08:02	51	65	FVAMDNNIAKPEIRT	Consensus	6.28	2133	6.28	2428.20	40.71	1.60	2.29
HLA-DRB1*08:02	62	76	EIRTFSNVLIKYNVV	Consensus	6.34	606	1.09	273.60	6.34	-0.30	8.66
HLA-DRB1*08:02	61	75	PEIRTFSNVLIKYNV	Consensus	6.55	271	0.28	281.50	6.55	-0.30	8.66
HLA-DRB1*08:02	7	21	ALISAVFLIGTCQAE	Consensus	6.86	2285	6.86	2405.80	40.48	1.90	1.57
HLA-DRB1*08:02	60	74	KPEIRTFSNVLIKYN	Consensus	7.09	251	0.25	299.90	7.09	-0.30	8.66

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*11:01	1	15	MKYLGLALISAVFLI	Consensus	0.49	274	0.49	194.90	20.04	3.30	0.43
HLA-DRB1*11:01	2	16	KYLGLALISAVFLIG	Consensus	0.71	340	0.71	345.60	26.41	3.30	0.43
HLA-DRB1*11:01	3	17	YLGLALISAVFLIGT	Consensus	0.71	340	0.71	1129.80	42.61	3.30	0.43
HLA-DRB1*11:01	81	95	KADIRKIMHECAKKV	Consensus	0.99	355	0.76	8.60	0.99	0.70	5.43
HLA-DRB1*11:01	82	96	ADIRKIMHECAKKVK	Consensus	1.42	246	0.42	10.40	1.42	0.70	5.43
HLA-DRB1*11:01	83	97	DIRKIMHECAKKVKK	Consensus	1.91	300	0.58	12.50	1.91	0.70	5.43
HLA-DRB1*11:01	80	94	LKADIRKIMHECAKK	Consensus	2.17	368	0.81	13.60	2.17	0.70	5.43
HLA-DRB1*11:01	84	98	IRKIMHECAKKVKKQ	Consensus	2.28	297	0.56	14.10	2.28	0.70	5.43
HLA-DRB1*11:01	6	20	LALISAVFLIGTCQA	Consensus	2.88	859	2.88	2396.20	54.98	2.50	1.08
HLA-DRB1*11:01	45	59	KYQYYGFVAMDNNIA	Consensus	2.90	357	0.77	313.60	25.29	1.50	2.90
HLA-DRB1*11:01	46	60	YQYYGFVAMDNNIAK	Consensus	2.90	345	0.73	170.30	18.63	1.50	2.90
HLA-DRB1*11:01	47	61	QYYGFVAMDNNIAKP	Consensus	2.90	363	0.79	116.90	15.08	1.50	2.90
HLA-DRB1*11:01	48	62	YYGFVAMDNNIAKPE	Consensus	2.90	359	0.78	84.20	12.33	1.50	2.90
HLA-DRB1*11:01	49	63	YGFVAMDNNIAKPEI	Consensus	2.90	357	0.77	141.10	16.82	1.50	2.90
HLA-DRB1*11:01	67	81	SNVLIKYNVVDKSLK	Consensus	3.13	220	0.36	154.30	17.67	1.40	3.13
HLA-DRB1*11:01	68	82	NVLIKYNVVDKSLKA	Consensus	3.13	215	0.35	84.70	12.38	1.40	3.13
HLA-DRB1*11:01	69	83	VLIKYNVVDKSLKAD	Consensus	3.13	218	0.35	84.90	12.40	1.40	3.13
HLA-DRB1*11:01	70	84	LIKYNVVDKSLKADI	Consensus	3.13	208	0.33	66.50	10.50	1.40	3.13
HLA-DRB1*11:01	71	85	IKYNVVDKSLKADIR	Consensus	3.13	217	0.35	99.60	13.69	1.40	3.13
HLA-DRB1*11:01	72	86	KYNVVDKSLKADIRK	Consensus	3.13	509	1.36	162.10	18.15	1.40	3.13
HLA-DRB1*11:01	73	87	YNVVDKSLKADIRKI	Consensus	3.13	521	1.41	261.30	23.19	1.40	3.13
HLA-DRB1*11:01	50	64	GFVAMDNNIAKPEIR	Consensus	3.40	972	3.40	270	23.55	1.50	2.90
HLA-DRB1*11:01	51	65	FVAMDNNIAKPEIRT	Consensus	3.72	1040	3.72	550.20	32.31	1.50	2.90
HLA-DRB1*11:01	114	128	YYRCILTDKRIGPQR	Consensus	3.80	367	0.80	21.10	3.80	0.20	7.59

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*11:01	79	93	SLKADIRKIMHECAK	Consensus	4.36	357	0.77	23.80	4.36	0.70	5.43
HLA-DRB1*11:01	115	129	YRCILTDKRIGPQRF	Consensus	4.81	388	0.88	26.10	4.81	0.20	7.59
HLA-DRB1*11:01	78	92	KSLKADIRKIMHECA	Consensus	5.43	182	0.27	29.70	5.46	0.70	5.43
HLA-DRB1*11:01	63	77	IRTFSNVLIKYNVVD	Consensus	5.43	231	0.38	29.50	5.43	0.10	8.05
HLA-DRB1*11:01	113	127	NYYRCILTDKRIGPQ	Consensus	6.52	277	0.50	36.20	6.52	0.20	7.59
HLA-DRB1*11:01	62	76	EIRTFSNVLIKYNVV	Consensus	6.73	227	0.37	37.50	6.73	0.10	8.05
HLA-DRB1*11:01	7	21	ALISAVFLIGTCQAE	Consensus	6.82	1650	6.82	1997.30	51.88	2.50	1.08
HLA-DRB1*11:01	116	130	RCILTDKRIGPQRFD	Consensus	6.88	629	1.83	38.40	6.88	-1.20	15.55

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*04:01/DQB1*04:02	1	15	MKYLGLALISAVFLI	Consensus	0.01	0.01	0.01	294	0.01	692.50	11.04
HLA-DQA1*04:01/DQB1*04:02	2	16	KYLGLALISAVFLIG	Consensus	0.01	0.01	0.01	299	0.01	905.70	14.40
HLA-DQA1*04:01/DQB1*04:02	3	17	YLGLALISAVFLIGT	Consensus	0.07	0.01	0.01	633	0.07	1037.60	16.36
HLA-DQA1*04:01/DQB1*04:02	4	18	LGLALISAVFLIGTC	Consensus	0.08	0.01	0.01	662	0.08	1546.20	23.27
HLA-DQA1*04:01/DQB1*04:02	7	21	ALISAVFLIGTCQAE	Consensus	0.52	0.01	0.01	1322	0.52	702.90	11.21
HLA-DQA1*04:01/DQB1*04:02	5	19	GLALISAVFLIGTCQ	Consensus	0.89	0.01	0.01	1663	0.89	3689.80	44.09
HLA-DQA1*04:01/DQB1*04:02	6	20	LALISAVFLIGTCQA	Consensus	3.75	0.01	0.01	3183	3.75	2475.70	33.66
HLA-DQA1*04:01/DQB1*04:02	42	56	PHCKYQYYGFVAMDN	Consensus	6.84	22495.02	56.75	2150	1.61	443.70	6.84

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*15:01	59	73	AKPEIRTFSNVLIKY	Consensus	0.81	74	0.81	21.60	1.72	6.30	0.04
HLA-DRB1*15:01	60	74	KPEIRTFSNVLIKYN	Consensus	0.83	75	0.83	22.60	1.84	6.30	0.04
HLA-DRB1*15:01	58	72	IAKPEIRTFSNVLIK	Consensus	0.85	76	0.85	28.90	2.61	6.30	0.04
HLA-DRB1*15:01	57	71	NIAKPEIRTFSNVLI	Consensus	0.86	77	0.86	37.10	3.59	6.30	0.04
HLA-DRB1*15:01	61	75	PEIRTFSNVLIKYNV	Consensus	1.01	86	1.01	28.20	2.52	6.30	0.04
HLA-DRB1*15:01	109	123	RTTINYYRCILTDKR	Consensus	2.48	162	2.48	579.70	31.31	5.40	0.23
HLA-DRB1*15:01	62	76	EIRTFSNVLIKYNVV	Consensus	2.50	163	2.50	32.80	3.08	6.30	0.04
HLA-DRB1*15:01	106	120	LNCRTTINYYRCILT	Consensus	2.50	163	2.50	166.30	14.88	5.40	0.23
HLA-DRB1*15:01	110	124	TTINYYRCILTDKRI	Consensus	2.58	167	2.58	438.70	27.15	5.40	0.23
HLA-DRB1*15:01	63	77	IRTFSNVLIKYNVVD	Consensus	2.60	168	2.60	126.10	12.06	6.30	0.04
HLA-DRB1*15:01	1	15	MKYLGLALISAVFLI	Consensus	2.61	82	0.94	193.10	16.57	3.30	2.61
HLA-DRB1*15:01	108	122	CRTTINYYRCILTDK	Consensus	2.64	170	2.64	504.50	29.19	5.40	0.23
HLA-DRB1*15:01	107	121	NCRTTINYYRCILTD	Consensus	2.71	173	2.71	205.70	17.27	5.40	0.23
HLA-DRB1*15:01	2	16	KYLGLALISAVFLIG	Consensus	5.61	87	1.02	272	20.66	2.40	5.61
HLA-DRB1*15:01	3	17	YLGLALISAVFLIGT	Consensus	5.61	87	1.02	349.30	23.97	2.40	5.61
HLA-DRB1*15:01	4	18	LGLALISAVFLIGTC	Consensus	5.61	90	1.08	377.90	25.06	2.40	5.61
HLA-DRB1*15:01	5	19	GLALISAVFLIGTCQ	Consensus	5.61	254	4.55	499.40	29.03	2.40	5.61
HLA-DRB1*15:01	6	20	LALISAVFLIGTCQA	Consensus	5.61	276	5.07	1920	51.63	2.40	5.61
HLA-DRB1*15:01	45	59	KYQYYGFVAMDNNIA	Consensus	5.84	311	5.84	184.20	16.02	3.58	2.13
HLA-DRB1*15:01	46	60	YQYYGFVAMDNNIAK	Consensus	6.22	328	6.22	532.60	30.01	3.58	2.13
HLA-DRB1*15:01	47	61	QYYGFVAMDNNIAKP	Consensus	7.14	371	7.14	529.70	29.92	3.50	2.16
HLA-DRB1*15:01	48	62	YYGFVAMDNNIAKPE	Consensus	7.22	375	7.22	587.40	31.51	3.50	2.16
HLA-DRB1*15:01	49	63	YGFVAMDNNIAKPEI	Consensus	7.43	386	7.43	736.10	35.08	3.50	2.16

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB3*01:01	28	42	EEKFKNDASKMACIP	Consensus	0.39	875.22	36.53	48	0.01	8.50	0.39
HLA-DRB3*01:01	27	41	CEEKFKNDASKMACI	Consensus	0.44	875.22	36.53	47	0.01	9.10	0.44
HLA-DRB3*01:01	26	40	KCEEKFKNDASKMAC	Consensus	0.63	875.22	36.53	49	0.01	11.40	0.63
HLA-DRB3*01:01	29	43	EKFKNDASKMACIPH	Consensus	0.66	20	19.64	49	0.01	11.80	0.66
HLA-DRB3*01:01	1	15	MKYLGLALISAVFLI	Consensus	0.80	0.01	0.01	2333	0.80	2415.70	30.99
HLA-DRB3*01:01	2	16	KYLGLALISAVFLIG	Consensus	0.83	0.01	0.01	2389	0.83	2415.40	30.99
HLA-DRB3*01:01	3	17	YLGLALISAVFLIGT	Consensus	0.83	0.01	0.01	2396	0.83	2302.70	30.07
HLA-DRB3*01:01	4	18	LGLALISAVFLIGTC	Consensus	0.85	0.01	0.01	2415	0.85	2218.20	29.36
HLA-DRB3*01:01	25	39	KKCEEKFKNDASKMA	Consensus	0.87	875.22	36.53	48	0.01	14.70	0.87
HLA-DRB3*01:01	5	19	GLALISAVFLIGTCQ	Consensus	1.08	0.01	0.01	2778	1.08	2685.10	33.11
HLA-DRB3*01:01	30	44	KFKNDASKMACIPHC	Consensus	1.13	20	19.64	146	0.01	18.40	1.13
HLA-DRB3*01:01	31	45	FKNDASKMACIPHCK	Consensus	2.08	20	19.64	150	0.01	34.70	2.08
HLA-DRB3*01:01	77	91	DKSLKADIRKIMHEC	Consensus	2.90	9.52	17.07	212	0.01	52.70	2.90
HLA-DRB3*01:01	76	90	VDKSLKADIRKIMHE	Consensus	3	9.52	17.07	211	0.01	55.10	3
HLA-DRB3*01:01	75	89	VVDKSLKADIRKIMH	Consensus	3.18	9.52	17.07	211	0.01	59.80	3.18
HLA-DRB3*01:01	74	88	NVVDKSLKADIRKIM	Consensus	3.39	9.52	17.07	209	0.01	65.40	3.39
HLA-DRB3*01:01	45	59	KYQYYGFVAMDNNIA	Consensus	3.58	0.01	3.58	3612	1.68	2904.40	34.79
HLA-DRB3*01:01	46	60	YQYYGFVAMDNNIAK	Consensus	3.58	0.01	3.58	1289	0.25	781.90	15.34
HLA-DRB3*01:01	47	61	QYYGFVAMDNNIAKP	Consensus	3.58	0.01	3.58	1329	0.27	723.10	14.63
HLA-DRB3*01:01	48	62	YYGFVAMDNNIAKPE	Consensus	3.58	0.01	3.58	1284	0.25	646.30	13.69
HLA-DRB3*01:01	49	63	YGFVAMDNNIAKPEI	Consensus	3.58	0.01	3.58	1250	0.23	592.20	12.98
HLA-DRB3*01:01	50	64	GFVAMDNNIAKPEIR	Consensus	3.58	0.01	3.58	1341	0.27	1394.70	21.91
HLA-DRB3*01:01	51	65	FVAMDNNIAKPEIRT	Consensus	3.58	0.01	3.58	3579	1.66	2760.20	33.68
HLA-DRB3*01:01	78	92	KSLKADIRKIMHECA	Consensus	4.67	9.52	17.07	211	0.01	106.70	4.67
HLA-DRB3*01:01	6	20	LALISAVFLIGTCQA	Consensus	4.89	0.01	0.01	7564	4.89	3462.10	38.78

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB3*01:01	83	97	DIRKIMHECAKKVKK	Consensus	4.94	1468.28	39.27	447	0.02	116.70	4.94
HLA-DRB3*01:01	84	98	IRKIMHECAKKVKKQ	Consensus	4.98	1468.28	39.27	450	0.02	118.10	4.98
HLA-DRB3*01:01	82	96	ADIRKIMHECAKKVK	Consensus	5.06	1468.28	39.27	441	0.02	121	5.06
HLA-DRB3*01:01	81	95	KADIRKIMHECAKKV	Consensus	5.38	1468.28	39.27	435	0.02	133.90	5.38
HLA-DRB3*01:01	7	21	ALISAVFLIGTCQAE	Consensus	5.40	0.01	0.01	8152	5.40	3719.90	40.52
HLA-DRB3*01:01	57	71	NIAKPEIRTFSNVLI	Consensus	6.87	0.14	6.87	2800	1.10	3959.10	42.06
HLA-DRB3*01:01	58	72	IAKPEIRTFSNVLIK	Consensus	6.87	0.14	6.87	2836	1.13	4031.30	42.51
HLA-DRB3*01:01	59	73	AKPEIRTFSNVLIKY	Consensus	6.87	0.14	6.87	2570	0.96	3250.60	37.31
HLA-DRB3*01:01	60	74	KPEIRTFSNVLIKYN	Consensus	6.87	0.14	6.87	2480	0.90	2608	32.51
HLA-DRB3*01:01	61	75	PEIRTFSNVLIKYNV	Consensus	6.87	0.14	6.87	2540	0.94	2824	34.17
HLA-DRB3*01:01	62	76	EIRTFSNVLIKYNVV	Consensus	6.87	0.14	6.87	4697	2.54	2561.10	32.14
HLA-DRB3*01:01	63	77	IRTFSNVLIKYNVVD	Consensus	6.87	0.14	6.87	4708	2.55	2781.70	33.85

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*01:01	46	60	YQYYGFVAMDNNIAK	Consensus	3.24	16.11	33.26	18	3.24	5	0.79
HLA-DRB1*01:01	45	59	KYQYYGFVAMDNNIA	Consensus	3.49	0.26	13.70	19	3.49	5.90	1.62
HLA-DRB1*01:01	47	61	QYYGFVAMDNNIAKP	Consensus	3.72	16.11	33.26	20	3.72	5.10	0.88
HLA-DRB1*01:01	48	62	YYGFVAMDNNIAKPE	Consensus	3.72	16.11	33.26	20	3.72	4.80	0.62
HLA-DRB1*01:01	49	63	YGFVAMDNNIAKPEI	Consensus	3.72	16.11	33.26	20	3.72	5.70	1.43
HLA-DRB1*01:01	1	15	MKYLGLALISAVFLI	Consensus	4.30	0.38	15.04	7	0.77	8.90	4.30
HLA-DRB1*01:01	2	16	KYLGLALISAVFLIG	Consensus	5.73	0.38	15.04	8	0.96	10.70	5.73

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	NN align IC50 (nM)	NN align rank	NetMHCIIpan IC50 (nM)	NetMHCIIpan rank	Sturniolo score	Sturniolo rank
HLA-DRB3*02:02	47	61	QYYGFVAMDNNIAKP	NetMHCIIpan	1.62	-	-	88.71	1.62	-	-
HLA-DRB3*02:02	48	62	YYGFVAMDNNIAKPE	NetMHCIIpan	1.66	-	-	92.09	1.66	-	-
HLA-DRB3*02:02	46	60	YQYYGFVAMDNNIAK	NetMHCIIpan	1.88	-	-	98.42	1.88	-	-
HLA-DRB3*02:02	49	63	YGFVAMDNNIAKPEI	NetMHCIIpan	2.12	-	-	105.92	2.12	-	-
HLA-DRB3*02:02	68	82	NVLIKYNVVDKSLKA	NetMHCIIpan	2.68	-	-	121.90	2.68	-	-
HLA-DRB3*02:02	69	83	VLIKYNVVDKSLKAD	NetMHCIIpan	2.91	-	-	129.06	2.91	-	-
HLA-DRB3*02:02	70	84	LIKYNVVDKSLKADI	NetMHCIIpan	3.68	-	-	146.99	3.68	-	-
HLA-DRB3*02:02	111	125	TINYYRCILTDKRIG	NetMHCIIpan	4.36	-	-	163.09	4.36	-	-
HLA-DRB3*02:02	67	81	SNVLIKYNVVDKSLK	NetMHCIIpan	4.46	-	-	165.53	4.46	-	-
HLA-DRB3*02:02	50	64	GFVAMDNNIAKPEIR	NetMHCIIpan	4.54	-	-	169.01	4.54	-	-
HLA-DRB3*02:02	110	124	TTINYYRCILTDKRI	NetMHCIIpan	4.63	-	-	174.16	4.63	-	-
HLA-DRB3*02:02	84	98	IRKIMHECAKKVKKQ	NetMHCIIpan	4.67	-	-	175.99	4.67	-	-
HLA-DRB3*02:02	112	126	INYYRCILTDKRIGP	NetMHCIIpan	4.70	-	-	176.84	4.70	-	-
HLA-DRB3*02:02	83	97	DIRKIMHECAKKVKK	NetMHCIIpan	4.99	-	-	181.98	4.99	-	-
HLA-DRB3*02:02	27	41	CEEKFKNDASKMACI	NetMHCIIpan	5.07	-	-	184.12	5.07	-	-
HLA-DRB3*02:02	28	42	EEKFKNDASKMACIP	NetMHCIIpan	6.15	-	-	211.60	6.15	-	-
HLA-DRB3*02:02	113	127	NYYRCILTDKRIGPQ	NetMHCIIpan	6.38	-	-	215.67	6.38	-	-
HLA-DRB3*02:02	29	43	EKFKNDASKMACIPH	NetMHCIIpan	6.77	-	-	222.16	6.77	-	-
HLA-DRB3*02:02	82	96	ADIRKIMHECAKKVK	NetMHCIIpan	6.80	-	-	222.85	6.80	-	-
HLA-DRB3*02:02	71	85	IKYNVVDKSLKADIR	NetMHCIIpan	7.39	-	-	237.76	7.39	-	-

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB5*01:01	110	124	TTINYYRCILTDKRI	Consensus	0.37	62	0.36	3.40	0.37	3.70	0.86
HLA-DRB5*01:01	111	125	TINYYRCILTDKRIG	Consensus	0.38	64	0.38	3.30	0.34	3.70	0.86
HLA-DRB5*01:01	112	126	INYYRCILTDKRIGP	Consensus	0.41	67	0.41	3.50	0.39	3.70	0.86
HLA-DRB5*01:01	113	127	NYYRCILTDKRIGPQ	Consensus	0.63	71	0.44	4.30	0.63	3.70	0.86
HLA-DRB5*01:01	109	123	RTTINYYRCILTDKR	Consensus	0.69	65	0.39	4.50	0.69	3.70	0.86
HLA-DRB5*01:01	114	128	YYRCILTDKRIGPQR	Consensus	1.15	206	1.64	6	1.15	3.70	0.86
HLA-DRB5*01:01	67	81	SNVLIKYNVVDKSLK	Consensus	1.16	98	0.66	22.90	5.51	3.40	1.16
HLA-DRB5*01:01	68	82	NVLIKYNVVDKSLKA	Consensus	1.16	95	0.62	19.40	4.74	3.40	1.16
HLA-DRB5*01:01	69	83	VLIKYNVVDKSLKAD	Consensus	1.16	97	0.64	20.40	4.97	3.40	1.16
HLA-DRB5*01:01	70	84	LIKYNVVDKSLKADI	Consensus	1.16	92	0.60	16.80	4.14	3.40	1.16
HLA-DRB5*01:01	71	85	IKYNVVDKSLKADIR	Consensus	1.16	91	0.59	29.50	6.77	3.40	1.16
HLA-DRB5*01:01	115	129	YRCILTDKRIGPQRF	Consensus	1.61	203	1.61	9.50	2.22	3.70	0.86
HLA-DRB5*01:01	72	86	KYNVVDKSLKADIRK	Consensus	1.67	209	1.67	41.10	8.72	3.40	1.16
HLA-DRB5*01:01	73	87	YNVVDKSLKADIRKI	Consensus	1.67	209	1.67	59.60	11.21	3.40	1.16
HLA-DRB5*01:01	46	60	YQYYGFVAMDNNIAK	Consensus	1.79	222	1.79	124.70	17.26	3	1.69
HLA-DRB5*01:01	45	59	KYQYYGFVAMDNNIA	Consensus	2.03	251	2.03	172	20.41	3	1.69
HLA-DRB5*01:01	47	61	QYYGFVAMDNNIAKP	Consensus	2.05	254	2.05	138.10	18.23	3	1.69
HLA-DRB5*01:01	48	62	YYGFVAMDNNIAKPE	Consensus	2.05	254	2.05	113.10	16.37	3	1.69
HLA-DRB5*01:01	49	63	YGFVAMDNNIAKPEI	Consensus	2.11	261	2.11	196.50	21.79	3	1.69
HLA-DRB5*01:01	1	15	MKYLGLALISAVFLI	Consensus	3.19	145	1.08	815	40.07	2.30	3.19
HLA-DRB5*01:01	2	16	KYLGLALISAVFLIG	Consensus	3.19	149	1.11	1243.70	46.75	2.30	3.19
HLA-DRB5*01:01	3	17	YLGLALISAVFLIGT	Consensus	3.19	148	1.11	1495.10	49.80	2.30	3.19
HLA-DRB5*01:01	4	18	LGLALISAVFLIGTC	Consensus	3.19	150	1.12	2060.20	55.42	2.30	3.19
HLA-DRB5*01:01	84	98	IRKIMHECAKKVKKQ	Consensus	3.27	132	0.98	13.30	3.27	0.70	9.44
HLA-DRB5*01:01	50	64	GFVAMDNNIAKPEIR	Consensus	3.28	391	3.28	256.80	24.80	3	1.69

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB5*01:01	5	19	GLALISAVFLIGTCQ	Consensus	3.67	434	3.67	3118.90	62.97	2.30	3.19
HLA-DRB5*01:01	83	97	DIRKIMHECAKKVKK	Consensus	3.76	132	0.98	15.20	3.76	0.70	9.44
HLA-DRB5*01:01	6	20	LALISAVFLIGTCQA	Consensus	3.89	460	3.89	3832.30	66.81	2.30	3.19
HLA-DRB5*01:01	51	65	FVAMDNNIAKPEIRT	Consensus	3.90	462	3.90	418.80	30.70	3	1.69
HLA-DRB5*01:01	85	99	RKIMHECAKKVKKQA	Consensus	4.10	138	1.02	16.60	4.10	0.70	9.44
HLA-DRB5*01:01	86	100	KIMHECAKKVKKQAR	Consensus	4.35	312	2.58	17.70	4.35	0.70	9.44
HLA-DRB5*01:01	105	119	WLNCRTTINYYRCIL	Consensus	5.08	602	5.08	18.10	4.45	1	7.95
HLA-DRB5*01:01	106	120	LNCRTTINYYRCILT	Consensus	6.17	573	4.83	26.20	6.17	1	7.95
HLA-DRB5*01:01	12	26	VFLIGTCQAENPSKK	Consensus	6.60	628	5.30	185.90	21.22	1.30	6.60
HLA-DRB5*01:01	82	96	ADIRKIMHECAKKVK	Consensus	6.67	139	1.03	28.90	6.67	0.70	9.44
HLA-DRB5*01:01	52	66	VAMDNNIAKPEIRTF	Consensus	7.46	612	5.16	1104	44.80	1.10	7.46
HLA-DRB5*01:01	104	118	HWLNCRTTINYYRCI	Consensus	7.46	653	5.51	33.30	7.46	1	7.95

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*13:02	57	71	NIAKPEIRTFSNVLI	Consensus	3.77	132	3.65	95.10	5.85	1.90	3.77
HLA-DRB1*13:02	58	72	IAKPEIRTFSNVLIK	Consensus	3.77	131	3.62	77.80	5.05	1.90	3.77
HLA-DRB1*13:02	59	73	AKPEIRTFSNVLIKY	Consensus	3.77	130	3.59	66.20	4.42	1.90	3.77
HLA-DRB1*13:02	60	74	KPEIRTFSNVLIKYN	Consensus	3.77	130	3.59	70.50	4.65	1.90	3.77
HLA-DRB1*13:02	61	75	PEIRTFSNVLIKYNV	Consensus	3.88	139	3.88	105.60	6.31	1.90	3.77
HLA-DRB1*13:02	84	98	IRKIMHECAKKVKKQ	Consensus	6.90	94	2.46	119.70	6.90	-0.30	16.04

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB4*01:01	81	95	KADIRKIMHECAKKV	Consensus	5.19	0.01	0.01	619	5.19	151.40	11.35
HLA-DRB4*01:01	82	96	ADIRKIMHECAKKVK	Consensus	5.34	0.01	0.01	634	5.34	280.40	18.54
HLA-DRB4*01:01	67	81	SNVLIKYNVVDKSLK	Consensus	5.45	44.38	25.40	445	3.48	70.30	5.45
HLA-DRB4*01:01	1	15	MKYLGLALISAVFLI	Consensus	5.57	0.32	7.40	168	0.95	71.70	5.57
HLA-DRB4*01:01	68	82	NVLIKYNVVDKSLKA	Consensus	5.61	44.38	25.40	446	3.49	72.20	5.61
HLA-DRB4*01:01	78	92	KSLKADIRKIMHECA	Consensus	6.15	0.01	0.01	720	6.15	139.50	10.57
HLA-DRB4*01:01	79	93	SLKADIRKIMHECAK	Consensus	6.56	0.01	0.01	763	6.56	137	10.40
HLA-DRB4*01:01	80	94	LKADIRKIMHECAKK	Consensus	6.84	0.01	0.01	794	6.84	127.30	9.74
HLA-DRB4*01:01	69	83	VLIKYNVVDKSLKAD	Consensus	7.19	44.38	25.40	483	3.85	92.30	7.19
HLA-DRB4*01:01	2	16	KYLGLALISAVFLIG	Consensus	7.40	0.32	7.40	274	1.87	695.30	33.36
HLA-DRB4*01:01	3	17	YLGLALISAVFLIGT	Consensus	7.40	0.32	7.40	358	2.65	1327.80	46.60
HLA-DRB4*01:01	4	18	LGLALISAVFLIGTC	Consensus	7.40	0.32	7.40	363	2.70	1229.90	44.93
HLA-DRB4*01:01	5	19	GLALISAVFLIGTCQ	Consensus	7.40	0.32	7.40	601	5.01	2238.80	58.38
HLA-DRB4*01:01	6	20	LALISAVFLIGTCQA	Consensus	7.40	0.32	7.40	574	4.75	1758.20	52.83
HLA-DRB4*01:01	7	21	ALISAVFLIGTCQAE	Consensus	7.40	0.32	7.40	780	6.72	1648.30	51.38

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*09:01	45	59	KYQYYGFVAMDNNIA	Consensus	3.97	89525.96	55.69	471	3.97	59.10	3.95
HLA-DRB1*09:01	46	60	YQYYGFVAMDNNIAK	Consensus	4.92	89525.96	55.69	475	4.01	72.30	4.92
HLA-DRB1*09:01	47	61	QYYGFVAMDNNIAKP	Consensus	7.01	5407.84	49.15	535	4.65	101.20	7.01

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*12:01	65	79	TFSNVLIKYNVVDKS	smm	3.53	-	-	388	3.53	-	-
HLA-DRB1*12:01	66	80	FSNVLIKYNVVDKSL	smm	3.73	-	-	403	3.73	-	-
HLA-DRB1*12:01	1	15	MKYLGLALISAVFLI	smm	4.39	-	-	451	4.39	-	-
HLA-DRB1*12:01	60	74	KPEIRTFSNVLIKYN	smm	5.13	-	-	505	5.13	-	-
HLA-DRB1*12:01	61	75	PEIRTFSNVLIKYNV	smm	5.45	-	-	529	5.45	-	-
HLA-DRB1*12:01	3	17	YLGLALISAVFLIGT	smm	5.91	-	-	562	5.91	-	-
HLA-DRB1*12:01	2	16	KYLGLALISAVFLIG	smm	6.06	-	-	573	6.06	-	-
HLA-DRB1*12:01	67	81	SNVLIKYNVVDKSLK	smm	6.26	-	-	587	6.26	-	-
HLA-DRB1*12:01	68	82	NVLIKYNVVDKSLKA	smm	6.66	-	-	617	6.66	-	-
HLA-DRB1*12:01	62	76	EIRTFSNVLIKYNVV	smm	6.90	-	-	634	6.90	-	-
HLA-DRB1*12:01	63	77	IRTFSNVLIKYNVVD	smm	7	-	-	641	7	-	-

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*05:01/DQB1*02:01	1	15	MKYLGLALISAVFLI	Consensus	5.52	0.01	0.01	856	5.52	1260.20	24.41
HLA-DQA1*05:01/DQB1*02:01	45	59	KYQYYGFVAMDNNIA	Consensus	5.64	0.01	0.01	1181	9.44	254.20	5.64
HLA-DQA1*05:01/DQB1*02:01	2	16	KYLGLALISAVFLIG	Consensus	6.39	0.01	0.01	931	6.39	1451.40	27.06
HLA-DQA1*05:01/DQB1*02:01	3	17	YLGLALISAVFLIGT	Consensus	6.46	0.01	0.01	937	6.46	1301	25

Supplementary data 5 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*07:01	1	15	MKYLGLALISAVFLI	Consensus	1.31	0.01	1.31	32	0.21	131.30	16.81
HLA-DRB1*07:01	2	16	KYLGLALISAVFLIG	Consensus	1.31	0.01	1.31	34	0.23	330.20	27.49
HLA-DRB1*07:01	3	17	YLGLALISAVFLIGT	Consensus	1.31	0.01	1.31	34	0.23	603	36.09
HLA-DRB1*07:01	4	18	LGLALISAVFLIGTC	Consensus	1.31	0.01	1.31	34	0.23	920.90	42.89
HLA-DRB1*07:01	5	19	GLALISAVFLIGTCQ	Consensus	1.31	0.01	1.31	105	0.96	739.10	39.29
HLA-DRB1*07:01	6	20	LALISAVFLIGTCQA	Consensus	1.31	0.01	1.31	110	1.01	777.20	40.13
HLA-DRB1*07:01	57	71	NIAKPEIRTFSNVLI	Consensus	2.34	0.10	3.88	71	0.60	13	2.34
HLA-DRB1*07:01	58	72	IAKPEIRTFSNVLIK	Consensus	3.88	0.10	3.88	72	0.61	20.90	4.03
HLA-DRB1*07:01	59	73	AKPEIRTFSNVLIKY	Consensus	3.88	0.10	3.88	70	0.59	33.70	6.31
HLA-DRB1*07:01	60	74	KPEIRTFSNVLIKYN	Consensus	3.88	0.10	3.88	71	0.60	51.50	8.93
HLA-DRB1*07:01	61	75	PEIRTFSNVLIKYNV	Consensus	3.88	0.10	3.88	82	0.74	80.60	12.28
HLA-DRB1*07:01	62	76	EIRTFSNVLIKYNVV	Consensus	3.88	0.10	3.88	207	2.25	101.30	14.31
HLA-DRB1*07:01	63	77	IRTFSNVLIKYNVVD	Consensus	3.88	0.10	3.88	228	2.50	153.60	18.47

Notes: The MHCII binding predictions were made on 06/25/2017 using the IEDB analysis resource Consensus tool. In terms of population coverage, the reference sets for class II should provide >99%. Results are presented by allele, and from lowest to highest percentile rank. Peptides predicted as good binders are all of those with a percentile ranking lower or equal to 10.

Supplementary data 6. PdSP15 in silico analysis: MHC-I restricted-epitopes prediction - Human (97% population coverage)

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*01:01	38	46	9	ISCLHHCKY	Consensus	0.3	156.89	0.3	306.58	0.3
HLA-A*01:01	34	42	9	NTDKISCLH	Consensus	0.5	312.25	0.3	642.02	0.7
HLA-A*01:01	40	49	10	CLHHCKYQYY	Consensus	0.85	4319.36	0.9	816.21	0.8
HLA-A*01:01	105	114	10	WLNCRTSINY	Consensus	0.9	885.35	0.5	1275.85	1.3
HLA-A*01:01	51	59	9	FIDVNYNIA	Consensus	0.95	1372.17	0.5	1447.27	1.4
HLA-A*01:01	23	31	9	PSQKCEEKY	Consensus	0.95	6628.84	0.8	1110.58	1.1

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-A*02:01	3	12	10	YLGLALISAV	Consensus	0.2	7.87	0.1	18.86	0.3	-	-
HLA-A*02:01	7	15	9	ALISAVFLI	Consensus	0.3	7.37	0.1	13.49	0.3	7.08e-06	0.5
HLA-A*02:01	46	54	9	YQYYGFIDV	Consensus	0.8	14.96	0.3	47.11	0.8	6.03e-05	3.5
HLA-A*02:01	3	11	9	YLGLALISA	Consensus	0.9	52.46	0.5	53.10	0.9	5.04e-05	2.9

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*02:03	3	12	10	YLGLALISAV	Consensus	0.15	4.51	0.1	5.39	0.2
HLA-A*02:03	7	15	9	ALISAVFLI	Consensus	0.6	18.44	0.6	16.79	0.6
HLA-A*02:03	3	11	9	YLGLALISA	Consensus	0.9	15.24	0.6	35.40	1.2
HLA-A*02:03	87	95	9	VMHECAKQV	Consensus	0.9	30.78	0.6	33.34	1.2
HLA-A*02:03	46	54	9	YQYYGFIDV	Consensus	0.95	7.71	0.4	43.35	1.5

Supplementary data 6 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*02:06	46	54	9	YQYYGFIDV	Consensus	0.1	3.18	0.1	2.39	0.1
HLA-A*02:06	3	12	10	YLGLALISAV	Consensus	0.25	15.52	0.2	24.15	0.3
HLA-A*02:06	7	15	9	ALISAVFLI	Consensus	0.95	16.90	0.6	37.63	1.3

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*03:01	122	131	10	KLIGPQRFDK	Consensus	0.4	13.67	0.4	43.37	0.4
HLA-A*03:01	37	45	9	KISCLHHCK	Consensus	0.45	124.28	0.3	146.49	0.6
HLA-A*03:01	78	86	9	KSKKRELKK	Consensus	0.45	168.87	0.3	143.81	0.6
HLA-A*03:01	69	78	10	VLMDYGVVDK	Consensus	0.65	51.33	0.5	73.82	0.8

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*11:01	37	45	9	KISCLHHCK	Consensus	0.5	31.25	0.4	53.74	0.6
HLA-A*11:01	122	131	10	KLIGPQRFDK	Consensus	0.5	96.45	0.3	62.21	0.7
HLA-A*11:01	69	78	10	VLMDYGVVDK	Consensus	0.75	32.58	0.3	117.45	1.2
HLA-A*11:01	123	131	9	LIGPQRFDK	Consensus	1.0	171.98	0.4	209.56	1.6

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*26:01	NO PREDICTED BINDERS									

Supplementary data 6 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*23:01	50	58	9	GFIDVNYNI	Consensus	0.35	44.48	0.2	117.07	0.5
HLA-A*23:01	30	38	9	KYKGNTDKI	Consensus	0.7	485.80	0.5	294.73	0.9
HLA-A*23:01	48	56	9	YYGFIDVNY	Consensus	0.8	216.50	0.4	402.19	1.2
HLA-A*23:01	114	123	10	YYRCVLTSKL	Consensus	0.85	68.67	0.4	232.65	1.3
HLA-A*23:01	49	58	10	YGFIDVNYNI	Consensus	0.9	29.05	0.4	253.93	1.4

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*24:02	30	38	9	KYKGNTDKI	Consensus	0.8	726.69	0.9	337.78	0.7
HLA-A*24:02	114	123	10	YYRCVLTSKL	Consensus	0.8	802.39	0.8	357.87	0.8
HLA-A*24:02	45	53	9	KYQYYGFID	Consensus	0.85	1498.84	1.2	259.20	0.5

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-A*30:01	78	86	9	KSKKRELKK	Consensus	0.2	7.97	0.2	7.31	0.2	0.000264	4.7
HLA-A*30:01	78	87	10	KSKKRELKKV	Consensus	0.6	377.92	0.5	299.19	0.7	-	-
HLA-A*30:01	114	122	9	YYRCVLTSK	Consensus	0.6	34.58	0.4	37.49	0.6	0.000157	3
HLA-A*30:01	24	32	9	SQKCEEKYK	Consensus	0.9	200.94	0.8	50.58	0.9	0.000417	7.2

Supplementary data 6 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*30:02	64	73	10	RKFSNVLMDY	Consensus	0.15	54.47	0.2	21.10	0.1
HLA-A*30:02	65	73	9	KFSNVLMDY	Consensus	0.35	46.15	0.2	120.63	0.5
HLA-A*30:02	47	56	10	QYYGFIDVNY	Consensus	0.6	289.78	0.5	143.67	0.7
HLA-A*30:02	37	46	10	KISCLHHCKY	Consensus	0.65	231.55	0.5	175.54	0.8
HLA-A*30:02	40	48	9	CLHHCKYQY	Consensus	0.95	355.57	0.8	237.94	1.1

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*31:01	120	128	9	TSKLIGPQR	Consensus	0.9	97.36	0.5	68.15	1.3
HLA-A*31:01	107	116	10	NCRTSINYYR	Consensus	0.95	179.53	0.9	83.25	1
HLA-A*31:01	108	116	9	CRTSINYYR	Consensus	0.95	263.45	0.6	77.35	1.3

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*33:01	113	122	10	NYYRCVLTSK	Consensus	0.4	121.08	0.2	143.05	0.6
HLA-A*33:01	55	64	10	NYNIAQSEIR	Consensus	0.45	654.72	0.6	61.73	0.3
HLA-A*33:01	108	116	9	CRTSINYYR	Consensus	0.6	153.30	0.4	130.93	0.8

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*68:01	120	128	9	TSKLIGPQR	Consensus	0.9	109.19	0.7	63.60	1.1

Supplementary data 6 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*08:01	62	70	9	EIRKFSNVL	Consensus	0.7	258.82	0.5	185.59	0.7	2.81e-05	1.1
HLA-B*08:01	111	119	9	SINYYRCVL	Consensus	0.7	117.48	0.2	185.59	0.7	8.43e-05	3.2
HLA-B*08:01	110	119	10	TSINYYRCVL	Consensus	0.85	439.27	0.6	757.91	1.1	-	-

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-A*68:02	NO PREDICTED BINDERS											

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-A*32:01	5	13	9	GLALISAVF	Consensus	0.9	261.12	0.5	111.16	0.9	0.000105	13

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*07:02	NO PREDICTED BINDERS											

Supplementary data 6 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*15:01	5	13	9	GLALISAVF	Consensus	0.3	38.34	0.1	51.05	0.3	3.31e-05	4.8
HLA-B*15:01	4	13	10	LGLALISAVF	Consensus	0.6	16.24	0.4	9.34	0.8	-	-
HLA-B*15:01	105	114	10	WLNCRTSINY	Consensus	0.7	54.61	0.5	9.94	0.9	-	-

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*51:01	6	15	10	LALISAVFLI	Consensus	0.6	1301.23	0.2	3963.24	1	-	-
HLA-B*51:01	54	63	10	VNYNIAQSEI	Consensus	0.75	11523.46	1	2594.48	0.5	-	-
HLA-B*51:01	1	9	9	MKYLGLALI	Consensus	0.8	4106	0.4	1512.27	0.8	4.25e-05	2.8
HLA-B*51:01	6	14	9	LALISAVFL	Consensus	0.8	2424.63	0.4	1966.21	1	1.43e-05	0.8

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*35:01	NO PREDICTED BINDERS											

Supplementary data 6 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-B*40:01	61	70	10	SEIRKFSNVL	Consensus	0.25	59.07	0.2	56.89	0.3
HLA-B*40:01	97	106	10	KEARKDSHWL	Consensus	0.9	490.90	0.9	295.82	0.9

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-B*40:01	61	70	10	SEIRKFSNVL	Consensus	0.25	59.07	0.2	56.89	0.3
HLA-B*40:01	97	106	10	KEARKDSHWL	Consensus	0.9	490.90	0.9	295.82	0.9

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-B*44:02	97	105	9	KEARKDSHW	Consensus	0.15	113.30	0.1	101.77	0.2
HLA-B*44:02	61	70	10	SEIRKFSNVL	Consensus	0.45	985.19	0.5	138.96	0.4

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-B*44:03	61	69	9	SEIRKFSNV	Consensus	0.7	549.07	0.5	481.38	0.9
HLA-B*44:03	97	105	9	KEARKDSHW	Consensus	0.7	1467.16	1	241.26	0.4
HLA-B*44:03	61	70	10	SEIRKFSNVL	Consensus	0.7	339.04	1.1	160.72	0.3

Supplementary data 6 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-B*57:01	58	66	9	IAQSEIRKF	Consensus	0.7	627.39	0.4	492.97	1

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*58:01	97	105	9	KEARKDSHW	Consensus	0.9	447.79	0.5	144.31	0.9	0.000163	11

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*53:01	NO PREDICTED BINDERS											

Note: The MHCI binding predictions were made on 11/25/2016 using the IEDB analysis resource Consensus tool which combines predictions from ANN aka NetMHC (4.0), SMM and Comblib. In terms of population coverage, the reference sets for class II should provide >97%. Results are presented by allele, and from lowest to highest percentile rank. Peptides predicted as good binders are all of those with a percentile ranking lower or equal to 1.

Supplementary data 7. LJL143 in silico analysis: murine MHC-II restricted-epitopes prediction - BALB/c (H2-IAd and H2-IEd)

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align core	SMM align IC50 (nM)	SMM align rank	NN align core	NN align IC50 (nM)	NN align rank
H2-IAd	74	88	NKKSCISAIKLTSYP	Consensus	1.45	KSCISAIKL	2533	1.63	NKKSCISAI	224.40	1.27
H2-IAd	73	87	TNKKSCISAIKLTSY	Consensus	1.52	KSCISAIKL	2442	1.49	NKKSCISAI	256.80	1.55
H2-IAd	272	286	DELMTYALVKVFTNP	Consensus	1.69	LMTYALVKV	2799	2.04	MTYALVKVF	231.40	1.33
H2-IAd	271	285	TDELMTYALVKVFTN	Consensus	2.02	LMTYALVKV	2669	1.84	MTYALVKVF	332.60	2.19
H2-IAd	270	284	DTDELMTYALVKVFT	Consensus	2.64	LMTYALVKV	2588	1.70	MTYALVKVF	490.30	3.58
H2-IAd	72	86	KTNKKSCISAIKLTS	Consensus	2.90	KSCISAIKL	2924	2.25	NKKSCISAI	486.20	3.55
H2-IAd	273	287	ELMTYALVKVFTNPE	Consensus	2.99	MTYALVKVF	4188	4.67	MTYALVKVF	228.80	1.31
H2-IAd	204	218	FSEAREITAKDIKKK	Consensus	3.36	AREITAKDI	2548	1.65	AREITAKDI	659	5.07
H2-IAd	71	85	FKTNKKSCISAIKLT	Consensus	3.58	KSCISAIKL	2988	2.36	NKKSCISAI	628.20	4.79
H2-IAd	205	219	SEAREITAKDIKKKF	Consensus	3.58	AREITAKDI	2762	1.98	AREITAKDI	672.20	5.18
H2-IAd	75	89	KKSCISAIKLTSYPK	Consensus	3.71	ISAIKLTSY	3484	3.25	SCISAIKLT	557.10	4.18
H2-IAd	76	90	KSCISAIKLTSYPKI	Consensus	4.38	ISAIKLTSY	4342	4.98	SCISAIKLT	513.30	3.78
H2-IAd	269	283	FDTDELMTYALVKVF	Consensus	5.13	LMTYALVKV	2830	2.08	MTYALVKVF	1025.60	8.18
H2-IAd	274	288	LMTYALVKVFTNPES	Consensus	5.35	MTYALVKVF	6287	9.46	MTYALVKVF	220.70	1.24
H2-IAd	203	217	SFSEAREITAKDIKK	Consensus	5.46	AREITAKDI	2964	2.32	AREITAKDI	1076.20	8.61
H2-IAd	118	132	KRKRQIAANIEIYGI	Consensus	6.67	KRQIAANIE	4743	5.86	KRKRQIAAN	940.10	7.47
H2-IAd	206	220	EAREITAKDIKKKFS	Consensus	6.83	AREITAKDI	4779	5.94	AREITAKDI	967.80	7.71
H2-IAd	202	216	DSFSEAREITAKDIK	Consensus	7.25	AREITAKDI	2886	2.18	AREITAKDI	1530.50	12.31
H2-IAd	207	221	AREITAKDIKKKFSI	Consensus	7.39	AREITAKDI	5315	7.17	AREITAKDI	957	7.62
H2-IAd	70	84	NFKTNKKSCISAIKL	Consensus	7.61	TNKKSCISA	4906	6.23	NKKSCISAI	1120.30	8.98
H2-IAd	117	131	KKRKRQIAANIEIYG	Consensus	7.62	KRQIAANIE	3996	4.27	KRQIAANIE	1365	10.97
H2-IAd	77	91	SCISAIKLTSYPKIN	Consensus	9.64	ISAIKLTSY	6604	10.22	SCISAIKLT	1130.70	9.06

Supplementary data 7 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align core	SMM align IC50 (nM)	SMM align rank	NN align core	NN align IC50 (nM)	NN align rank
H2-IEd	110	124	DNCKLVFKKRKRQIA	smm	4.68	KLVFKKRKR	264	4.68	-	-	-
H2-IEd	109	123	TDNCKLVFKKRKRQI	smm	4.85	KLVFKKRKR	277	4.85	-	-	-
H2-IEd	111	125	NCKLVFKKRKRQIAA	smm	5.40	KLVFKKRKR	318	5.40	-	-	-
H2-IEd	108	122	GTDNCKLVFKKRKRQ	smm	5.78	KLVFKKRKR	348	5.78	-	-	-
H2-IEd	107	121	IGTDNCKLVFKKRKR	smm	6.17	CKLVFKKRK	380	6.17	-	-	-
H2-IEd	112	126	CKLVFKKRKRQIAAN	smm	7.92	KLVFKKRKR	533	7.92	-	-	-
H2-IEd	233	247	FLDVYWFQETMRKKP	smm	8.98	WFQETMRKK	635	8.98	-	-	-
H2-IEd	234	248	LDVYWFQETMRKKPK	smm	9.30	WFQETMRKK	667	9.30	-	-	-
H2-IEd	235	249	DVYWFQETMRKKPKY	smm	9.32	WFQETMRKK	669	9.32	-	-	-
H2-IEd	232	246	SFLDVYWFQETMRKK	smm	9.87	YWFQETMRK	725	9.87	-	-	-

Note: The MHCII binding predictions were made on 11/25/2016 using the IEDB analysis resources Consensus tool (H2-IAd), or SMM-Align (ver. 1.1) tool (H2-IEd).

Supplementary data 8. LJL143 in silico analysis: murine MHC-II restricted-epitopes prediction - C57BL/6 (H2-IAb)

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align core	SMM align IC50 (nM)	SMM align rank	NN align core	NN align IC50 (nM)	NN align rank
H2-IAb	139	153	FKDRYIGADPLHVDS	Consensus	2.48	YIGADPLHV	215	2.15	YIGADPLHV	171.40	2.80
H2-IAb	138	152	SFKDRYIGADPLHVD	Consensus	2.67	YIGADPLHV	216	2.16	YIGADPLHV	195.80	3.18
H2-IAb	137	151	CSFKDRYIGADPLHV	Consensus	2.81	DRYIGADPL	215	2.15	YIGADPLHV	215.90	3.48
H2-IAb	140	154	KDRYIGADPLHVDSY	Consensus	2.84	YIGADPLHV	288	2.94	YIGADPLHV	168.60	2.75
H2-IAb	141	155	DRYIGADPLHVDSYG	Consensus	3.41	YIGADPLHV	295	3.01	YIGADPLHV	238	3.80
H2-IAb	142	156	RYIGADPLHVDSYGL	Consensus	7.74	YIGADPLHV	1104	10.55	YIGADPLHV	317.10	4.93

Note: The MHCII binding predictions were made on 11/15/2016 using the IEDB analysis resource Consensus tool. Results are presented from lowest to highest percentile rank. Peptides predicted as good binders are all of those with a percentile ranking lower or equal to 10.

Supplementary data 9. LJL143 in silico analysis: murine MHC-I restricted-epitopes prediction - BALB/c (H-2-Dd, H-2-Kd and H-2-Ld)

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Dd	127	139	13	IEIYGIPAKKCSF	ann	0.2	4454.38	0.2	-	-	-	-
H-2-Dd	128	140	13	EIYGIPAKKCSFK	ann	0.2	4822.10	0.2	-	-	-	-
H-2-Dd	129	141	13	IYGIPAKKCSFKD	ann	0.2	5194.58	0.2	-	-	-	-
H-2-Dd	130	142	13	YGIPAKKCSFKDR	ann	0.2	5863.80	0.2	-	-	-	-
H-2-Dd	154	166	13	YGLPYQFDQEHGW	ann	0.2	3263.29	0.2	-	-	-	-
H-2-Dd	155	167	13	GLPYQFDQEHGWN	ann	0.2	5392.21	0.2	-	-	-	-
H-2-Dd	162	174	13	QEHGWNVERYNIF	ann	0.2	3874.77	0.2	-	-	-	-
H-2-Dd	163	175	13	EHGWNVERYNIFK	ann	0.2	4245.09	0.2	-	-	-	-
H-2-Dd	164	176	13	HGWNVERYNIFKD	ann	0.2	5044.65	0.2	-	-	-	-
H-2-Dd	128	139	12	EIYGIPAKKCSF	ann	0.2	4594.78	0.2	-	-	-	-
H-2-Dd	129	140	12	IYGIPAKKCSFK	ann	0.2	5065.21	0.2	-	-	-	-
H-2-Dd	155	166	12	GLPYQFDQEHGW	ann	0.2	5336.32	0.2	-	-	-	-
H-2-Dd	163	174	12	EHGWNVERYNIF	ann	0.2	4097.48	0.2	-	-	-	-
H-2-Dd	164	175	12	HGWNVERYNIFK	ann	0.2	4934.09	0.2	-	-	-	-
H-2-Dd	129	139	11	IYGIPAKKCSF	ann	0.2	4832.91	0.2	-	-	-	-
H-2-Dd	130	140	11	YGIPAKKCSFK	ann	0.2	5700.28	0.2	-	-	-	-
H-2-Dd	153	163	11	SYGLPYQFDQE	ann	0.2	9676.71	0.2	-	-	-	-
H-2-Dd	164	174	11	HGWNVERYNIF	ann	0.2	4781.05	0.2	-	-	-	-
H-2-Dd	49	56	8	RDPCQIVL	ann	0.2	9360.25	0.2	-	-	-	-
H-2-Dd	155	162	8	GLPYQFDQ	ann	0.2	9629.29	0.2	-	-	-	-
H-2-Dd	245	252	8	KKPKYPYI	ann	0.2	6153.77	0.2	-	-	-	-
H-2-Dd	131	139	9	GIPAKKCSF	Consensus	0.3	2888.17	0.3	420.03	0.2	3.34e-05	0.8
H-2-Dd	151	163	13	VDSYGLPYQFDQE	ann	0.3	9080.93	0.3	-	-	-	-
H-2-Dd	152	164	13	DSYGLPYQFDQEH	ann	0.3	9701.66	0.3	-	-	-	-

Supplementary data 9 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Dd	130	141	12	YGIPAKKCSFKD	ann	0.3	5837.46	0.3	-	-	-	-
H-2-Dd	152	163	12	DSYGLPYQFDQE	ann	0.3	9298.38	0.3	-	-	-	-
H-2-Dd	154	164	11	YGLPYQFDQEH	ann	0.3	11005.32	0.3	-	-	-	-
H-2-Dd	126	139	14	NIEIYGIPAKKCSF	ann	0.4	4367.43	0.4	-	-	-	-
H-2-Dd	127	140	14	IEIYGIPAKKCSFK	ann	0.4	4679.06	0.4	-	-	-	-
H-2-Dd	128	141	14	EIYGIPAKKCSFKD	ann	0.4	4948.26	0.4	-	-	-	-
H-2-Dd	129	142	14	IYGIPAKKCSFKDR	ann	0.4	5220.16	0.4	-	-	-	-
H-2-Dd	153	166	14	SYGLPYQFDQEHGW	ann	0.4	2970.92	0.4	-	-	-	-
H-2-Dd	154	167	14	YGLPYQFDQEHGWN	ann	0.4	3302.25	0.4	-	-	-	-
H-2-Dd	155	168	14	GLPYQFDQEHGWNV	ann	0.4	5454.88	0.4	-	-	-	-
H-2-Dd	161	174	14	DQEHGWNVERYNIF	ann	0.4	3747.73	0.4	-	-	-	-
H-2-Dd	162	175	14	QEHGWNVERYNIFK	ann	0.4	4019.75	0.4	-	-	-	-
H-2-Dd	163	176	14	EHGWNVERYNIFKD	ann	0.4	4342.37	0.4	-	-	-	-
H-2-Dd	164	177	14	HGWNVERYNIFKDT	ann	0.4	5058.97	0.4	-	-	-	-
H-2-Dd	153	165	13	SYGLPYQFDQEHG	ann	0.4	10271.92	0.4	-	-	-	-
H-2-Dd	153	164	12	SYGLPYQFDQEH	ann	0.4	10073.04	0.4	-	-	-	-
H-2-Dd	130	143	14	YGIPAKKCSFKDRY	ann	0.5	5840.69	0.5	-	-	-	-
H-2-Dd	29	42	14	FIGKYKEKDETLFF	ann	0.6	9574.75	0.6	-	-	-	-
H-2-Dd	150	163	14	HVDSYGLPYQFDQE	ann	0.6	8943.25	0.6	-	-	-	-
H-2-Dd	151	164	14	VDSYGLPYQFDQEH	ann	0.6	9488.42	0.6	-	-	-	-
H-2-Dd	152	165	14	DSYGLPYQFDQEHG	ann	0.6	9898.13	0.6	-	-	-	-
H-2-Dd	166	174	9	WNVERYNIF	Consensus	0.6	5798.88	0.4	5275.70	5.6	2.62e-05	0.6

Supplementary data 9 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Dd	30	42	13	IGKYKEKDETLFF	ann	0.6	10560.19	0.6	-	-	-	-
H-2-Dd	154	165	12	YGLPYQFDQEHG	ann	0.6	11219.21	0.6	-	-	-	-
H-2-Dd	132	139	8	IPAKKCSF	ann	0.6	12125.41	0.6	-	-	-	-
H-2-Dd	165	175	11	GWNVERYNIFK	ann	0.8	13202.14	0.8	-	-	-	-
H-2-Dd	30	43	14	IGKYKEKDETLFFA	ann	0.9	10573.10	0.9	-	-	-	-
H-2-Dd	10	18	9	VGLISFGFI	Consensus	0.9	13076.61	0.9	3208.34	2.8	2.37e-05	0.5
H-2-Dd	29	41	13	FIGKYKEKDETLF	ann	0.9	11486.12	0.9	-	-	-	-

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Kd	184	195	12	FYHKNGLFNTQI	ann	0.1	57.20	0.1	-	-	-	-
H-2-Kd	83	96	14	KLTSYPKINQNSDL	ann	0.1	371.17	0.1	-	-	-	-
H-2-Kd	182	195	14	EVFYHKNGLFNTQI	ann	0.1	71.01	0.1	-	-	-	-
H-2-Kd	183	196	14	VFYHKNGLFNTQIT	ann	0.1	135.53	0.1	-	-	-	-
H-2-Kd	184	197	14	FYHKNGLFNTQITY	ann	0.1	147.55	0.1	-	-	-	-
H-2-Kd	84	96	13	LTSYPKINQNSDL	ann	0.1	361.13	0.1	-	-	-	-
H-2-Kd	183	195	13	VFYHKNGLFNTQI	ann	0.1	66.39	0.1	-	-	-	-
H-2-Kd	184	196	13	FYHKNGLFNTQIT	ann	0.1	114	0.1	-	-	-	-
H-2-Kd	85	97	13	TSYPKINQNSDLT	ann	0.2	760.80	0.2	-	-	-	-
H-2-Kd	86	98	13	SYPKINQNSDLTK	ann	0.2	715.91	0.2	-	-	-	-
H-2-Kd	85	96	12	TSYPKINQNSDL	ann	0.3	338.57	0.3	-	-	-	-
H-2-Kd	84	97	14	LTSYPKINQNSDLT	ann	0.3	810.02	0.3	-	-	-	-
H-2-Kd	85	98	14	TSYPKINQNSDLTK	ann	0.3	856.15	0.3	-	-	-	-

Supplementary data 9 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Kd	86	99	14	SYPKINQNSDLTKN	ann	0.3	958.40	0.3	-	-	-	-
H-2-Kd	170	183	14	RYNIFKDTRFSTEV	ann	0.3	970.78	0.3	-	-	-	-
H-2-Kd	185	198	14	YHKNGLFNTQITYL	ann	0.3	787.23	0.3	-	-	-	-
H-2-Kd	57	68	12	GYKCSNNQTHFV	ann	0.4	1097.29	0.4	-	-	-	-
H-2-Kd	86	97	12	SYPKINQNSDLT	ann	0.4	633.91	0.4	-	-	-	-
H-2-Kd	142	151	10	RYIGADPLHV	Consensus	0.45	1836.57	0.4	638.94	0.5	-	-
H-2-Kd	56	69	14	LGYKCSNNQTHFVL	ann	0.5	1308.90	0.5	-	-	-	-
H-2-Kd	57	69	13	GYKCSNNQTHFVL	ann	0.5	1106.90	0.5	-	-	-	-
H-2-Kd	27	40	14	EYFIGKYKEKDETL	ann	0.6	1349.50	0.6	-	-	-	-
H-2-Kd	227	236	10	EYKRISFLDV	Consensus	0.6	10669.18	1	453.38	0.2	-	-
H-2-Kd	55	68	14	VLGYKCSNNQTHFV	ann	0.7	1411.66	0.7	-	-	-	-
H-2-Kd	56	68	13	LGYKCSNNQTHFV	ann	0.7	1307.84	0.7	-	-	-	-
H-2-Kd	184	191	8	FYHKNGLF	Consensus	0.85	11728.87	1.4	581.38	0.3	-	-
H-2-Kd	41	54	14	FFASYGLKRDPCQI	ann	1.0	2530.25	1	-	-	-	-
H-2-Kd	57	70	14	GYKCSNNQTHFVLN	ann	1.0	2228.45	1	-	-	-	-
H-2-Kd	138	151	14	SFKDRYIGADPLHV	ann	1.0	2271.51	1	-	-	-	-
H-2-Kd	142	155	14	RYIGADPLHVDSYG	ann	1.0	2581.66	1	-	-	-	-
H-2-Kd	181	194	14	TEVFYHKNGLFNTQ	ann	1.0	2462.22	1	-	-	-	-
H-2-Kd	194	207	14	QITYLAEEDSFSEA	ann	1.0	2202.87	1	-	-	-	-
H-2-Kd	239	252	14	FQETMRKKPKYPYI	ann	1.0	2794.50	1	-	-	-	-

Supplementary data 9 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Ld	132	139	8	IPAKKCSF	ann	0.2	3148.58	0.2	-	-	-	-
H-2-Ld	87	96	10	YPKINQNSDL	ann	0.3	5278.04	0.3	-	-	-	-
H-2-Ld	156	168	13	LPYQFDQEHGWNV	ann	0.3	543.87	0.3	-	-	-	-
H-2-Ld	249	256	8	YPYIHYNG	ann	0.4	10736.92	0.4	-	-	-	-
H-2-Ld	155	168	14	GLPYQFDQEHGWNV	ann	0.6	461.15	0.6	-	-	-	-
H-2-Ld	156	169	14	LPYQFDQEHGWNVE	ann	0.6	397.72	0.6	-	-	-	-
H-2-Ld	222	234	13	ILPNEEYKRISFL	ann	0.7	1643.53	0.7	-	-	-	-
H-2-Ld	9	17	9	IVGLISFGF	Consensus	0.75	2454.98	0.9	142.50	0.6	-	-

Note: The MHCI binding predictions were made on 01/27/2017 using the IEDB analysis resource Consensus tool which combines predictions from ANN aka NetMHC (4.0), SMM and Comblib. Results are presented by allele, and from lowest to highest percentile rank. Peptides predicted as good binders are all of those with a percentile ranking lower or equal to 1.

Supplementary data 10. LJL143 in silico analysis: murine MHC-I restricted-epitopes prediction - C57BL/6 (H-2-Db and H-2-Kb)

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Db	187	198	12	KNGLFNTQITYL	ann	0.1	649.90	0.1	-	-	-	-
H-2-Db	185	198	14	YHKNGLFNTQITYL	ann	0.2	731.30	0.2	-	-	-	-
H-2-Db	2	9	8	NSINFLSI	Consensus	0.25	2812	0.3	9022.98	0.2	-	-
H-2-Db	10	18	9	VGLISFGFI	Consensus	0.3	1150.05	0.2	2764.52	2.8	3.95e-06	0.3
H-2-Db	186	198	13	HKNGLFNTQITYL	ann	0.3	709.96	0.3	-	-	-	-
H-2-Db	258	267	10	CSNENKTCEL	Consensus	0.5	2538.80	0.6	2024.18	0.4	-	-
H-2-Db	191	198	8	FNTQITYL	Consensus	0.55	10696.34	0.5	14566.33	0.6	-	-
H-2-Db	79	90	12	ISAIKLTSYPKI	ann	0.6	1992.32	0.6	-	-	-	-
H-2-Db	80	91	12	SAIKLTSYPKIN	ann	0.6	4544.35	0.6	-	-	-	-
H-2-Db	188	199	12	NGLFNTQITYLA	ann	0.6	1789.40	0.6	-	-	-	-
H-2-Db	232	243	12	SFLDVYWFQETM	ann	0.6	4527.61	0.6	-	-	-	-
H-2-Db	236	243	8	VYWFQETM	Consensus	0.6	10365.71	0.5	15008.94	0.7	-	-
H-2-Db	19	30	12	VAVKCDGDEYFI	ann	0.7	5125.75	0.7	-	-	-	-
H-2-Db	7	18	12	LSIVGLISFGFI	ann	0.8	6108.60	0.8	-	-	-	-
H-2-Db	220	231	12	SIILPNEEYKRI	ann	0.8	6838.87	0.8	-	-	-	-
H-2-Db	233	244	12	FLDVYWFQETMR	ann	0.8	7660.18	0.8	-	-	-	-
H-2-Db	77	90	14	SCISAIKLTSYPKI	ann	0.8	2239.52	0.8	-	-	-	-
H-2-Db	186	199	14	HKNGLFNTQITYLA	ann	0.8	2428.51	0.8	-	-	-	-
H-2-Db	188	201	14	NGLFNTQITYLAEE	ann	0.8	3203.91	0.8	-	-	-	-
H-2-Db	5	18	14	NFLSIVGLISFGFI	ann	0.9	5323	0.9	-	-	-	-
H-2-Db	78	91	14	CISAIKLTSYPKIN	ann	0.9	5998.56	0.9	-	-	-	-
H-2-Db	187	200	14	KNGLFNTQITYLAE	ann	0.9	3351.34	0.9	-	-	-	-
H-2-Db	218	231	14	KFSIILPNEEYKRI	ann	0.9	4465.92	0.9	-	-	-	-
H-2-Db	221	234	14	IILPNEEYKRISFL	ann	0.9	5670.51	0.9	-	-	-	-
H-2-Db	230	243	14	RISFLDVYWFQETM	ann	0.9	3623.88	0.9	-	-	-	-

Supplementary data 10 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Db	184	195	12	FYHKNGLFNTQI	ann	1.0	10038.44	1	-	-	-	-
H-2-Db	17	30	14	FIVAVKCDGDEYFI	ann	1.0	6733.14	1	-	-	-	-
H-2-Db	80	93	14	SAIKLTSYPKINQN	ann	1.0	7220.13	1	-	-	-	-
H-2-Db	231	244	14	ISFLDVYWFQETMR	ann	1.0	6593.50	1	-	-	-	-
H-2-Db	239	252	14	FQETMRKKPKYPYI	ann	1.0	7206.86	1	-	-	-	-

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Kb	231	243	13	ISFLDVYWFQETM	ann	0.2	195.26	0.2	-	-	-	-
H-2-Kb	230	243	14	RISFLDVYWFQETM	ann	0.2	338.71	0.2	-	-	-	-
H-2-Kb	13	21	9	ISFGFIVAV	Consensus	0.25	31.62	0.2	85.14	0.3	-	-
H-2-Kb	10	21	12	VGLISFGFIVAV	ann	0.3	674.01	0.3	-	-	-	-
H-2-Kb	232	243	12	SFLDVYWFQETM	ann	0.3	689.41	0.3	-	-	-	-
H-2-Kb	8	21	14	SIVGLISFGFIVAV	ann	0.3	764.77	0.3	-	-	-	-
H-2-Kb	221	234	14	IILPNEEYKRISFL	ann	0.3	720.03	0.3	-	-	-	-
H-2-Kb	231	244	14	ISFLDVYWFQETMR	ann	0.3	421.71	0.3	-	-	-	-
H-2-Kb	4	12	9	INFLSIVGL	Consensus	0.3	67.55	0.2	139.67	0.4	-	-
H-2-Kb	9	21	13	IVGLISFGFIVAV	ann	0.4	770.48	0.4	-	-	-	-
H-2-Kb	183	195	13	VFYHKNGLFNTQI	ann	0.4	907.97	0.4	-	-	-	-

Supplementary data 10 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Kb	13	25	13	ISFGFIVAVKCDG	ann	0.5	1227.49	0.5	-	-	-	-
H-2-Kb	183	191	9	VFYHKNGLF	Consensus	0.5	198.06	0.6	132.16	0.4	-	-
H-2-Kb	3	12	10	SINFLSIVGL	Consensus	0.55	531.21	0.3	2384.13	0.8	-	-
H-2-Kb	183	192	10	VFYHKNGLFN	Consensus	0.55	1369.49	0.8	1528.73	0.3	-	-
H-2-Kb	1	12	12	MNSINFLSIVGL	ann	0.6	1191.21	0.6	-	-	-	-
H-2-Kb	4	15	12	INFLSIVGLISF	ann	0.6	1133	0.6	-	-	-	-
H-2-Kb	13	24	12	ISFGFIVAVKCD	ann	0.6	1159.95	0.6	-	-	-	-
H-2-Kb	4	17	14	INFLSIVGLISFGF	ann	0.6	1158.70	0.6	-	-	-	-
H-2-Kb	267	280	14	LVFDTDELMTYALV	ann	0.6	1100.15	0.6	-	-	-	-
H-2-Kb	10	22	13	VGLISFGFIVAVK	ann	0.7	1316.93	0.7	-	-	-	-
H-2-Kb	13	26	14	ISFGFIVAVKCDGD	ann	0.7	1274.44	0.7	-	-	-	-
H-2-Kb	166	174	9	WNVERYNIF	Consensus	0.75	773.71	0.7	232.33	0.8	-	-
H-2-Kb	13	20	8	ISFGFIVA	Consensus	0.75	447.26	0.9	118.78	0.6	-	-
H-2-Kb	183	190	8	VFYHKNGL	Consensus	0.75	218.55	0.8	152.31	0.7	-	-
H-2-Kb	232	244	13	SFLDVYWFQETMR	ann	0.8	1436.17	0.8	-	-	-	-
H-2-Kb	4	13	10	INFLSIVGLI	Consensus	0.8	571.38	0.3	2827.03	1.3	-	-
H-2-Kb	275	282	8	MTYALVKV	Consensus	0.85	291.49	0.8	186.53	0.9	-	-
H-2-Kb	1	13	13	MNSINFLSIVGLI	ann	0.9	1882.30	0.9	-	-	-	-
H-2-Kb	2	14	13	NSINFLSIVGLIS	ann	0.9	2238.18	0.9	-	-	-	-
H-2-Kb	3	15	13	SINFLSIVGLISF	ann	0.9	1696.19	0.9	-	-	-	-
H-2-Kb	4	16	13	INFLSIVGLISFG	ann	0.9	1505.54	0.9	-	-	-	-
H-2-Kb	11	23	13	GLISFGFIVAVKC	ann	0.9	1968.57	0.9	-	-	-	-
H-2-Kb	12	24	13	LISFGFIVAVKCD	ann	0.9	1827.20	0.9	-	-	-	-
H-2-Kb	78	90	13	CISAIKLTSYPKI	ann	0.9	4607.77	0.9	-	-	-	-
H-2-Kb	167	179	13	NVERYNIFKDTRF	ann	0.9	4688.69	0.9	-	-	-	-

Supplementary data 10 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Kb	171	183	13	YNIFKDTRFSTEV	ann	0.9	4055.93	0.9	-	-	-	-
H-2-Kb	178	190	13	RFSTEVFYHKNGL	ann	0.9	1707.37	0.9	-	-	-	-
H-2-Kb	179	191	13	FSTEVFYHKNGLF	ann	0.9	1587.50	0.9	-	-	-	-
H-2-Kb	180	192	13	STEVFYHKNGLFN	ann	0.9	1926.08	0.9	-	-	-	-
H-2-Kb	181	193	13	TEVFYHKNGLFNT	ann	0.9	2984.23	0.9	-	-	-	-
H-2-Kb	182	194	13	EVFYHKNGLFNTQ	ann	0.9	3983.69	0.9	-	-	-	-
H-2-Kb	195	207	13	ITYLAEEDSFSEA	ann	0.9	4223.92	0.9	-	-	-	-
H-2-Kb	222	234	13	ILPNEEYKRISFL	ann	0.9	3829.96	0.9	-	-	-	-
H-2-Kb	230	242	13	RISFLDVYWFQET	ann	0.9	2292.79	0.9	-	-	-	-
H-2-Kb	233	245	13	FLDVYWFQETMRK	ann	0.9	4267.79	0.9	-	-	-	-
H-2-Kb	234	246	13	LDVYWFQETMRKK	ann	0.9	4509.42	0.9	-	-	-	-
H-2-Kb	267	279	13	LVFDTDELMTYAL	ann	0.9	4004.86	0.9	-	-	-	-
H-2-Kb	268	280	13	VFDTDELMTYALV	ann	0.9	1834.96	0.9	-	-	-	-
H-2-Kb	40	47	8	LFFASYGL	Consensus	0.9	412.49	0.9	190.87	0.9	-	-
H-2-Kb	235	243	9	DVYWFQETM	Consensus	0.95	403.51	0.6	361.50	1.3	-	-
H-2-Kb	179	190	12	FSTEVFYHKNGL	ann	1.0	1297.77	1	-	-	-	-
H-2-Kb	180	191	12	STEVFYHKNGLF	ann	1.0	1312.36	1	-	-	-	-
H-2-Kb	67	79	13	FVLNFKTNKKSCI	ann	1.0	5284.72	1	-	-	-	-
H-2-Kb	137	149	13	CSFKDRYIGADPL	ann	1.0	5127.30	1	-	-	-	-
H-2-Kb	281	293	13	KVFTNPESDGSRL	ann	1.0	4886.43	1	-	-	-	-
H-2-Kb	2	15	14	NSINFLSIVGLISF	ann	1.0	1903	1	-	-	-	-
H-2-Kb	3	16	14	SINFLSIVGLISFG	ann	1.0	2258.18	1	-	-	-	-

Supplementary data 10 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
H-2-Kb	3	16	14	SINFLSIVGLISFG	ann	1.0	2258.18	1	-	-	-	-
H-2-Kb	9	22	14	IVGLISFGFIVAVK	ann	1.0	1480.50	1	-	-	-	-
H-2-Kb	10	23	14	VGLISFGFIVAVKC	ann	1.0	1620.10	1	-	-	-	-
H-2-Kb	11	24	14	GLISFGFIVAVKCD	ann	1.0	2146.54	1	-	-	-	-
H-2-Kb	12	25	14	LISFGFIVAVKCDG	ann	1.0	1926.66	1	-	-	-	-
H-2-Kb	177	190	14	TRFSTEVFYHKNGL	ann	1.0	1692.49	1	-	-	-	-
H-2-Kb	178	191	14	RFSTEVFYHKNGLF	ann	1.0	1793.37	1	-	-	-	-
H-2-Kb	180	193	14	STEVFYHKNGLFNT	ann	1.0	2186.32	1	-	-	-	-
H-2-Kb	182	195	14	EVFYHKNGLFNTQI	ann	1.0	1415.24	1	-	-	-	-
H-2-Kb	183	196	14	VFYHKNGLFNTQIT	ann	1.0	1631.52	1	-	-	-	-
H-2-Kb	232	245	14	SFLDVYWFQETMRK	ann	1.0	1848.29	1	-	-	-	-

Note: The MHCI binding predictions were made on 06/20/2017 using the IEDB analysis resource Consensus tool which combines predictions from ANN aka NetMHC (4.0), SMM and Comblib. Results are presented by allele, and from lowest to highest percentile rank. Peptides predicted as good binders are all of those with a percentile ranking lower or equal to 1.

Supplementary data 11. LJL143 in silico analysis: MHC-II restricted-epitopes prediction - Human (99% population coverage)

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*01/DPB1*04:01	225	239	NEEYKRISFLDVYWF	Consensus	0.35	0.15	7.30	67	0.02	7.40	0.35
HLA-DPA1*01/DPB1*04:01	224	238	PNEEYKRISFLDVYW	Consensus	0.37	0.15	7.30	68	0.02	7.70	0.37
HLA-DPA1*01/DPB1*04:01	226	240	EEYKRISFLDVYWFQ	Consensus	0.40	0.15	7.30	70	0.02	8.10	0.40
HLA-DPA1*01/DPB1*04:01	223	237	LPNEEYKRISFLDVY	Consensus	0.46	0.15	7.30	68	0.02	8.90	0.46
HLA-DPA1*01/DPB1*04:01	171	185	YNIFKDTRFSTEVFY	Consensus	0.51	1.69	14.01	66	0.02	9.50	0.51
HLA-DPA1*01/DPB1*04:01	170	184	RYNIFKDTRFSTEVF	Consensus	0.79	1.69	14.01	67	0.02	13.20	0.79
HLA-DPA1*01/DPB1*04:01	173	187	IFKDTRFSTEVFYHK	Consensus	0.82	0.02	3.80	70	0.02	13.60	0.82
HLA-DPA1*01/DPB1*04:01	227	241	EYKRISFLDVYWFQE	Consensus	0.84	0.15	7.30	156	0.13	13.80	0.84
HLA-DPA1*01/DPB1*04:01	169	183	ERYNIFKDTRFSTEV	Consensus	0.91	79.23	32.11	68	0.02	14.80	0.91
HLA-DPA1*01/DPB1*04:01	172	186	NIFKDTRFSTEVFYH	Consensus	1	0.02	3.80	74	0.02	16	1
HLA-DPA1*01/DPB1*04:01	39	53	TLFFASYGLKRDPCQ	Consensus	1.10	3.75	17	108	0.06	17.30	1.10
HLA-DPA1*01/DPB1*04:01	174	188	FKDTRFSTEVFYHKN	Consensus	1.10	0.02	3.80	83	0.03	17.40	1.10
HLA-DPA1*01/DPB1*04:01	38	52	ETLFFASYGLKRDPC	Consensus	1.13	3.75	17	103	0.05	17.80	1.13
HLA-DPA1*01/DPB1*04:01	228	242	YKRISFLDVYWFQET	Consensus	1.14	0.15	7.30	144	0.11	17.90	1.14
HLA-DPA1*01/DPB1*04:01	37	51	DETLFFASYGLKRDP	Consensus	1.19	3.75	17	101	0.05	18.60	1.19
HLA-DPA1*01/DPB1*04:01	36	50	KDETLFFASYGLKRD	Consensus	1.32	3.75	17	100	0.05	20.40	1.32
HLA-DPA1*01/DPB1*04:01	176	190	DTRFSTEVFYHKNGL	Consensus	1.34	0.02	3.80	115	0.06	20.70	1.34
HLA-DPA1*01/DPB1*04:01	177	191	TRFSTEVFYHKNGLF	Consensus	1.46	0.02	3.80	121	0.07	22.40	1.46
HLA-DPA1*01/DPB1*04:01	168	182	VERYNIFKDTRFSTE	Consensus	1.50	79.23	32.11	69	0.02	23	1.50
HLA-DPA1*01/DPB1*04:01	35	49	EKDETLFFASYGLKR	Consensus	1.51	3.75	17	98	0.04	23.20	1.51
HLA-DPA1*01/DPB1*04:01	229	243	KRISFLDVYWFQETM	Consensus	1.51	0.15	7.30	436	1.25	23.10	1.51
HLA-DPA1*01/DPB1*04:01	175	189	KDTRFSTEVFYHKNG	Consensus	1.52	0.02	3.80	118	0.07	23.30	1.52
HLA-DPA1*01/DPB1*04:01	222	236	ILPNEEYKRISFLDV	Consensus	1.67	3417.29	55.84	68	0.02	25.60	1.67
HLA-DPA1*01/DPB1*04:01	230	244	RISFLDVYWFQETMR	Consensus	1.76	0.88	11.85	415	1.13	26.90	1.76

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*01/DPB1*04:01	40	54	LFFASYGLKRDPCQI	Consensus	2.41	59.62	30.49	304	0.59	37.50	2.41
HLA-DPA1*01/DPB1*04:01	231	245	ISFLDVYWFQETMRK	Consensus	2.48	2.29	15.09	431	1.22	38.80	2.48
HLA-DPA1*01/DPB1*04:01	232	246	SFLDVYWFQETMRKK	Consensus	2.87	2.29	15.09	564	2.04	46	2.87
HLA-DPA1*01/DPB1*04:01	178	192	RFSTEVFYHKNGLFN	Consensus	3.80	0.02	3.80	321	0.67	105.70	5.52
HLA-DPA1*01/DPB1*04:01	6	20	FLSIVGLISFGFIVA	Consensus	4.15	0.02	4.15	549	1.94	500.40	14.90
HLA-DPA1*01/DPB1*04:01	7	21	LSIVGLISFGFIVAV	Consensus	4.15	0.02	4.15	495	1.59	490.60	14.74
HLA-DPA1*01/DPB1*04:01	8	22	SIVGLISFGFIVAVK	Consensus	4.15	0.02	4.15	507	1.67	643.10	17.15
HLA-DPA1*01/DPB1*04:01	9	23	IVGLISFGFIVAVKC	Consensus	4.15	0.02	4.15	654	2.68	593.60	16.41
HLA-DPA1*01/DPB1*04:01	10	24	VGLISFGFIVAVKCD	Consensus	4.15	0.02	4.15	725	3.22	715.90	18.18
HLA-DPA1*01/DPB1*04:01	233	247	FLDVYWFQETMRKKP	Consensus	5.84	2.29	15.09	854	4.31	114.80	5.84
HLA-DPA1*01/DPB1*04:01	34	48	KEKDETLFFASYGLK	Consensus	7.02	3.75	17	780	3.67	149.30	7.02

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*12:01	269	283	FDTDELMTYALVKVF	smm	1.06	-	-	191	1.06	-	-
HLA-DRB1*12:01	271	285	TDELMTYALVKVFTN	smm	1.07	-	-	192	1.07	-	-
HLA-DRB1*12:01	272	286	DELMTYALVKVFTNP	smm	1.12	-	-	196	1.12	-	-
HLA-DRB1*12:01	270	284	DTDELMTYALVKVFT	smm	1.13	-	-	197	1.13	-	-
HLA-DRB1*12:01	185	199	YHKNGLFNTQITYLA	smm	1.70	-	-	248	1.70	-	-
HLA-DRB1*12:01	187	201	KNGLFNTQITYLAEE	smm	1.95	-	-	268	1.95	-	-
HLA-DRB1*12:01	186	200	HKNGLFNTQITYLAE	smm	1.97	-	-	269	1.97	-	-
HLA-DRB1*12:01	188	202	NGLFNTQITYLAEED	smm	2.26	-	-	292	2.26	-	-
HLA-DRB1*12:01	273	287	ELMTYALVKVFTNPE	smm	2.62	-	-	320	2.62	-	-
HLA-DRB1*12:01	189	203	GLFNTQITYLAEEDS	smm	3	-	-	348	3	-	-
HLA-DRB1*12:01	274	288	LMTYALVKVFTNPES	smm	3.41	-	-	379	3.41	-	-
HLA-DRB1*12:01	268	282	VFDTDELMTYALVKV	smm	4.16	-	-	434	4.16	-	-

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*01:02/DQB1*06:02	188	202	NGLFNTQITYLAEED	Consensus	3.67	0.69	9.64	196	3.67	58.40	3.66
HLA-DQA1*01:02/DQB1*06:02	187	201	KNGLFNTQITYLAEE	Consensus	4.27	0.69	9.64	198	3.73	65.50	4.27
HLA-DQA1*01:02/DQB1*06:02	189	203	GLFNTQITYLAEEDS	Consensus	5.03	0.69	9.64	211	4.08	74.50	5.03
HLA-DQA1*01:02/DQB1*06:02	10	24	VGLISFGFIVAVKCD	Consensus	5.29	0.01	2.11	254	5.29	282.70	18.48
HLA-DQA1*01:02/DQB1*06:02	11	25	GLISFGFIVAVKCDG	Consensus	5.32	0.01	2.11	255	5.32	211.80	14.68
HLA-DQA1*01:02/DQB1*06:02	9	23	IVGLISFGFIVAVKC	Consensus	5.55	0.01	2.11	263	5.55	1003.30	40.26
HLA-DQA1*01:02/DQB1*06:02	8	22	SIVGLISFGFIVAVK	Consensus	5.81	0.01	2.11	272	5.81	1768	52.08
HLA-DQA1*01:02/DQB1*06:02	190	204	LFNTQITYLAEEDSF	Consensus	6.54	0.69	9.64	231	4.64	92.70	6.54
HLA-DQA1*01:02/DQB1*06:02	74	88	NKKSCISAIKLTSYP	Consensus	6.95	0.14	5.64	312	6.95	160.80	11.56
HLA-DQA1*01:02/DQB1*06:02	73	87	TNKKSCISAIKLTSY	Consensus	7.01	0.14	5.64	314	7.01	243.90	16.48
HLA-DQA1*01:02/DQB1*06:02	75	89	KKSCISAIKLTSYPK	Consensus	7.41	0.14	5.64	328	7.41	169.90	12.14
HLA-DQA1*01:02/DQB1*06:02	1	15	MNSINFLSIVGLISF	Consensus	7.46	2.80	14.55	188	3.46	104.20	7.46

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*05:01/DQB1*03:01	9	23	IVGLISFGFIVAVKC	Consensus	6.15	0.01	0.01	53	15.19	32.50	6.15
HLA-DQA1*05:01/DQB1*03:01	10	24	VGLISFGFIVAVKCD	Consensus	6.27	0.01	0.01	56	15.49	33.20	6.27
HLA-DQA1*05:01/DQB1*03:01	8	22	SIVGLISFGFIVAVK	Consensus	6.70	0.01	0.01	52	15.09	35.60	6.70
HLA-DQA1*05:01/DQB1*03:01	11	25	GLISFGFIVAVKCDG	Consensus	6.95	0.01	0.01	62	16.06	37.10	6.95

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*13:02	115	129	VFKKRKRQIAANIEI	Consensus	3.48	634	15.47	50	3.48	3.70	0.47
HLA-DRB1*13:02	116	130	FKKRKRQIAANIEIY	Consensus	3.77	628	15.35	37.40	2.68	1.90	3.77
HLA-DRB1*13:02	68	82	VLNFKTNKKSCISAI	Consensus	4.69	357	9.75	71.30	4.69	2.20	2.80
HLA-DRB1*13:02	66	80	HFVLNFKTNKKSCIS	Consensus	4.92	376	10.18	75.30	4.92	3.40	0.70
HLA-DRB1*13:02	67	81	FVLNFKTNKKSCISA	Consensus	5	390	10.50	76.80	5	3.40	0.70
HLA-DRB1*13:02	65	79	THFVLNFKTNKKSCI	Consensus	5.29	368	10.01	82.60	5.29	3.40	0.70
HLA-DRB1*13:02	69	83	LNFKTNKKSCISAIK	Consensus	5.97	364	9.92	97.80	5.97	2.20	2.80
HLA-DRB1*13:02	78	92	CISAIKLTSYPKINQ	Consensus	6.55	229	6.55	61.40	4.16	0.20	12.58
HLA-DRB1*13:02	79	93	ISAIKLTSYPKINQN	Consensus	6.55	229	6.55	68.30	4.54	0.20	12.58
HLA-DRB1*13:02	77	91	SCISAIKLTSYPKIN	Consensus	6.57	230	6.57	59.70	4.07	-1.30	22.85
HLA-DRB1*13:02	76	90	KSCISAIKLTSYPKI	Consensus	6.63	232	6.63	65.10	4.35	-1.30	22.85
HLA-DRB1*13:02	179	193	FSTEVFYHKNGLFNT	Consensus	6.70	636	15.51	80.90	5.21	1.20	6.70
HLA-DRB1*13:02	180	194	STEVFYHKNGLFNTQ	Consensus	6.70	635	15.49	70.40	4.65	1.20	6.70
HLA-DRB1*13:02	181	195	TEVFYHKNGLFNTQI	Consensus	6.70	652	15.79	89	5.59	1.20	6.70
HLA-DRB1*13:02	70	84	NFKTNKKSCISAIKL	Consensus	6.87	740	17.38	118.80	6.87	2.20	2.80
HLA-DRB1*13:02	140	154	KDRYIGADPLHVDSY	Consensus	6.99	246	6.99	970.30	25.50	2.50	2.06
HLA-DRB1*13:02	80	94	SAIKLTSYPKINQNS	Consensus	7	236	6.73	121.90	7	0.20	12.58
HLA-DRB1*13:02	139	153	FKDRYIGADPLHVDS	Consensus	7.05	248	7.05	1039.70	26.48	2.50	2.06
HLA-DRB1*13:02	141	155	DRYIGADPLHVDSYG	Consensus	7.05	248	7.05	1384.10	31.03	2.50	2.06
HLA-DRB1*13:02	137	151	CSFKDRYIGADPLHV	Consensus	7.08	249	7.08	1141.50	27.89	2.50	2.06
HLA-DRB1*13:02	138	152	SFKDRYIGADPLHVD	Consensus	7.08	249	7.08	1066.90	26.86	2.50	2.06

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*01:03/DPB1*02:01	226	240	EEYKRISFLDVYWFQ	Consensus	0.02	0.01	0.01	37	0.02	8.30	0.90
HLA-DPA1*01:03/DPB1*02:01	228	242	YKRISFLDVYWFQET	Consensus	0.03	0.01	0.01	53	0.03	7.90	0.84
HLA-DPA1*01:03/DPB1*02:01	227	241	EYKRISFLDVYWFQE	Consensus	0.04	0.01	0.01	65	0.04	8.80	0.97
HLA-DPA1*01:03/DPB1*02:01	229	243	KRISFLDVYWFQETM	Consensus	0.08	0.01	0.01	97	0.08	7.70	0.82
HLA-DPA1*01:03/DPB1*02:01	230	244	RISFLDVYWFQETMR	Consensus	0.08	0.01	0.01	96	0.08	7.60	0.80
HLA-DPA1*01:03/DPB1*02:01	231	245	ISFLDVYWFQETMRK	Consensus	0.10	0.01	0.01	106	0.10	8.90	0.98
HLA-DPA1*01:03/DPB1*02:01	171	185	YNIFKDTRFSTEVFY	Consensus	0.16	3	12.73	66	0.04	3.30	0.16
HLA-DPA1*01:03/DPB1*02:01	232	246	SFLDVYWFQETMRKK	Consensus	0.21	0.01	0.01	153	0.21	10.20	1.16
HLA-DPA1*01:03/DPB1*02:01	172	186	NIFKDTRFSTEVFYH	Consensus	0.22	0.02	2.63	73	0.04	3.70	0.22
HLA-DPA1*01:03/DPB1*02:01	173	187	IFKDTRFSTEVFYHK	Consensus	0.23	0.02	2.63	63	0.03	3.80	0.23
HLA-DPA1*01:03/DPB1*02:01	170	184	RYNIFKDTRFSTEVF	Consensus	0.30	3	12.73	67	0.04	4.20	0.30
HLA-DPA1*01:03/DPB1*02:01	169	183	ERYNIFKDTRFSTEV	Consensus	0.32	3	12.73	69	0.04	4.40	0.32
HLA-DPA1*01:03/DPB1*02:01	168	182	VERYNIFKDTRFSTE	Consensus	0.49	3	12.73	71	0.04	5.50	0.49
HLA-DPA1*01:03/DPB1*02:01	174	188	FKDTRFSTEVFYHKN	Consensus	0.85	0.02	2.63	71	0.04	8	0.85
HLA-DPA1*01:03/DPB1*02:01	225	239	NEEYKRISFLDVYWF	Consensus	0.91	622.64	40.75	39	0.02	8.40	0.91
HLA-DPA1*01:03/DPB1*02:01	224	238	PNEEYKRISFLDVYW	Consensus	0.96	3464.51	52.86	40	0.02	8.70	0.96
HLA-DPA1*01:03/DPB1*02:01	223	237	LPNEEYKRISFLDVY	Consensus	0.97	160.81	32.22	40	0.02	8.80	0.97
HLA-DPA1*01:03/DPB1*02:01	222	236	ILPNEEYKRISFLDV	Consensus	1.25	160.81	32.22	41	0.02	10.80	1.25
HLA-DPA1*01:03/DPB1*02:01	233	247	FLDVYWFQETMRKKP	Consensus	2.26	111.05	30	294	0.58	18.90	2.26
HLA-DPA1*01:03/DPB1*02:01	175	189	KDTRFSTEVFYHKNG	Consensus	2.38	0.02	2.63	81	0.06	20	2.38
HLA-DPA1*01:03/DPB1*02:01	176	190	DTRFSTEVFYHKNGL	Consensus	2.59	0.02	2.63	78	0.05	21.90	2.59
HLA-DPA1*01:03/DPB1*02:01	177	191	TRFSTEVFYHKNGLF	Consensus	2.63	0.02	2.63	80	0.05	32.30	3.65
HLA-DPA1*01:03/DPB1*02:01	178	192	RFSTEVFYHKNGLFN	Consensus	2.63	0.02	2.63	224	0.39	129.20	9.84
HLA-DPA1*01:03/DPB1*02:01	36	50	KDETLFFASYGLKRD	Consensus	3.34	0.11	4.79	140	0.18	29.10	3.34

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*01:03/DPB1*02:01	37	51	DETLFFASYGLKRDP	Consensus	3.47	5.21	14.85	142	0.18	30.40	3.47
HLA-DPA1*01:03/DPB1*02:01	35	49	EKDETLFFASYGLKR	Consensus	3.51	0.11	4.79	139	0.17	30.80	3.51
HLA-DPA1*01:03/DPB1*02:01	38	52	ETLFFASYGLKRDPC	Consensus	3.77	5.21	14.85	143	0.18	33.60	3.77
HLA-DPA1*01:03/DPB1*02:01	265	279	CELVFDTDELMTYAL	Consensus	4.66	575.58	40.27	613	2.21	44.10	4.66
HLA-DPA1*01:03/DPB1*02:01	266	280	ELVFDTDELMTYALV	Consensus	4.74	2.28	11.76	598	2.12	45.10	4.74
HLA-DPA1*01:03/DPB1*02:01	31	45	GKYKEKDETLFFASY	Consensus	4.79	0.11	4.79	866	3.85	331.90	17.22
HLA-DPA1*01:03/DPB1*02:01	32	46	KYKEKDETLFFASYG	Consensus	4.79	0.11	4.79	880	3.93	299.60	16.26
HLA-DPA1*01:03/DPB1*02:01	33	47	YKEKDETLFFASYGL	Consensus	4.79	0.11	4.79	860	3.81	223.80	13.76
HLA-DPA1*01:03/DPB1*02:01	34	48	KEKDETLFFASYGLK	Consensus	4.79	0.11	4.79	275	0.52	103.50	8.54
HLA-DPA1*01:03/DPB1*02:01	76	90	KSCISAIKLTSYPKI	Consensus	4.79	0.11	4.79	223	0.38	328.80	17.13
HLA-DPA1*01:03/DPB1*02:01	77	91	SCISAIKLTSYPKIN	Consensus	4.79	0.11	4.79	224	0.39	320.80	16.90
HLA-DPA1*01:03/DPB1*02:01	78	92	CISAIKLTSYPKINQ	Consensus	4.79	0.11	4.79	224	0.39	308.90	16.54
HLA-DPA1*01:03/DPB1*02:01	79	93	ISAIKLTSYPKINQN	Consensus	4.79	0.11	4.79	226	0.39	361.40	18.05
HLA-DPA1*01:03/DPB1*02:01	80	94	SAIKLTSYPKINQNS	Consensus	4.79	0.11	4.79	237	0.42	500.90	21.51
HLA-DPA1*01:03/DPB1*02:01	81	95	AIKLTSYPKINQNSD	Consensus	4.79	0.11	4.79	683	2.67	1510.50	37.08
HLA-DPA1*01:03/DPB1*02:01	82	96	IKLTSYPKINQNSDL	Consensus	4.79	0.11	4.79	713	2.87	3398.70	52.66
HLA-DPA1*01:03/DPB1*02:01	39	53	TLFFASYGLKRDPCQ	Consensus	4.83	5.21	14.85	186	0.29	46.10	4.83
HLA-DPA1*01:03/DPB1*02:01	197	211	YLAEEDSFSEAREIT	Consensus	5.13	0.02	2.77	1081	5.13	1325.30	34.86
HLA-DPA1*01:03/DPB1*02:01	264	278	TCELVFDTDELMTYA	Consensus	5.33	575.58	40.27	631	2.33	52.50	5.33
HLA-DPA1*01:03/DPB1*02:01	30	44	IGKYKEKDETLFFAS	Consensus	5.46	0.11	4.79	1140	5.46	835.80	27.88
HLA-DPA1*01:03/DPB1*02:01	263	277	KTCELVFDTDELMTY	Consensus	5.90	575.58	40.27	641	2.40	60.30	5.90
HLA-DPA1*01:03/DPB1*02:01	267	281	LVFDTDELMTYALVK	Consensus	5.91	2.28	11.76	667	2.57	60.50	5.91
HLA-DPA1*01:03/DPB1*02:01	40	54	LFFASYGLKRDPCQI	Consensus	7.28	5.21	14.85	418	1.07	81.30	7.28

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*02:01/DPB1*01:01	170	184	RYNIFKDTRFSTEVF	Consensus	0.59	59.84	17.78	108	0.14	11.50	0.59
HLA-DPA1*02:01/DPB1*01:01	171	185	YNIFKDTRFSTEVFY	Consensus	0.60	59.84	17.78	110	0.14	11.60	0.60
HLA-DPA1*02:01/DPB1*01:01	230	244	RISFLDVYWFQETMR	Consensus	0.78	48.93	16.38	201	0.44	13.10	0.78
HLA-DPA1*02:01/DPB1*01:01	232	246	SFLDVYWFQETMRKK	Consensus	0.95	48.93	16.38	230	0.56	14.70	0.95
HLA-DPA1*02:01/DPB1*01:01	231	245	ISFLDVYWFQETMRK	Consensus	1	48.93	16.38	206	0.46	15.10	1
HLA-DPA1*02:01/DPB1*01:01	169	183	ERYNIFKDTRFSTEV	Consensus	1.15	59.84	17.78	109	0.14	16.40	1.15
HLA-DPA1*02:01/DPB1*01:01	233	247	FLDVYWFQETMRKKP	Consensus	1.25	65.86	18.52	263	0.72	17.20	1.25
HLA-DPA1*02:01/DPB1*01:01	172	186	NIFKDTRFSTEVFYH	Consensus	1.28	0.40	1.28	146	0.25	17.60	1.30
HLA-DPA1*02:01/DPB1*01:01	173	187	IFKDTRFSTEVFYHK	Consensus	1.28	0.40	1.28	148	0.25	18.90	1.45
HLA-DPA1*02:01/DPB1*01:01	174	188	FKDTRFSTEVFYHKN	Consensus	1.28	0.40	1.28	182	0.37	42.50	4.40
HLA-DPA1*02:01/DPB1*01:01	175	189	KDTRFSTEVFYHKNG	Consensus	1.28	0.40	1.28	227	0.55	81.30	8.66
HLA-DPA1*02:01/DPB1*01:01	176	190	DTRFSTEVFYHKNGL	Consensus	1.28	0.40	1.28	223	0.53	88.70	9.37
HLA-DPA1*02:01/DPB1*01:01	177	191	TRFSTEVFYHKNGLF	Consensus	1.28	0.40	1.28	249	0.65	110.50	11.31
HLA-DPA1*02:01/DPB1*01:01	228	242	YKRISFLDVYWFQET	Consensus	1.36	48.93	16.38	139	0.23	18.10	1.36
HLA-DPA1*02:01/DPB1*01:01	227	241	EYKRISFLDVYWFQE	Consensus	1.37	48.93	16.38	147	0.25	18.20	1.37
HLA-DPA1*02:01/DPB1*01:01	229	243	KRISFLDVYWFQETM	Consensus	1.42	48.93	16.38	375	1.42	16.60	1.18
HLA-DPA1*02:01/DPB1*01:01	225	239	NEEYKRISFLDVYWF	Consensus	1.65	898.98	41.52	61	0.04	20.50	1.65
HLA-DPA1*02:01/DPB1*01:01	224	238	PNEEYKRISFLDVYW	Consensus	1.74	5892.17	61.34	62	0.04	21.20	1.74
HLA-DPA1*02:01/DPB1*01:01	168	182	VERYNIFKDTRFSTE	Consensus	1.82	59.84	17.78	114	0.16	21.80	1.82
HLA-DPA1*02:01/DPB1*01:01	226	240	EEYKRISFLDVYWFQ	Consensus	1.93	48.93	16.38	59	0.04	22.70	1.93
HLA-DPA1*02:01/DPB1*01:01	223	237	LPNEEYKRISFLDVY	Consensus	2.26	488.23	35.67	61	0.04	25.40	2.26
HLA-DPA1*02:01/DPB1*01:01	186	200	HKNGLFNTQITYLAE	Consensus	2.31	11.28	8.83	44	0.02	25.80	2.31
HLA-DPA1*02:01/DPB1*01:01	187	201	KNGLFNTQITYLAEE	Consensus	2.31	11.28	8.83	43	0.02	25.80	2.31
HLA-DPA1*02:01/DPB1*01:01	178	192	RFSTEVFYHKNGLFN	Consensus	2.58	0.40	1.28	521	2.58	187.40	16.96

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*02:01/DPB1*01:01	185	199	YHKNGLFNTQITYLA	Consensus	2.70	11.28	8.83	44	0.02	28.80	2.70
HLA-DPA1*02:01/DPB1*01:01	234	248	LDVYWFQETMRKKPK	Consensus	2.92	65.86	18.52	340	1.18	30.50	2.92
HLA-DPA1*02:01/DPB1*01:01	188	202	NGLFNTQITYLAEED	Consensus	3	11.28	8.83	50	0.03	31.10	3
HLA-DPA1*02:01/DPB1*01:01	189	203	GLFNTQITYLAEEDS	Consensus	4	11.28	8.83	129	0.20	39.20	4
HLA-DPA1*02:01/DPB1*01:01	235	249	DVYWFQETMRKKPKY	Consensus	4.17	22771.80	73.61	544	2.77	40.60	4.17
HLA-DPA1*02:01/DPB1*01:01	190	204	LFNTQITYLAEEDSF	Consensus	4.62	11.28	8.83	143	0.24	44.40	4.62
HLA-DPA1*02:01/DPB1*01:01	273	287	ELMTYALVKVFTNPE	Consensus	4.92	3.57	4.92	776	4.73	232.80	19.64
HLA-DPA1*02:01/DPB1*01:01	274	288	LMTYALVKVFTNPES	Consensus	4.92	3.57	4.92	737	4.38	243.30	20.21
HLA-DPA1*02:01/DPB1*01:01	184	198	FYHKNGLFNTQITYL	Consensus	5.17	11.28	8.83	45	0.02	49	5.17
HLA-DPA1*02:01/DPB1*01:01	222	236	ILPNEEYKRISFLDV	Consensus	5.26	488.23	35.67	61	0.04	49.80	5.26
HLA-DPA1*02:01/DPB1*01:01	37	51	DETLFFASYGLKRDP	Consensus	5.62	16.55	10.48	151	0.26	52.90	5.62
HLA-DPA1*02:01/DPB1*01:01	36	50	KDETLFFASYGLKRD	Consensus	5.77	16.55	10.48	143	0.24	54.20	5.77
HLA-DPA1*02:01/DPB1*01:01	38	52	ETLFFASYGLKRDPC	Consensus	5.86	16.55	10.48	155	0.28	55	5.86
HLA-DPA1*02:01/DPB1*01:01	236	250	VYWFQETMRKKPKYP	Consensus	6.20	22771.80	73.61	651	3.66	58.10	6.20
HLA-DPA1*02:01/DPB1*01:01	210	224	ITAKDIKKKFSIILP	Consensus	6.32	5.76	6.32	652	3.67	349	25.21
HLA-DPA1*02:01/DPB1*01:01	211	225	TAKDIKKKFSIILPN	Consensus	6.32	5.76	6.32	618	3.38	168.70	15.72
HLA-DPA1*02:01/DPB1*01:01	212	226	AKDIKKKFSIILPNE	Consensus	6.32	5.76	6.32	499	2.40	97	10.12
HLA-DPA1*02:01/DPB1*01:01	213	227	KDIKKKFSIILPNEE	Consensus	6.32	5.76	6.32	436	1.89	95	9.95
HLA-DPA1*02:01/DPB1*01:01	214	228	DIKKKFSIILPNEEY	Consensus	6.32	5.76	6.32	595	3.19	105.70	10.89
HLA-DPA1*02:01/DPB1*01:01	215	229	IKKKFSIILPNEEYK	Consensus	6.32	5.76	6.32	685	3.94	114.70	11.67
HLA-DPA1*02:01/DPB1*01:01	39	53	TLFFASYGLKRDPCQ	Consensus	7.01	16.55	10.48	231	0.57	65.50	7.01
HLA-DPA1*02:01/DPB1*01:01	209	223	EITAKDIKKKFSIIL	Consensus	7.31	5.76	6.32	1046	7.31	592.90	33.51
HLA-DPA1*02:01/DPB1*01:01	35	49	EKDETLFFASYGLKR	Consensus	7.40	16.55	10.48	141	0.23	69	7.40

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*02:01/DPB1*05:01	30	44	IGKYKEKDETLFFAS	Consensus	0.31	0.02	0.31	626	3.54	25.10	0.23
HLA-DPA1*02:01/DPB1*05:01	31	45	GKYKEKDETLFFASY	Consensus	0.31	0.02	0.31	604	3.35	21.10	0.16
HLA-DPA1*02:01/DPB1*05:01	32	46	KYKEKDETLFFASYG	Consensus	0.31	0.02	0.31	637	3.63	20.90	0.16
HLA-DPA1*02:01/DPB1*05:01	33	47	YKEKDETLFFASYGL	Consensus	0.31	0.02	0.31	697	4.14	23.60	0.20
HLA-DPA1*02:01/DPB1*05:01	34	48	KEKDETLFFASYGLK	Consensus	0.31	0.02	0.31	882	5.77	27.10	0.27
HLA-DPA1*02:01/DPB1*05:01	173	187	IFKDTRFSTEVFYHK	Consensus	0.58	2.77	5.05	206	0.58	40.50	0.54
HLA-DPA1*02:01/DPB1*05:01	29	43	FIGKYKEKDETLFFA	Consensus	0.71	0.02	0.31	741	4.52	48	0.71
HLA-DPA1*02:01/DPB1*05:01	174	188	FKDTRFSTEVFYHKN	Consensus	0.76	2.77	5.05	241	0.76	39.50	0.52
HLA-DPA1*02:01/DPB1*05:01	175	189	KDTRFSTEVFYHKNG	Consensus	1.03	2.77	5.05	287	1.03	41	0.55
HLA-DPA1*02:01/DPB1*05:01	176	190	DTRFSTEVFYHKNGL	Consensus	1.18	2.77	5.05	312	1.18	47.60	0.70
HLA-DPA1*02:01/DPB1*05:01	172	186	NIFKDTRFSTEVFYH	Consensus	1.20	2.77	5.05	179	0.45	68.30	1.20
HLA-DPA1*02:01/DPB1*05:01	28	42	YFIGKYKEKDETLFF	Consensus	1.37	0.02	0.31	1934	15.58	74.70	1.37
HLA-DPA1*02:01/DPB1*05:01	171	185	YNIFKDTRFSTEVFY	Consensus	1.92	179.81	23.45	161	0.37	96.80	1.92
HLA-DPA1*02:01/DPB1*05:01	35	49	EKDETLFFASYGLKR	Consensus	2.72	0.35	1.81	845	5.44	129	2.72
HLA-DPA1*02:01/DPB1*05:01	177	191	TRFSTEVFYHKNGLF	Consensus	2.84	2.77	5.05	542	2.84	65.90	1.14
HLA-DPA1*02:01/DPB1*05:01	225	239	NEEYKRISFLDVYWF	Consensus	3.41	1428.47	40.33	467	2.26	157.10	3.41
HLA-DPA1*02:01/DPB1*05:01	226	240	EEYKRISFLDVYWFQ	Consensus	3.41	5.81	6.89	611	3.41	147.10	3.17
HLA-DPA1*02:01/DPB1*05:01	170	184	RYNIFKDTRFSTEVF	Consensus	4.09	179.81	23.45	164	0.38	185.70	4.09
HLA-DPA1*02:01/DPB1*05:01	224	238	PNEEYKRISFLDVYW	Consensus	4.82	1428.47	40.33	462	2.22	218.20	4.82
HLA-DPA1*02:01/DPB1*05:01	168	182	VERYNIFKDTRFSTE	Consensus	4.90	179.81	23.45	174	0.42	221.40	4.90
HLA-DPA1*02:01/DPB1*05:01	178	192	RFSTEVFYHKNGLFN	Consensus	5.05	2.77	5.05	698	4.15	636.70	12.66
HLA-DPA1*02:01/DPB1*05:01	167	181	NVERYNIFKDTRFST	Consensus	5.29	179.81	23.45	496	2.48	238.80	5.29
HLA-DPA1*02:01/DPB1*05:01	211	225	TAKDIKKKFSIILPN	Consensus	5.38	4.52	6.22	838	5.38	59.20	0.98
HLA-DPA1*02:01/DPB1*05:01	212	226	AKDIKKKFSIILPNE	Consensus	5.46	4.52	6.22	847	5.46	50.90	0.78

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*02:01/DPB1*05:01	169	183	ERYNIFKDTRFSTEV	Consensus	5.48	179.81	23.45	168	0.40	247.40	5.48
HLA-DPA1*02:01/DPB1*05:01	213	227	KDIKKKFSIILPNEE	Consensus	6.15	4.52	6.22	924	6.15	44.70	0.63
HLA-DPA1*02:01/DPB1*05:01	210	224	ITAKDIKKKFSIILP	Consensus	6.22	4.52	6.22	1010	6.95	108.70	2.22
HLA-DPA1*02:01/DPB1*05:01	214	228	DIKKKFSIILPNEEY	Consensus	6.22	4.52	6.22	1344	10.06	47.50	0.70
HLA-DPA1*02:01/DPB1*05:01	215	229	IKKKFSIILPNEEYK	Consensus	6.22	4.52	6.22	1769	14.05	53.50	0.84
HLA-DPA1*02:01/DPB1*05:01	223	237	LPNEEYKRISFLDVY	Consensus	6.65	1428.47	40.33	453	2.15	302.90	6.65
HLA-DPA1*02:01/DPB1*05:01	227	241	EYKRISFLDVYWFQE	Consensus	6.89	5.81	6.89	1027	7.11	184.50	4.06
HLA-DPA1*02:01/DPB1*05:01	228	242	YKRISFLDVYWFQET	Consensus	6.89	5.81	6.89	1107	7.84	204.20	4.51
HLA-DPA1*02:01/DPB1*05:01	229	243	KRISFLDVYWFQETM	Consensus	6.89	5.81	6.89	1838	14.69	272.80	6.02
HLA-DPA1*02:01/DPB1*05:01	36	50	KDETLFFASYGLKRD	Consensus	7.26	0.35	1.81	1043	7.26	372.20	8.03

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*03:01/DPB1*04:02	38	52	ETLFFASYGLKRDPC	Consensus	1.59	71.51	26.35	146	0.16	15	1.59
HLA-DPA1*03:01/DPB1*04:02	39	53	TLFFASYGLKRDPCQ	Consensus	2.11	71.51	26.35	152	0.17	18.70	2.11
HLA-DPA1*03:01/DPB1*04:02	37	51	DETLFFASYGLKRDP	Consensus	2.14	71.51	26.35	145	0.15	18.90	2.14
HLA-DPA1*03:01/DPB1*04:02	184	198	FYHKNGLFNTQITYL	Consensus	2.67	0.07	2.67	82	0.05	44.30	5.19
HLA-DPA1*03:01/DPB1*04:02	185	199	YHKNGLFNTQITYLA	Consensus	2.67	0.07	2.67	79	0.04	31.40	3.75
HLA-DPA1*03:01/DPB1*04:02	186	200	HKNGLFNTQITYLAE	Consensus	2.67	0.07	2.67	78	0.04	55.70	6.30
HLA-DPA1*03:01/DPB1*04:02	187	201	KNGLFNTQITYLAEE	Consensus	2.67	0.07	2.67	77	0.04	56.90	6.41
HLA-DPA1*03:01/DPB1*04:02	188	202	NGLFNTQITYLAEED	Consensus	2.67	0.07	2.67	81	0.04	71.90	7.68
HLA-DPA1*03:01/DPB1*04:02	189	203	GLFNTQITYLAEEDS	Consensus	2.67	0.07	2.67	218	0.32	105.80	10.05

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*03:01/DPB1*04:02	190	204	LFNTQITYLAEEDSF	Consensus	2.67	0.07	2.67	217	0.32	159.40	12.97
HLA-DPA1*03:01/DPB1*04:02	40	54	LFFASYGLKRDPCQI	Consensus	2.79	71.51	26.35	360	0.78	23.60	2.79
HLA-DPA1*03:01/DPB1*04:02	36	50	KDETLFFASYGLKRD	Consensus	3.18	71.51	26.35	143	0.15	26.60	3.18
HLA-DPA1*03:01/DPB1*04:02	265	279	CELVFDTDELMTYAL	Consensus	3.74	407.62	38.84	222	0.33	31.30	3.74
HLA-DPA1*03:01/DPB1*04:02	266	280	ELVFDTDELMTYALV	Consensus	3.89	89.77	27.76	259	0.43	32.60	3.89
HLA-DPA1*03:01/DPB1*04:02	230	244	RISFLDVYWFQETMR	Consensus	4.14	120.26	29.97	589	1.99	34.80	4.14
HLA-DPA1*03:01/DPB1*04:02	172	186	NIFKDTRFSTEVFYH	Consensus	4.36	0.23	4.36	244	0.39	96.10	9.41
HLA-DPA1*03:01/DPB1*04:02	173	187	IFKDTRFSTEVFYHK	Consensus	4.36	0.23	4.36	391	0.92	113.40	10.51
HLA-DPA1*03:01/DPB1*04:02	174	188	FKDTRFSTEVFYHKN	Consensus	4.36	0.23	4.36	409	1	341.50	19.64
HLA-DPA1*03:01/DPB1*04:02	175	189	KDTRFSTEVFYHKNG	Consensus	4.36	0.23	4.36	719	2.77	438.20	22.19
HLA-DPA1*03:01/DPB1*04:02	176	190	DTRFSTEVFYHKNGL	Consensus	4.36	0.23	4.36	687	2.58	433.50	22.07
HLA-DPA1*03:01/DPB1*04:02	177	191	TRFSTEVFYHKNGLF	Consensus	4.36	0.23	4.36	720	2.78	539.90	24.47
HLA-DPA1*03:01/DPB1*04:02	231	245	ISFLDVYWFQETMRK	Consensus	4.36	120.26	29.97	558	1.80	36.70	4.36
HLA-DPA1*03:01/DPB1*04:02	232	246	SFLDVYWFQETMRKK	Consensus	4.56	120.26	29.97	670	2.48	38.60	4.56
HLA-DPA1*03:01/DPB1*04:02	267	281	LVFDTDELMTYALVK	Consensus	4.79	89.77	27.76	320	0.62	40.60	4.79
HLA-DPA1*03:01/DPB1*04:02	35	49	EKDETLFFASYGLKR	Consensus	4.85	98.79	28.49	156	0.18	41.20	4.85
HLA-DPA1*03:01/DPB1*04:02	228	242	YKRISFLDVYWFQET	Consensus	4.86	120.26	29.97	201	0.28	41.30	4.86
HLA-DPA1*03:01/DPB1*04:02	233	247	FLDVYWFQETMRKKP	Consensus	4.91	120.26	29.97	743	2.92	41.70	4.91
HLA-DPA1*03:01/DPB1*04:02	264	278	TCELVFDTDELMTYA	Consensus	5	407.62	38.84	231	0.35	42.60	5
HLA-DPA1*03:01/DPB1*04:02	229	243	KRISFLDVYWFQETM	Consensus	5.01	120.26	29.97	633	2.25	42.70	5.01
HLA-DPA1*03:01/DPB1*04:02	227	241	EYKRISFLDVYWFQE	Consensus	5.21	120.26	29.97	217	0.32	44.60	5.21
HLA-DPA1*03:01/DPB1*04:02	41	55	FFASYGLKRDPCQIV	Consensus	5.49	71.51	26.35	450	1.20	47.40	5.49
HLA-DPA1*03:01/DPB1*04:02	168	182	VERYNIFKDTRFSTE	Consensus	5.74	0.48	5.74	241	0.38	197.90	14.71
HLA-DPA1*03:01/DPB1*04:02	169	183	ERYNIFKDTRFSTEV	Consensus	5.74	0.48	5.74	231	0.35	105.50	10.03
HLA-DPA1*03:01/DPB1*04:02	170	184	RYNIFKDTRFSTEVF	Consensus	5.74	0.48	5.74	229	0.35	68	7.35

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*03:01/DPB1*04:02	171	185	YNIFKDTRFSTEVFY	Consensus	5.74	0.48	5.74	228	0.35	66.50	7.23
HLA-DPA1*03:01/DPB1*04:02	6	20	FLSIVGLISFGFIVA	Consensus	6.19	0.59	6.19	187	0.25	279	17.70
HLA-DPA1*03:01/DPB1*04:02	7	21	LSIVGLISFGFIVAV	Consensus	6.19	0.59	6.19	214	0.31	332.60	19.37
HLA-DPA1*03:01/DPB1*04:02	8	22	SIVGLISFGFIVAVK	Consensus	6.19	0.59	6.19	210	0.30	289.60	18.05
HLA-DPA1*03:01/DPB1*04:02	9	23	IVGLISFGFIVAVKC	Consensus	6.19	0.59	6.19	222	0.33	333.50	19.40
HLA-DPA1*03:01/DPB1*04:02	10	24	VGLISFGFIVAVKCD	Consensus	6.19	0.59	6.19	231	0.35	507.70	23.78
HLA-DPA1*03:01/DPB1*04:02	11	25	GLISFGFIVAVKCDG	Consensus	6.19	0.59	6.19	597	2.04	786.10	28.83
HLA-DPA1*03:01/DPB1*04:02	12	26	LISFGFIVAVKCDGD	Consensus	6.19	0.59	6.19	659	2.41	1684.50	39.15
HLA-DPA1*03:01/DPB1*04:02	268	282	VFDTDELMTYALVKV	Consensus	6.55	89.77	27.76	269	0.46	58.50	6.55
HLA-DPA1*03:01/DPB1*04:02	225	239	NEEYKRISFLDVYWF	Consensus	6.75	1709.96	50.72	87	0.05	60.80	6.75
HLA-DPA1*03:01/DPB1*04:02	226	240	EEYKRISFLDVYWFQ	Consensus	6.99	1709.96	50.72	87	0.05	63.80	6.99
HLA-DPA1*03:01/DPB1*04:02	234	248	LDVYWFQETMRKKPK	Consensus	7.43	140	31.12	1112	5.30	68.90	7.43

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*01:01/DQB1*05:01	229	243	KRISFLDVYWFQETM	Consensus	0.05	0.01	0.01	422	0.05	22	0.29
HLA-DQA1*01:01/DQB1*05:01	228	242	YKRISFLDVYWFQET	Consensus	0.12	0.01	0.01	599	0.12	47.70	0.92
HLA-DQA1*01:01/DQB1*05:01	227	241	EYKRISFLDVYWFQE	Consensus	0.20	0.01	0.01	737	0.20	59	1.21
HLA-DQA1*01:01/DQB1*05:01	230	244	RISFLDVYWFQETMR	Consensus	0.28	8.18	11.15	443	0.06	21.30	0.28
HLA-DQA1*01:01/DQB1*05:01	231	245	ISFLDVYWFQETMRK	Consensus	0.29	11.02	12.27	544	0.10	21.80	0.29
HLA-DQA1*01:01/DQB1*05:01	226	240	EEYKRISFLDVYWFQ	Consensus	0.33	0.01	0.01	919	0.33	70.30	1.50
HLA-DQA1*01:01/DQB1*05:01	225	239	NEEYKRISFLDVYWF	Consensus	0.35	0.01	0.01	949	0.35	88	1.93

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*01:01/DQB1*05:01	224	238	PNEEYKRISFLDVYW	Consensus	0.45	0.01	0.01	1069	0.45	377.30	7.32
HLA-DQA1*01:01/DQB1*05:01	232	246	SFLDVYWFQETMRKK	Consensus	0.46	11.02	12.27	636	0.14	29.10	0.46
HLA-DQA1*01:01/DQB1*05:01	233	247	FLDVYWFQETMRKKP	Consensus	0.71	11.02	12.27	831	0.26	39.30	0.71
HLA-DQA1*01:01/DQB1*05:01	160	174	FDQEHGWNVERYNIF	Consensus	1.26	1263.98	40.26	391	0.05	60.90	1.26
HLA-DQA1*01:01/DQB1*05:01	234	248	LDVYWFQETMRKKPK	Consensus	1.50	11.02	12.27	1684	1.14	70.50	1.50
HLA-DQA1*01:01/DQB1*05:01	161	175	DQEHGWNVERYNIFK	Consensus	1.91	1263.98	40.26	479	0.07	87.10	1.91
HLA-DQA1*01:01/DQB1*05:01	159	173	QFDQEHGWNVERYNI	Consensus	1.99	1263.98	40.26	532	0.09	90.60	1.99
HLA-DQA1*01:01/DQB1*05:01	158	172	YQFDQEHGWNVERYN	Consensus	2.60	1263.98	40.26	501	0.08	117.60	2.60
HLA-DQA1*01:01/DQB1*05:01	162	176	QEHGWNVERYNIFKD	Consensus	2.64	1263.98	40.26	930	0.34	119.90	2.64
HLA-DQA1*01:01/DQB1*05:01	264	278	TCELVFDTDELMTYA	Consensus	2.75	28.60	16.37	1113	0.49	124.70	2.75
HLA-DQA1*01:01/DQB1*05:01	10	24	VGLISFGFIVAVKCD	Consensus	2.79	2.06	6.90	863	0.29	126.70	2.79
HLA-DQA1*01:01/DQB1*05:01	149	163	LHVDSYGLPYQFDQE	Consensus	2.82	110.58	23.59	2229	2	127.80	2.82
HLA-DQA1*01:01/DQB1*05:01	263	277	KTCELVFDTDELMTY	Consensus	2.91	28.60	16.37	1032	0.42	131.80	2.91
HLA-DQA1*01:01/DQB1*05:01	9	23	IVGLISFGFIVAVKC	Consensus	3.02	2.06	6.90	843	0.27	136.70	3.02
HLA-DQA1*01:01/DQB1*05:01	150	164	HVDSYGLPYQFDQEH	Consensus	3.07	110.58	23.59	2459	2.41	138.60	3.07
HLA-DQA1*01:01/DQB1*05:01	148	162	PLHVDSYGLPYQFDQ	Consensus	3.32	158.13	25.76	2908	3.28	150.30	3.32
HLA-DQA1*01:01/DQB1*05:01	262	276	NKTCELVFDTDELMT	Consensus	3.37	28.60	16.37	1027	0.42	152.50	3.37
HLA-DQA1*01:01/DQB1*05:01	223	237	LPNEEYKRISFLDVY	Consensus	3.41	0.01	0.01	2969	3.41	1566.50	19.90
HLA-DQA1*01:01/DQB1*05:01	265	279	CELVFDTDELMTYAL	Consensus	3.44	28.60	16.37	1849	1.38	155.70	3.44
HLA-DQA1*01:01/DQB1*05:01	11	25	GLISFGFIVAVKCDG	Consensus	3.48	2.06	6.90	1550	0.97	157.20	3.48
HLA-DQA1*01:01/DQB1*05:01	235	249	DVYWFQETMRKKPKY	Consensus	3.54	11.02	12.27	3032	3.54	123.40	2.72
HLA-DQA1*01:01/DQB1*05:01	12	26	LISFGFIVAVKCDGD	Consensus	3.70	2.06	6.90	2009	1.63	168.20	3.70
HLA-DQA1*01:01/DQB1*05:01	151	165	VDSYGLPYQFDQEHG	Consensus	3.73	110.58	23.59	2370	2.25	169.20	3.73
HLA-DQA1*01:01/DQB1*05:01	147	161	DPLHVDSYGLPYQFD	Consensus	4.02	158.13	25.76	3081	3.64	183.90	4.02

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*01:01/DQB1*05:01	8	22	SIVGLISFGFIVAVK	Consensus	4.09	2.06	6.90	845	0.27	187.20	4.09
HLA-DQA1*01:01/DQB1*05:01	7	21	LSIVGLISFGFIVAV	Consensus	4.32	2.06	6.90	898	0.31	199.60	4.32
HLA-DQA1*01:01/DQB1*05:01	261	275	ENKTCELVFDTDELM	Consensus	4.44	28.60	16.37	1029	0.42	205.90	4.44
HLA-DQA1*01:01/DQB1*05:01	146	160	ADPLHVDSYGLPYQF	Consensus	5.18	158.13	25.76	3462	4.48	246.40	5.18
HLA-DQA1*01:01/DQB1*05:01	266	280	ELVFDTDELMTYALV	Consensus	5.22	59.24	20.07	2700	2.86	248.60	5.22
HLA-DQA1*01:01/DQB1*05:01	168	182	VERYNIFKDTRFSTE	Consensus	5.33	1424.65	41.16	2348	2.21	255	5.33
HLA-DQA1*01:01/DQB1*05:01	1	15	MNSINFLSIVGLISF	Consensus	5.58	0.69	4.52	3928	5.58	2176.40	24.29
HLA-DQA1*01:01/DQB1*05:01	167	181	NVERYNIFKDTRFST	Consensus	6.19	1424.65	41.16	2483	2.45	306.20	6.19
HLA-DQA1*01:01/DQB1*05:01	157	171	PYQFDQEHGWNVERY	Consensus	6.22	1263.98	40.26	483	0.07	307.50	6.22
HLA-DQA1*01:01/DQB1*05:01	169	183	ERYNIFKDTRFSTEV	Consensus	6.28	1424.65	41.16	2605	2.68	311.10	6.28
HLA-DQA1*01:01/DQB1*05:01	163	177	EHGWNVERYNIFKDT	Consensus	6.69	1263.98	40.26	1019	0.41	336.70	6.69
HLA-DQA1*01:01/DQB1*05:01	166	180	WNVERYNIFKDTRFS	Consensus	6.75	1424.65	41.16	1977	1.58	340.40	6.75
HLA-DQA1*01:01/DQB1*05:01	13	27	ISFGFIVAVKCDGDE	Consensus	6.90	2.06	6.90	6771	13.24	256.30	5.35
HLA-DQA1*01:01/DQB1*05:01	170	184	RYNIFKDTRFSTEVF	Consensus	7.17	88.48	22.30	2892	3.24	368.10	7.17
HLA-DQA1*01:01/DQB1*05:01	2	16	NSINFLSIVGLISFG	Consensus	7.43	0.69	4.52	4663	7.43	2581.50	26.95

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*03:01/DQB1*03:02	190	204	LFNTQITYLAEEDSF	Consensus	0.88	0.01	0.01	544	0.88	120.50	1.23
HLA-DQA1*03:01/DQB1*03:02	189	203	GLFNTQITYLAEEDS	Consensus	0.93	0.01	0.01	558	0.93	122.10	1.26
HLA-DQA1*03:01/DQB1*03:02	188	202	NGLFNTQITYLAEED	Consensus	0.94	0.01	0.01	561	0.94	123.70	1.29
HLA-DQA1*03:01/DQB1*03:02	191	205	FNTQITYLAEEDSFS	Consensus	1.20	0.01	0.01	635	1.20	141.20	1.60

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*03:01/DQB1*03:02	193	207	TQITYLAEEDSFSEA	Consensus	1.80	0.01	0.01	1302	4.46	151.30	1.80
HLA-DQA1*03:01/DQB1*03:02	187	201	KNGLFNTQITYLAEE	Consensus	2	400.75	49.07	773	1.76	162	2
HLA-DQA1*03:01/DQB1*03:02	192	206	NTQITYLAEEDSFSE	Consensus	2.08	0.01	0.01	845	2.08	176.40	2.27
HLA-DQA1*03:01/DQB1*03:02	194	208	QITYLAEEDSFSEAR	Consensus	2.52	0.01	0.01	938	2.52	228	3.29
HLA-DQA1*03:01/DQB1*03:02	196	210	TYLAEEDSFSEAREI	Consensus	2.64	1.65	20.70	858	2.14	195.40	2.64
HLA-DQA1*03:01/DQB1*03:02	195	209	ITYLAEEDSFSEARE	Consensus	2.68	0.04	8.56	930	2.48	197.30	2.68
HLA-DQA1*03:01/DQB1*03:02	197	211	YLAEEDSFSEAREIT	Consensus	2.75	80.02	40.51	909	2.38	201.30	2.75
HLA-DQA1*03:01/DQB1*03:02	198	212	LAEEDSFSEAREITA	Consensus	3.37	11472.41	67.40	906	2.37	232.20	3.37
HLA-DQA1*03:01/DQB1*03:02	199	213	AEEDSFSEAREITAK	Consensus	5.87	16722.11	69.14	1539	5.87	332.40	5.36

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*04:01/DQB1*04:02	188	202	NGLFNTQITYLAEED	Consensus	1.40	170.35	35.67	508	0.04	134.40	1.40
HLA-DQA1*04:01/DQB1*04:02	187	201	KNGLFNTQITYLAEE	Consensus	1.42	170.35	35.67	534	0.05	135.80	1.42
HLA-DQA1*04:01/DQB1*04:02	189	203	GLFNTQITYLAEEDS	Consensus	1.64	170.35	35.67	515	0.04	149.40	1.64
HLA-DQA1*04:01/DQB1*04:02	190	204	LFNTQITYLAEEDSF	Consensus	1.79	170.35	35.67	507	0.04	158.10	1.79
HLA-DQA1*04:01/DQB1*04:02	191	205	FNTQITYLAEEDSFS	Consensus	3	3.20	20.51	576	0.06	226.30	3
HLA-DQA1*04:01/DQB1*04:02	192	206	NTQITYLAEEDSFSE	Consensus	3.79	3.20	20.51	1269	0.47	271.80	3.79
HLA-DQA1*04:01/DQB1*04:02	196	210	TYLAEEDSFSEAREI	Consensus	4.05	3.20	20.51	1640	0.86	286.30	4.05
HLA-DQA1*04:01/DQB1*04:02	214	228	DIKKKFSIILPNEEY	Consensus	4.13	1000000	72.30	1691	0.93	291	4.13
HLA-DQA1*04:01/DQB1*04:02	193	207	TQITYLAEEDSFSEA	Consensus	5.33	3.20	20.51	1509	0.71	358.40	5.33
HLA-DQA1*04:01/DQB1*04:02	197	211	YLAEEDSFSEAREIT	Consensus	5.51	3.20	20.51	1719	0.96	368.40	5.51

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*04:01/DQB1*04:02	215	229	IKKKFSIILPNEEYK	Consensus	5.85	1000000	72.30	1710	0.95	387.50	5.85
HLA-DQA1*04:01/DQB1*04:02	172	186	NIFKDTRFSTEVFYH	Consensus	6.66	0.01	0.01	4256	6.66	1868.90	27.12
HLA-DQA1*04:01/DQB1*04:02	213	227	KDIKKKFSIILPNEE	Consensus	6.80	1000000	72.30	2127	1.57	441.20	6.80
HLA-DQA1*04:01/DQB1*04:02	198	212	LAEEDSFSEAREITA	Consensus	7.44	6.18	22.72	1805	1.08	478.50	7.44

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*05:01/DQB1*02:01	263	277	KTCELVFDTDELMTY	Consensus	0.22	95.46	27.03	196	0.17	31.90	0.22
HLA-DQA1*05:01/DQB1*02:01	264	278	TCELVFDTDELMTYA	Consensus	0.27	95.46	27.03	196	0.17	34.70	0.27
HLA-DQA1*05:01/DQB1*02:01	261	275	ENKTCELVFDTDELM	Consensus	0.34	95.46	27.03	244	0.32	39.30	0.34
HLA-DQA1*05:01/DQB1*02:01	262	276	NKTCELVFDTDELMT	Consensus	0.35	95.46	27.03	223	0.25	39.60	0.35
HLA-DQA1*05:01/DQB1*02:01	191	205	FNTQITYLAEEDSFS	Consensus	0.67	1178.56	40.76	324	0.67	36.40	0.29
HLA-DQA1*05:01/DQB1*02:01	265	279	CELVFDTDELMTYAL	Consensus	0.76	95.46	27.03	339	0.76	37.90	0.32
HLA-DQA1*05:01/DQB1*02:01	192	206	NTQITYLAEEDSFSE	Consensus	0.77	8558.51	53.12	342	0.77	42.40	0.40
HLA-DQA1*05:01/DQB1*02:01	190	204	LFNTQITYLAEEDSF	Consensus	1.02	1178.56	40.76	382	1.02	34.10	0.26
HLA-DQA1*05:01/DQB1*02:01	266	280	ELVFDTDELMTYALV	Consensus	1.35	95.46	27.03	431	1.35	45.40	0.46
HLA-DQA1*05:01/DQB1*02:01	193	207	TQITYLAEEDSFSEA	Consensus	1.41	8558.51	53.12	439	1.41	54.30	0.65
HLA-DQA1*05:01/DQB1*02:01	194	208	QITYLAEEDSFSEAR	Consensus	1.71	8558.51	53.12	478	1.71	67.70	0.96
HLA-DQA1*05:01/DQB1*02:01	189	203	GLFNTQITYLAEEDS	Consensus	2.32	1178.56	40.76	549	2.32	69.20	0.99
HLA-DQA1*05:01/DQB1*02:01	195	209	ITYLAEEDSFSEARE	Consensus	5.24	8558.51	53.12	831	5.24	105.40	1.87
HLA-DQA1*05:01/DQB1*02:01	188	202	NGLFNTQITYLAEED	Consensus	5.83	1178.56	40.76	609	2.87	262.10	5.83
HLA-DQA1*05:01/DQB1*02:01	260	274	NENKTCELVFDTDEL	Consensus	5.87	1068.76	40.27	421	1.28	263.70	5.87
HLA-DQA1*05:01/DQB1*02:01	229	243	KRISFLDVYWFQETM	Consensus	7.26	0.16	6.51	1310	11.05	322.20	7.26

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*03:01	41	55	FFASYGLKRDPCQIV	Consensus	0.70	637	0.70	169.10	7.22	4.30	0.60
HLA-DRB1*03:01	167	181	NVERYNIFKDTRFST	Consensus	0.78	192	0.09	86.80	4.47	4.10	0.78
HLA-DRB1*03:01	168	182	VERYNIFKDTRFSTE	Consensus	0.78	191	0.09	58	3.22	4.10	0.78
HLA-DRB1*03:01	169	183	ERYNIFKDTRFSTEV	Consensus	0.78	184	0.08	39.70	2.34	4.10	0.78
HLA-DRB1*03:01	170	184	RYNIFKDTRFSTEVF	Consensus	0.78	179	0.06	29.40	1.72	4.10	0.78
HLA-DRB1*03:01	171	185	YNIFKDTRFSTEVFY	Consensus	0.78	184	0.08	59.40	3.29	4.10	0.78
HLA-DRB1*03:01	172	186	NIFKDTRFSTEVFYH	Consensus	0.78	545	0.57	129.40	6.01	4.10	0.78
HLA-DRB1*03:01	173	187	IFKDTRFSTEVFYHK	Consensus	0.78	548	0.58	268.20	9.65	4.10	0.78
HLA-DRB1*03:01	42	56	FASYGLKRDPCQIVL	Consensus	0.86	725	0.86	126.20	5.90	4.30	0.60
HLA-DRB1*03:01	44	58	SYGLKRDPCQIVLGY	Consensus	0.92	754	0.92	57.30	3.19	4.30	0.60
HLA-DRB1*03:01	45	59	YGLKRDPCQIVLGYK	Consensus	0.93	760	0.93	96.60	4.90	4.30	0.60
HLA-DRB1*03:01	43	57	ASYGLKRDPCQIVLG	Consensus	0.94	765	0.94	98	4.95	4.30	0.60
HLA-DRB1*03:01	104	118	TGGIGTDNCKLVFKK	Consensus	0.96	344	0.22	17.30	0.96	2.20	6.07
HLA-DRB1*03:01	261	275	ENKTCELVFDTDELM	Consensus	1.28	91	0.02	759.50	17.75	3.70	1.28
HLA-DRB1*03:01	262	276	NKTCELVFDTDELMT	Consensus	1.28	90	0.02	349.90	11.35	3.70	1.28
HLA-DRB1*03:01	263	277	KTCELVFDTDELMTY	Consensus	1.28	88	0.02	30.90	1.81	3.70	1.28
HLA-DRB1*03:01	264	278	TCELVFDTDELMTYA	Consensus	1.28	88	0.02	24.10	1.39	3.70	1.28
HLA-DRB1*03:01	265	279	CELVFDTDELMTYAL	Consensus	1.28	88	0.02	22.70	1.32	3.70	1.28
HLA-DRB1*03:01	266	280	ELVFDTDELMTYALV	Consensus	1.28	214	0.11	24.30	1.40	3.70	1.28
HLA-DRB1*03:01	267	281	LVFDTDELMTYALVK	Consensus	1.28	217	0.11	60.20	3.33	3.70	1.28
HLA-DRB1*03:01	103	117	QTGGIGTDNCKLVFK	Consensus	1.53	351	0.24	26.30	1.53	2.20	6.07
HLA-DRB1*03:01	105	119	GGIGTDNCKLVFKKR	Consensus	1.53	346	0.22	26.40	1.53	2.20	6.07
HLA-DRB1*03:01	61	75	SNNQTHFVLNFKTNK	Consensus	1.99	573	0.62	626.10	15.95	3.30	1.99
HLA-DRB1*03:01	62	76	NNQTHFVLNFKTNKK	Consensus	1.99	562	0.60	287.60	10.08	3.30	1.99
HLA-DRB1*03:01	63	77	NQTHFVLNFKTNKKS	Consensus	1.99	562	0.60	162.10	7	3.30	1.99
HLA-DRB1*03:01	64	78	QTHFVLNFKTNKKSC	Consensus	1.99	564	0.61	95.10	4.85	3.30	1.99
HLA-DRB1*03:01	65	79	THFVLNFKTNKKSCI	Consensus	1.99	565	0.61	156.10	6.83	3.30	1.99

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*03:01	66	80	HFVLNFKTNKKSCIS	Consensus	2.17	1428	2.17	261.20	9.48	3.30	1.99
HLA-DRB1*03:01	67	81	FVLNFKTNKKSCISA	Consensus	2.21	1445	2.21	563.10	15.04	3.30	1.99
HLA-DRB1*03:01	106	120	GIGTDNCKLVFKKRK	Consensus	2.72	1026	1.40	47.20	2.72	2.20	6.07
HLA-DRB1*03:01	102	116	CQTGGIGTDNCKLVF	Consensus	2.79	358	0.25	48.40	2.79	2.20	6.07
HLA-DRB1*03:01	208	222	REITAKDIKKKFSII	Consensus	3.08	533	0.56	54.80	3.08	-1.40	29.05
HLA-DRB1*03:01	237	251	YWFQETMRKKPKYPY	Consensus	3.65	1769	2.84	7657.20	54.65	2.75	3.65
HLA-DRB1*03:01	238	252	WFQETMRKKPKYPYI	Consensus	3.66	2176	3.66	6870.20	52.12	2.75	3.65
HLA-DRB1*03:01	239	253	FQETMRKKPKYPYIH	Consensus	3.67	2178	3.67	6580.50	51.12	2.75	3.65
HLA-DRB1*03:01	46	60	GLKRDPCQIVLGYKC	Consensus	3.83	2254	3.83	185.60	7.65	4.30	0.60
HLA-DRB1*03:01	47	61	LKRDPCQIVLGYKCS	Consensus	3.85	2265	3.85	403.20	12.42	4.30	0.60
HLA-DRB1*03:01	240	254	QETMRKKPKYPYIHY	Consensus	4.02	2344	4.02	5323.40	46.57	2.75	3.65
HLA-DRB1*03:01	241	255	ETMRKKPKYPYIHYN	Consensus	4.05	2361	4.05	9102.60	58.67	2.75	3.65
HLA-DRB1*03:01	140	154	KDRYIGADPLHVDSY	Consensus	4.12	2171	3.65	78	4.12	1.60	8.98
HLA-DRB1*03:01	273	287	ELMTYALVKVFTNPE	Consensus	4.36	1497	2.32	3632.30	39.10	2.51	4.36
HLA-DRB1*03:01	1	15	MNSINFLSIVGLISF	Consensus	4.68	2682	4.68	8365.60	56.67	2.70	3.93
HLA-DRB1*03:01	38	52	ETLFFASYGLKRDPC	Consensus	4.70	496	0.51	91.80	4.70	0.50	16.60
HLA-DRB1*03:01	3	17	SINFLSIVGLISFGF	Consensus	4.76	2721	4.76	6427.60	50.64	3.60	1.42
HLA-DRB1*03:01	207	221	AREITAKDIKKKFSI	Consensus	4.82	559	0.60	94.50	4.82	-1.40	29.05
HLA-DRB1*03:01	2	16	NSINFLSIVGLISFG	Consensus	5.01	2830	5.01	8436.10	56.86	2.70	3.93
HLA-DRB1*03:01	15	29	FGFIVAVKCDGDEYF	Consensus	5.24	938	1.25	302.20	10.39	2.30	5.24
HLA-DRB1*03:01	16	30	GFIVAVKCDGDEYFI	Consensus	5.24	1003	1.37	325.80	10.89	2.30	5.24
HLA-DRB1*03:01	17	31	FIVAVKCDGDEYFIG	Consensus	5.24	1057	1.45	395	12.26	2.30	5.24
HLA-DRB1*03:01	107	121	IGTDNCKLVFKKRKR	Consensus	5.24	1031	1.41	106.10	5.24	2.20	6.07
HLA-DRB1*03:01	68	82	VLNFKTNKKSCISAI	Consensus	5.59	2361	4.05	1533.10	25.79	2.27	5.59
HLA-DRB1*03:01	69	83	LNFKTNKKSCISAIK	Consensus	5.59	2477	4.29	2954.20	35.32	2.27	5.59
HLA-DRB1*03:01	209	223	EITAKDIKKKFSIIL	Consensus	5.65	547	0.58	118.40	5.65	0.36	17.96
HLA-DRB1*03:01	101	115	YCQTGGIGTDNCKLV	Consensus	6.06	367	0.26	131.10	6.06	2.20	6.07

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*03:01	37	51	DETLFFASYGLKRDP	Consensus	6.42	494	0.51	141.50	6.42	0.50	16.60
HLA-DRB1*03:01	141	155	DRYIGADPLHVDSYG	Consensus	6.56	2239	3.80	146.60	6.56	1.60	8.98
HLA-DRB1*03:01	14	28	SFGFIVAVKCDGDEY	Consensus	6.67	3611	6.67	439.40	13.07	2.30	5.24
HLA-DRB1*03:01	177	191	TRFSTEVFYHKNGLF	Consensus	6.71	1701	2.71	2762.20	34.19	2	6.71
HLA-DRB1*03:01	178	192	RFSTEVFYHKNGLFN	Consensus	6.71	1736	2.79	2424.30	32.10	2	6.71
HLA-DRB1*03:01	179	193	FSTEVFYHKNGLFNT	Consensus	6.71	1716	2.75	1536.60	25.82	2	6.71
HLA-DRB1*03:01	180	194	STEVFYHKNGLFNTQ	Consensus	6.71	1704	2.72	1002.10	20.68	2	6.71
HLA-DRB1*03:01	181	195	TEVFYHKNGLFNTQI	Consensus	6.71	1716	2.75	1863.50	28.32	2	6.71
HLA-DRB1*03:01	269	283	FDTDELMTYALVKVF	Consensus	6.71	973	1.31	736.90	17.47	2	6.71
HLA-DRB1*03:01	270	284	DTDELMTYALVKVFT	Consensus	6.71	1454	2.23	3991.10	40.87	2	6.71
HLA-DRB1*03:01	271	285	TDELMTYALVKVFTN	Consensus	6.71	1450	2.22	3489.80	38.33	2	6.71
HLA-DRB1*03:01	272	286	DELMTYALVKVFTNP	Consensus	6.71	1524	2.36	3817.60	40.02	2	6.71
HLA-DRB1*03:01	139	153	FKDRYIGADPLHVDS	Consensus	6.77	2202	3.72	153.80	6.77	1.60	8.98
HLA-DRB1*03:01	274	288	LMTYALVKVFTNPES	Consensus	6.83	3686	6.83	2937.40	35.22	2.51	4.36
HLA-DRB1*03:01	13	27	ISFGFIVAVKCDGDE	Consensus	6.92	3730	6.92	909.50	19.62	2.30	5.24
HLA-DRB1*03:01	12	26	LISFGFIVAVKCDGD	Consensus	7	3766	7	1452.20	25.07	2.30	5.24
HLA-DRB1*03:01	11	25	GLISFGFIVAVKCDG	Consensus	7.01	3773	7.01	2598.70	33.22	2.30	5.24
HLA-DRB1*03:01	143	157	YIGADPLHVDSYGLP	Consensus	7.08	3578	6.61	839.50	18.72	2	7.08
HLA-DRB1*03:01	144	158	IGADPLHVDSYGLPY	Consensus	7.08	2885	5.13	3441.60	38.06	2	7.08
HLA-DRB1*03:01	145	159	GADPLHVDSYGLPYQ	Consensus	7.08	2989	5.34	2730.60	34.01	2	7.08
HLA-DRB1*03:01	146	160	ADPLHVDSYGLPYQF	Consensus	7.08	2913	5.19	1301.70	23.74	2	7.08
HLA-DRB1*03:01	147	161	DPLHVDSYGLPYQFD	Consensus	7.08	2898	5.16	1820.10	28.01	2	7.08
HLA-DRB1*03:01	275	289	MTYALVKVFTNPESD	Consensus	7.16	3844	7.16	2110.40	30.06	2.51	4.36
HLA-DRB1*03:01	230	244	RISFLDVYWFQETMR	Consensus	7.40	3961	7.40	1722.20	27.32	2.55	4.35

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:01	234	248	LDVYWFQETMRKKPK	Consensus	1.59	1000	10.76	26.70	1.59	3.90	0.25
HLA-DRB1*04:01	185	199	YHKNGLFNTQITYLA	Consensus	1.64	52	0.36	84.70	6.87	2.30	1.64
HLA-DRB1*04:01	186	200	HKNGLFNTQITYLAE	Consensus	1.64	51	0.35	53.70	4.16	2.30	1.64
HLA-DRB1*04:01	187	201	KNGLFNTQITYLAEE	Consensus	1.64	51	0.35	38.20	2.68	2.30	1.64
HLA-DRB1*04:01	188	202	NGLFNTQITYLAEED	Consensus	1.64	51	0.35	33	2.19	2.30	1.64
HLA-DRB1*04:01	189	203	GLFNTQITYLAEEDS	Consensus	1.64	52	0.36	41.60	3	2.30	1.64
HLA-DRB1*04:01	190	204	LFNTQITYLAEEDSF	Consensus	1.64	155	1.49	40.30	2.88	2.30	1.64
HLA-DRB1*04:01	191	205	FNTQITYLAEEDSFS	Consensus	1.64	126	1.19	29.30	1.83	2.30	1.64
HLA-DRB1*04:01	1	15	MNSINFLSIVGLISF	Consensus	2.36	68	0.51	854.10	39.46	1.90	2.36
HLA-DRB1*04:01	2	16	NSINFLSIVGLISFG	Consensus	2.36	67	0.50	852.70	39.42	1.90	2.36
HLA-DRB1*04:01	3	17	SINFLSIVGLISFGF	Consensus	2.36	66	0.49	332.80	21.99	1.90	2.36
HLA-DRB1*04:01	4	18	INFLSIVGLISFGFI	Consensus	2.36	68	0.51	437.30	26.43	1.90	2.36
HLA-DRB1*04:01	5	19	NFLSIVGLISFGFIV	Consensus	2.36	159	1.54	467.20	27.62	1.90	2.36
HLA-DRB1*04:01	6	20	FLSIVGLISFGFIVA	Consensus	2.36	151	1.45	516.60	29.43	1.90	2.36
HLA-DRB1*04:01	194	208	QITYLAEEDSFSEAR	Consensus	2.36	286	3	30.40	1.94	1.90	2.36
HLA-DRB1*04:01	264	278	TCELVFDTDELMTYA	Consensus	2.47	298	3.13	35.90	2.47	2	2.16
HLA-DRB1*04:01	233	247	FLDVYWFQETMRKKP	Consensus	2.50	1019	10.94	36.20	2.50	3.90	0.25
HLA-DRB1*04:01	235	249	DVYWFQETMRKKPKY	Consensus	2.58	1133	12.05	37.10	2.58	3.90	0.25
HLA-DRB1*04:01	193	207	TQITYLAEEDSFSEA	Consensus	2.72	273	2.85	38.60	2.72	1.90	2.36
HLA-DRB1*04:01	192	206	NTQITYLAEEDSFSE	Consensus	2.93	280	2.93	48.70	3.68	1.90	2.36
HLA-DRB1*04:01	195	209	ITYLAEEDSFSEARE	Consensus	3.04	290	3.04	48.80	3.69	1.90	2.36
HLA-DRB1*04:01	53	67	QIVLGYKCSNNQTHF	Consensus	3.10	295	3.10	234	16.95	3.28	0.61
HLA-DRB1*04:01	52	66	CQIVLGYKCSNNQTH	Consensus	3.14	299	3.14	260.20	18.37	3.28	0.61
HLA-DRB1*04:01	265	279	CELVFDTDELMTYAL	Consensus	3.14	299	3.14	44.90	3.31	2	2.16
HLA-DRB1*04:01	232	246	SFLDVYWFQETMRKK	Consensus	3.19	1366	14.23	43.60	3.19	3.90	0.25
HLA-DRB1*04:01	263	277	KTCELVFDTDELMTY	Consensus	3.25	308	3.25	56.20	4.39	2	2.16
HLA-DRB1*04:01	54	68	IVLGYKCSNNQTHFV	Consensus	3.29	313	3.29	229.30	16.69	3.28	0.61
HLA-DRB1*04:01	65	79	THFVLNFKTNKKSCI	Consensus	3.33	316	3.33	86.50	7.02	1.80	2.56

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:01	274	288	LMTYALVKVFTNPES	Consensus	3.34	198	2	45.20	3.34	1	4.78
HLA-DRB1*04:01	276	290	TYALVKVFTNPESDG	Consensus	3.35	281	2.94	45.30	3.35	1	4.78
HLA-DRB1*04:01	249	263	YPYIHYNGECSNENK	Consensus	3.39	322	3.39	235.70	17.04	2.70	1.19
HLA-DRB1*04:01	66	80	HFVLNFKTNKKSCIS	Consensus	3.43	325	3.43	162.40	12.63	1.80	2.56
HLA-DRB1*04:01	248	262	KYPYIHYNGECSNEN	Consensus	3.44	326	3.44	234.80	16.99	2.70	1.19
HLA-DRB1*04:01	63	77	NQTHFVLNFKTNKKS	Consensus	3.46	328	3.46	109.30	8.84	1.80	2.56
HLA-DRB1*04:01	64	78	QTHFVLNFKTNKKSC	Consensus	3.59	338	3.59	86.20	6.99	1.80	2.56
HLA-DRB1*04:01	275	289	MTYALVKVFTNPESD	Consensus	3.69	206	2.09	48.80	3.69	1	4.78
HLA-DRB1*04:01	262	276	NKTCELVFDTDELMT	Consensus	3.93	366	3.93	124.70	9.99	2	2.16
HLA-DRB1*04:01	55	69	VLGYKCSNNQTHFVL	Consensus	4.03	374	4.03	186.30	14.15	3.28	0.61
HLA-DRB1*04:01	62	76	NNQTHFVLNFKTNKK	Consensus	4.05	376	4.05	158.20	12.35	1.80	2.56
HLA-DRB1*04:01	250	264	PYIHYNGECSNENKT	Consensus	4.12	382	4.12	205.70	15.34	2.70	1.19
HLA-DRB1*04:01	261	275	ENKTCELVFDTDELM	Consensus	4.15	385	4.15	238.60	17.20	2	2.16
HLA-DRB1*04:01	251	265	YIHYNGECSNENKTC	Consensus	4.20	389	4.20	197.70	14.85	2.70	1.19
HLA-DRB1*04:01	266	280	ELVFDTDELMTYALV	Consensus	4.33	538	5.88	55.50	4.33	2	2.16
HLA-DRB1*04:01	7	21	LSIVGLISFGFIVAV	Consensus	4.47	279	2.92	606.10	32.44	1.10	4.47
HLA-DRB1*04:01	56	70	LGYKCSNNQTHFVLN	Consensus	4.50	415	4.50	401.70	25	3.28	0.61
HLA-DRB1*04:01	9	23	IVGLISFGFIVAVKC	Consensus	4.52	417	4.52	689.30	34.95	2.18	1.95
HLA-DRB1*04:01	252	266	IHYNGECSNENKTCE	Consensus	4.60	423	4.60	333.70	22.03	2.70	1.19
HLA-DRB1*04:01	236	250	VYWFQETMRKKPKYP	Consensus	4.72	1644	16.61	59.70	4.72	3.90	0.25
HLA-DRB1*04:01	273	287	ELMTYALVKVFTNPE	Consensus	4.78	252	2.61	74.90	6.05	1	4.78
HLA-DRB1*04:01	277	291	YALVKVFTNPESDGS	Consensus	4.78	278	2.91	63.70	5.07	1	4.78
HLA-DRB1*04:01	278	292	ALVKVFTNPESDGSR	Consensus	4.78	403	4.36	133.60	10.65	1	4.78
HLA-DRB1*04:01	267	281	LVFDTDELMTYALVK	Consensus	6.09	557	6.09	102.10	8.28	2	2.16
HLA-DRB1*04:01	196	210	TYLAEEDSFSEAREI	Consensus	6.10	813	8.82	75.50	6.10	1.90	2.36
HLA-DRB1*04:01	231	245	ISFLDVYWFQETMRK	Consensus	6.12	1383	14.38	75.70	6.12	3.90	0.25
HLA-DRB1*04:01	8	22	SIVGLISFGFIVAVK	Consensus	6.38	584	6.38	514	29.34	1.10	4.47
HLA-DRB1*04:01	51	65	PCQIVLGYKCSNNQT	Consensus	7.17	659	7.17	858	39.56	0.70	5.83

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:01	67	81	FVLNFKTNKKSCISA	Consensus	7.17	659	7.17	274.80	19.12	1.80	2.56
HLA-DRB1*04:01	10	24	VGLISFGFIVAVKCD	Consensus	7.34	675	7.34	1070.80	44.54	2.18	1.95
HLA-DRB1*04:01	50	64	DPCQIVLGYKCSNNQ	Consensus	7.38	679	7.38	1028.50	43.60	0.70	5.83
HLA-DRB1*04:01	36	50	KDETLFFASYGLKRD	Consensus	7.43	682	7.43	41.50	2.99	-0.20	9.97

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*01:01	140	154	KDRYIGADPLHVDSY	Consensus	7.28	137.81	46.47	37	7.28	7.90	3.45
HLA-DRB1*01:01	141	155	DRYIGADPLHVDSYG	Consensus	7.48	137.81	46.47	38	7.48	11.70	6.46

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	NetMHCIIpan IC50 (nM)	NetMHCIIpan rank
HLA-DPA1*02:01/DPB1*14:01	232	246	SFLDVYWFQETMRKK	NetMHCIIpan	1.32	-	-	-	-	14.81	1.32
HLA-DPA1*02:01/DPB1*14:01	227	241	EYKRISFLDVYWFQE	NetMHCIIpan	1.65	-	-	-	-	16.42	1.65
HLA-DPA1*02:01/DPB1*14:01	231	245	ISFLDVYWFQETMRK	NetMHCIIpan	1.65	-	-	-	-	16.51	1.65
HLA-DPA1*02:01/DPB1*14:01	36	50	KDETLFFASYGLKRD	NetMHCIIpan	1.76	-	-	-	-	17	1.76
HLA-DPA1*02:01/DPB1*14:01	37	51	DETLFFASYGLKRDP	NetMHCIIpan	1.99	-	-	-	-	18.02	1.99
HLA-DPA1*02:01/DPB1*14:01	230	244	RISFLDVYWFQETMR	NetMHCIIpan	2.07	-	-	-	-	18.53	2.07
HLA-DPA1*02:01/DPB1*14:01	228	242	YKRISFLDVYWFQET	NetMHCIIpan	2.08	-	-	-	-	18.55	2.08
HLA-DPA1*02:01/DPB1*14:01	226	240	EEYKRISFLDVYWFQ	NetMHCIIpan	2.21	-	-	-	-	19	2.21
HLA-DPA1*02:01/DPB1*14:01	38	52	ETLFFASYGLKRDPC	NetMHCIIpan	2.23	-	-	-	-	19.04	2.23

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	NetMHCIIpan IC50 (nM)	NetMHCIIpan rank
HLA-DPA1*02:01/DPB1*14:01	229	243	KRISFLDVYWFQETM	NetMHCIIpan	3	-	-	-	-	21.80	3
HLA-DPA1*02:01/DPB1*14:01	233	247	FLDVYWFQETMRKKP	NetMHCIIpan	3.18	-	-	-	-	22.74	3.18
HLA-DPA1*02:01/DPB1*14:01	39	53	TLFFASYGLKRDPCQ	NetMHCIIpan	3.21	-	-	-	-	23	3.21
HLA-DPA1*02:01/DPB1*14:01	225	239	NEEYKRISFLDVYWF	NetMHCIIpan	3.58	-	-	-	-	24.40	3.58
HLA-DPA1*02:01/DPB1*14:01	224	238	PNEEYKRISFLDVYW	NetMHCIIpan	5.19	-	-	-	-	31.57	5.19
HLA-DPA1*02:01/DPB1*14:01	234	248	LDVYWFQETMRKKPK	NetMHCIIpan	5.37	-	-	-	-	32.19	5.37
HLA-DPA1*02:01/DPB1*14:01	271	285	TDELMTYALVKVFTN	NetMHCIIpan	6.02	-	-	-	-	34.80	6.02
HLA-DPA1*02:01/DPB1*14:01	170	184	RYNIFKDTRFSTEVF	NetMHCIIpan	6.07	-	-	-	-	34.92	6.07
HLA-DPA1*02:01/DPB1*14:01	40	54	LFFASYGLKRDPCQI	NetMHCIIpan	6.23	-	-	-	-	35.48	6.23
HLA-DPA1*02:01/DPB1*14:01	223	237	LPNEEYKRISFLDVY	NetMHCIIpan	6.55	-	-	-	-	37.09	6.55
HLA-DPA1*02:01/DPB1*14:01	272	286	DELMTYALVKVFTNP	NetMHCIIpan	6.82	-	-	-	-	38.46	6.82
HLA-DPA1*02:01/DPB1*14:01	173	187	IFKDTRFSTEVFYHK	NetMHCIIpan	6.88	-	-	-	-	39.01	6.88
HLA-DPA1*02:01/DPB1*14:01	215	229	IKKKFSIILPNEEYK	NetMHCIIpan	7.11	-	-	-	-	40.08	7.11
HLA-DPA1*02:01/DPB1*14:01	171	185	YNIFKDTRFSTEVFY	NetMHCIIpan	7.38	-	-	-	-	41.11	7.38
HLA-DPA1*02:01/DPB1*14:01	187	201	KNGLFNTQITYLAEE	NetMHCIIpan	7.40	-	-	-	-	41.21	7.40
HLA-DPA1*02:01/DPB1*14:01	1	15	MNSINFLSIVGLISF	NetMHCIIpan	7.45	-	-	-	-	41.32	7.45

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:05	275	289	MTYALVKVFTNPESD	Consensus	0.09	37	0.42	7.20	0.09	5.50	0.07
HLA-DRB1*04:05	276	290	TYALVKVFTNPESDG	Consensus	0.10	40	0.46	7.30	0.10	5.50	0.07
HLA-DRB1*04:05	277	291	YALVKVFTNPESDGS	Consensus	0.17	40	0.46	8.60	0.17	5.50	0.07

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:05	274	288	LMTYALVKVFTNPES	Consensus	0.18	38	0.43	8.70	0.18	5.50	0.07
HLA-DRB1*04:05	214	228	DIKKKFSIILPNEEY	Consensus	0.30	48	0.58	8.80	0.18	4.30	0.30
HLA-DRB1*04:05	215	229	IKKKFSIILPNEEYK	Consensus	0.30	48	0.58	7.60	0.11	4.30	0.30
HLA-DRB1*04:05	216	230	KKKFSIILPNEEYKR	Consensus	0.30	51	0.63	7.30	0.10	4.30	0.30
HLA-DRB1*04:05	217	231	KKFSIILPNEEYKRI	Consensus	0.30	53	0.67	9.30	0.21	4.30	0.30
HLA-DRB1*04:05	278	292	ALVKVFTNPESDGSR	Consensus	0.32	110	1.46	10.60	0.32	5.50	0.07
HLA-DRB1*04:05	273	287	ELMTYALVKVFTNPE	Consensus	0.34	39	0.45	10.90	0.34	5.50	0.07
HLA-DRB1*04:05	213	227	KDIKKKFSIILPNEE	Consensus	0.36	48	0.58	11.20	0.36	4.30	0.30
HLA-DRB1*04:05	218	232	KFSIILPNEEYKRIS	Consensus	0.59	162	2.30	13.50	0.59	4.30	0.30
HLA-DRB1*04:05	3	17	SINFLSIVGLISFGF	Consensus	1.14	43	0.50	348.80	23.84	3.15	1.14
HLA-DRB1*04:05	4	18	INFLSIVGLISFGFI	Consensus	1.14	44	0.52	411.40	26.26	3.15	1.14
HLA-DRB1*04:05	279	293	LVKVFTNPESDGSRL	Consensus	1.48	123	1.68	21.70	1.48	5.50	0.07
HLA-DRB1*04:05	5	19	NFLSIVGLISFGFIV	Consensus	1.62	119	1.62	423.80	26.72	3.15	1.14
HLA-DRB1*04:05	6	20	FLSIVGLISFGFIVA	Consensus	1.63	120	1.63	448.10	27.57	3.15	1.14
HLA-DRB1*04:05	219	233	FSIILPNEEYKRISF	Consensus	2.43	169	2.43	37.20	3.38	4.30	0.30
HLA-DRB1*04:05	185	199	YHKNGLFNTQITYLA	Consensus	3.64	247	3.64	88.50	8.65	2.50	2.01
HLA-DRB1*04:05	186	200	HKNGLFNTQITYLAE	Consensus	3.77	257	3.77	109.10	10.37	2.50	2.01
HLA-DRB1*04:05	1	15	MNSINFLSIVGLISF	Consensus	3.83	45	0.54	346.40	23.74	1.80	3.83
HLA-DRB1*04:05	2	16	NSINFLSIVGLISFG	Consensus	3.83	45	0.54	401.90	25.91	1.80	3.83
HLA-DRB1*04:05	187	201	KNGLFNTQITYLAEE	Consensus	3.86	263	3.86	104.20	9.97	2.50	2.01
HLA-DRB1*04:05	168	182	VERYNIFKDTRFSTE	Consensus	3.87	264	3.87	125.20	11.63	2.50	2.17
HLA-DRB1*04:05	224	238	PNEEYKRISFLDVYW	Consensus	3.87	259	3.80	276.20	20.62	1.70	3.87
HLA-DRB1*04:05	225	239	NEEYKRISFLDVYWF	Consensus	3.87	257	3.77	260.20	19.82	1.70	3.87
HLA-DRB1*04:05	188	202	NGLFNTQITYLAEED	Consensus	3.88	265	3.88	118.40	11.11	2.50	2.01
HLA-DRB1*04:05	169	183	ERYNIFKDTRFSTEV	Consensus	3.94	269	3.94	155.50	13.76	2.50	2.17
HLA-DRB1*04:05	189	203	GLFNTQITYLAEEDS	Consensus	3.95	270	3.95	165.70	14.44	2.50	2.01

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:05	226	240	EEYKRISFLDVYWFQ	Consensus	3.95	270	3.95	315.80	22.46	1.70	3.87
HLA-DRB1*04:05	167	181	NVERYNIFKDTRFST	Consensus	4.11	281	4.11	134.60	12.32	2.50	2.17
HLA-DRB1*04:05	222	236	ILPNEEYKRISFLDV	Consensus	4.12	282	4.12	458.40	27.92	1.70	3.87
HLA-DRB1*04:05	223	237	LPNEEYKRISFLDVY	Consensus	4.12	282	4.12	396.50	25.71	1.70	3.87
HLA-DRB1*04:05	166	180	WNVERYNIFKDTRFS	Consensus	4.13	283	4.13	117.20	11.02	2.50	2.17
HLA-DRB1*04:05	165	179	GWNVERYNIFKDTRF	Consensus	4.17	285	4.17	112.80	10.67	2.50	2.17
HLA-DRB1*04:05	7	21	LSIVGLISFGFIVAV	Consensus	4.30	295	4.30	813.30	37.56	3.15	1.14
HLA-DRB1*04:05	271	285	TDELMTYALVKVFTN	Consensus	4.93	335	4.93	507	29.50	2.95	1.38
HLA-DRB1*04:05	272	286	DELMTYALVKVFTNP	Consensus	5.13	348	5.13	415.60	26.42	2.95	1.38
HLA-DRB1*04:05	248	262	KYPYIHYNGECSNEN	Consensus	6.57	447	6.57	278.90	20.75	2.20	2.81
HLA-DRB1*04:05	249	263	YPYIHYNGECSNENK	Consensus	6.59	448	6.59	282.40	20.92	2.20	2.81
HLA-DRB1*04:05	227	241	EYKRISFLDVYWFQE	Consensus	7.28	498	7.28	142.10	12.85	1.70	3.87
HLA-DRB1*04:05	228	242	YKRISFLDVYWFQET	Consensus	7.39	506	7.39	157	13.86	1.70	3.87

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*07:01	165	179	GWNVERYNIFKDTRF	Consensus	1.43	65.54	31.19	121	1.14	9.10	1.43
HLA-DRB1*07:01	166	180	WNVERYNIFKDTRFS	Consensus	1.50	9680.90	66.36	122	1.16	9.40	1.50
HLA-DRB1*07:01	167	181	NVERYNIFKDTRFST	Consensus	1.69	0.54	7.82	121	1.14	10.20	1.69
HLA-DRB1*07:01	168	182	VERYNIFKDTRFSTE	Consensus	2.39	0.54	7.82	121	1.14	13.20	2.39
HLA-DRB1*07:01	169	183	ERYNIFKDTRFSTEV	Consensus	3.63	0.54	7.82	127	1.22	19.10	3.63
HLA-DRB1*07:01	70	84	NFKTNKKSCISAIKL	Consensus	4.10	1.03	9.71	264	2.97	21.20	4.10
HLA-DRB1*07:01	76	90	KSCISAIKLTSYPKI	Consensus	4.79	10.24	20.07	134	1.31	24.80	4.79

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*07:01	137	151	CSFKDRYIGADPLHV	Consensus	4.90	6836.88	63.92	145	1.45	25.40	4.90
HLA-DRB1*07:01	170	184	RYNIFKDTRFSTEVF	Consensus	4.90	0.54	7.82	365	4.26	25.40	4.90
HLA-DRB1*07:01	71	85	FKTNKKSCISAIKLT	Consensus	5.19	1.03	9.71	267	3.02	27	5.19
HLA-DRB1*07:01	35	49	EKDETLFFASYGLKR	Consensus	5.84	2.08	12.46	129	1.24	30.80	5.84
HLA-DRB1*07:01	138	152	SFKDRYIGADPLHVD	Consensus	6.33	6836.88	63.92	148	1.48	33.80	6.33
HLA-DRB1*07:01	36	50	KDETLFFASYGLKRD	Consensus	6.91	2.08	12.46	132	1.28	38	6.91
HLA-DRB1*07:01	72	86	KTNKKSCISAIKLTS	Consensus	7.25	1.03	9.71	441	5.23	40.30	7.25
HLA-DRB1*07:01	77	91	SCISAIKLTSYPKIN	Consensus	7.28	10.24	20.07	136	1.33	40.50	7.28
HLA-DRB1*07:01	171	185	YNIFKDTRFSTEVFY	Consensus	7.43	0.54	7.82	379	4.44	41.50	7.43

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*08:02	276	290	TYALVKVFTNPESDG	Consensus	0.62	247	0.24	57.70	0.62	0.20	6.30
HLA-DRB1*08:02	277	291	YALVKVFTNPESDGS	Consensus	0.64	247	0.24	58.70	0.64	0.20	6.30
HLA-DRB1*08:02	278	292	ALVKVFTNPESDGSR	Consensus	0.90	405	0.57	71.80	0.90	0	7.28
HLA-DRB1*08:02	275	289	MTYALVKVFTNPESD	Consensus	1.27	255	0.25	88.20	1.27	0.20	6.30
HLA-DRB1*08:02	77	91	SCISAIKLTSYPKIN	Consensus	1.74	418	0.60	104.80	1.74	-2.80	23.90
HLA-DRB1*08:02	78	92	CISAIKLTSYPKINQ	Consensus	1.85	870	1.88	109	1.85	2.40	0.96
HLA-DRB1*08:02	274	288	LMTYALVKVFTNPES	Consensus	1.89	249	0.24	110.60	1.89	0.70	4.45
HLA-DRB1*08:02	76	90	KSCISAIKLTSYPKI	Consensus	1.90	403	0.57	110.80	1.90	-2.80	23.90
HLA-DRB1*08:02	79	93	ISAIKLTSYPKINQN	Consensus	1.93	886	1.93	150.60	3.02	2.40	0.96
HLA-DRB1*08:02	114	128	LVFKKRKRQIAANIE	Consensus	2.01	908	2.01	931.30	21.23	4.90	0.02
HLA-DRB1*08:02	113	127	KLVFKKRKRQIAANI	Consensus	2.09	935	2.09	638.30	15.31	4.90	0.02
HLA-DRB1*08:02	123	137	IAANIEIYGIPAKKC	Consensus	2.18	960	2.18	464.60	11.29	2	1.56
HLA-DRB1*08:02	124	138	AANIEIYGIPAKKCS	Consensus	2.24	976	2.24	499.90	12.15	2.20	1.29

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*08:02	125	139	ANIEIYGIPAKKCSF	Consensus	2.51	1056	2.51	563.50	13.67	2.20	1.29
HLA-DRB1*08:02	1	15	MNSINFLSIVGLISF	Consensus	2.97	405	0.57	1997.30	36.20	1.20	2.97
HLA-DRB1*08:02	2	16	NSINFLSIVGLISFG	Consensus	2.97	390	0.53	1628.90	31.78	1.20	2.97
HLA-DRB1*08:02	3	17	SINFLSIVGLISFGF	Consensus	2.97	393	0.55	1607.70	31.50	1.20	2.97
HLA-DRB1*08:02	4	18	INFLSIVGLISFGFI	Consensus	2.97	394	0.55	1867.20	34.69	1.20	2.97
HLA-DRB1*08:02	5	19	NFLSIVGLISFGFIV	Consensus	2.97	724	1.43	2086.50	37.21	1.20	2.97
HLA-DRB1*08:02	6	20	FLSIVGLISFGFIVA	Consensus	2.97	1121	2.72	3882.20	52.30	1.20	2.97
HLA-DRB1*08:02	112	126	CKLVFKKRKRQIAAN	Consensus	3.04	1215	3.04	659.50	15.75	4.90	0.02
HLA-DRB1*08:02	115	129	VFKKRKRQIAANIEI	Consensus	3.25	1277	3.25	829.70	19.24	3.20	0.38
HLA-DRB1*08:02	121	135	RQIAANIEIYGIPAK	Consensus	3.36	1308	3.36	386.50	9.33	2	1.56
HLA-DRB1*08:02	111	125	NCKLVFKKRKRQIAA	Consensus	3.41	1322	3.41	639.50	15.33	4.90	0.02
HLA-DRB1*08:02	116	130	FKKRKRQIAANIEIY	Consensus	3.63	1385	3.63	889.60	20.44	3.20	0.38
HLA-DRB1*08:02	126	140	NIEIYGIPAKKCSFK	Consensus	3.78	1429	3.78	555.10	13.49	2.20	1.29
HLA-DRB1*08:02	209	223	EITAKDIKKKFSIIL	Consensus	4.10	681	1.30	482.50	11.74	0.90	4.10
HLA-DRB1*08:02	210	224	ITAKDIKKKFSIILP	Consensus	4.10	654	1.23	331.80	7.93	0.90	4.10
HLA-DRB1*08:02	110	124	DNCKLVFKKRKRQIA	Consensus	4.12	1521	4.12	1099.50	24.01	4.90	0.02
HLA-DRB1*08:02	65	79	THFVLNFKTNKKSCI	Consensus	4.28	1569	4.28	694.70	16.50	2.20	1.29
HLA-DRB1*08:02	75	89	KKSCISAIKLTSYPK	Consensus	4.30	411	0.59	196.90	4.30	-4.20	30.50
HLA-DRB1*08:02	212	226	AKDIKKKFSIILPNE	Consensus	4.32	711	1.39	197.60	4.32	0.50	5.47
HLA-DRB1*08:02	122	136	QIAANIEIYGIPAKK	Consensus	4.45	1619	4.45	420.30	10.19	2	1.56
HLA-DRB1*08:02	268	282	VFDTDELMTYALVKV	Consensus	4.45	906	2	4114.50	53.75	0.70	4.45
HLA-DRB1*08:02	269	283	FDTDELMTYALVKVF	Consensus	4.45	682	1.30	2481	41.21	0.70	4.45
HLA-DRB1*08:02	270	284	DTDELMTYALVKVFT	Consensus	4.45	671	1.27	1852.20	34.50	0.70	4.45
HLA-DRB1*08:02	271	285	TDELMTYALVKVFTN	Consensus	4.45	659	1.24	1207.60	25.77	0.70	4.45
HLA-DRB1*08:02	272	286	DELMTYALVKVFTNP	Consensus	4.45	663	1.25	896.10	20.58	0.70	4.45
HLA-DRB1*08:02	273	287	ELMTYALVKVFTNPE	Consensus	4.45	420	0.61	224.80	5.08	0.70	4.45
HLA-DRB1*08:02	127	141	IEIYGIPAKKCSFKD	Consensus	4.52	1641	4.52	1161.40	25.04	2.20	1.29
HLA-DRB1*08:02	279	293	LVKVFTNPESDGSRL	Consensus	4.80	722	1.42	215.30	4.80	0	7.28

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*08:02	211	225	TAKDIKKKFSIILPN	Consensus	4.87	706	1.37	217.50	4.87	0.50	5.47
HLA-DRB1*08:02	184	198	FYHKNGLFNTQITYL	Consensus	4.88	1749	4.88	1510.30	30.20	1	3.51
HLA-DRB1*08:02	66	80	HFVLNFKTNKKSCIS	Consensus	4.89	1751	4.89	697.60	16.58	2.20	1.29
HLA-DRB1*08:02	63	77	NQTHFVLNFKTNKKS	Consensus	5.29	1862	5.29	1337	27.75	2.20	1.29
HLA-DRB1*08:02	81	95	AIKLTSYPKINQNSD	Consensus	5.31	1867	5.31	339.90	8.13	2.40	0.96
HLA-DRB1*08:02	82	96	IKLTSYPKINQNSDL	Consensus	5.31	1867	5.31	966.80	21.88	2.40	0.96
HLA-DRB1*08:02	213	227	KDIKKKFSIILPNEE	Consensus	5.47	708	1.38	278	6.46	0.50	5.47
HLA-DRB1*08:02	214	228	DIKKKFSIILPNEEY	Consensus	5.47	1742	4.86	424.10	10.28	0.50	5.47
HLA-DRB1*08:02	215	229	IKKKFSIILPNEEYK	Consensus	5.47	1782	5.01	665.30	15.88	0.50	5.47
HLA-DRB1*08:02	64	78	QTHFVLNFKTNKKSC	Consensus	5.54	1928	5.54	929.20	21.19	2.20	1.29
HLA-DRB1*08:02	181	195	TEVFYHKNGLFNTQI	Consensus	5.59	1941	5.59	814.10	18.95	1	3.51
HLA-DRB1*08:02	62	76	NNQTHFVLNFKTNKK	Consensus	5.70	1969	5.70	2146.70	37.85	1.70	1.93
HLA-DRB1*08:02	80	94	SAIKLTSYPKINQNS	Consensus	5.74	2112	6.20	249.40	5.74	2.40	0.96
HLA-DRB1*08:02	69	83	LNFKTNKKSCISAIK	Consensus	6.33	2144	6.33	2209.40	38.50	2.20	1.29
HLA-DRB1*08:02	84	98	LTSYPKINQNSDLTK	Consensus	6.51	2196	6.51	2666.80	42.98	2.40	0.96
HLA-DRB1*08:02	9	23	IVGLISFGFIVAVKC	Consensus	7.10	2355	7.10	4066.40	53.46	0.50	5.13
HLA-DRB1*08:02	10	24	VGLISFGFIVAVKCD	Consensus	7.28	2403	7.28	4947.80	58.59	0.50	5.13
HLA-DRB1*08:02	67	81	FVLNFKTNKKSCISA	Consensus	7.28	2399	7.28	1176.70	25.28	2.20	1.29
HLA-DRB1*08:02	280	294	VKVFTNPESDGSRLK	Consensus	7.28	2310	6.95	1840.50	34.36	0	7.28

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*09:01	140	154	KDRYIGADPLHVDSY	Consensus	1.10	1000000	61.16	194	1.10	10.70	0.20
HLA-DRB1*09:01	139	153	FKDRYIGADPLHVDS	Consensus	1.12	1000000	61.16	196	1.12	12.40	0.29
HLA-DRB1*09:01	138	152	SFKDRYIGADPLHVD	Consensus	1.13	1000000	61.16	197	1.13	15.70	0.49
HLA-DRB1*09:01	137	151	CSFKDRYIGADPLHV	Consensus	1.14	1000000	61.16	198	1.14	20.70	0.83
HLA-DRB1*09:01	141	155	DRYIGADPLHVDSYG	Consensus	1.15	1000000	61.16	200	1.15	14	0.39
HLA-DRB1*09:01	142	156	RYIGADPLHVDSYGL	Consensus	5.30	1000000	61.16	594	5.30	19.80	0.77
HLA-DRB1*09:01	143	157	YIGADPLHVDSYGLP	Consensus	5.44	1000000	61.16	606	5.44	36.10	2.06
HLA-DRB1*09:01	79	93	ISAIKLTSYPKINQN	Consensus	5.53	2267.63	47.09	391	3.05	80.30	5.53
HLA-DRB1*09:01	225	239	NEEYKRISFLDVYWF	Consensus	6.63	1.38	29.92	412	3.28	95.90	6.63
HLA-DRB1*09:01	271	285	TDELMTYALVKVFTN	Consensus	6.83	0.01	0.01	719	6.83	1319.70	45.21
HLA-DRB1*09:01	78	92	CISAIKLTSYPKINQ	Consensus	6.87	2267.63	47.09	387	3	99.30	6.87
HLA-DRB1*09:01	272	286	DELMTYALVKVFTNP	Consensus	6.97	0.01	0.01	732	6.97	1559.50	49.30
HLA-DRB1*09:01	214	228	DIKKKFSIILPNEEY	Consensus	7.27	8.35	33.95	758	7.27	103.20	7.12
HLA-DRB1*09:01	215	229	IKKKFSIILPNEEYK	Consensus	7.32	39410.81	53.80	762	7.32	75.30	5.18
HLA-DRB1*09:01	80	94	SAIKLTSYPKINQNS	Consensus	7.48	2267.63	47.09	415	3.31	108.80	7.48

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*11:01	1	15	MNSINFLSIVGLISF	Consensus	0.29	63	0.04	190.70	19.82	3.60	0.29
HLA-DRB1*11:01	2	16	NSINFLSIVGLISFG	Consensus	0.29	61	0.03	116	15.02	3.60	0.29
HLA-DRB1*11:01	3	17	SINFLSIVGLISFGF	Consensus	0.29	58	0.03	62.50	10.04	3.60	0.29
HLA-DRB1*11:01	4	18	INFLSIVGLISFGFI	Consensus	0.29	59	0.03	103.10	13.98	3.60	0.29
HLA-DRB1*11:01	5	19	NFLSIVGLISFGFIV	Consensus	0.29	153	0.19	170.60	18.65	3.60	0.29
HLA-DRB1*11:01	6	20	FLSIVGLISFGFIVA	Consensus	0.29	154	0.19	313.40	25.28	3.60	0.29
HLA-DRB1*11:01	111	125	NCKLVFKKRKRQIAA	Consensus	0.50	23	0.01	6.40	0.50	2.70	0.89

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*11:01	110	124	DNCKLVFKKRKRQIA	Consensus	0.61	24	0.01	6.90	0.61	2.70	0.89
HLA-DRB1*11:01	109	123	TDNCKLVFKKRKRQI	Consensus	0.74	39	0.02	7.50	0.74	2.70	0.89
HLA-DRB1*11:01	112	126	CKLVFKKRKRQIAAN	Consensus	0.74	24	0.01	7.50	0.74	2.70	0.89
HLA-DRB1*11:01	61	75	SNNQTHFVLNFKTNK	Consensus	0.78	161	0.21	44.90	7.84	2.80	0.78
HLA-DRB1*11:01	62	76	NNQTHFVLNFKTNKK	Consensus	0.78	155	0.19	25.70	4.73	2.80	0.78
HLA-DRB1*11:01	63	77	NQTHFVLNFKTNKKS	Consensus	0.78	129	0.13	21.50	3.89	2.80	0.78
HLA-DRB1*11:01	64	78	QTHFVLNFKTNKKSC	Consensus	0.78	132	0.14	20.80	3.75	2.80	0.78
HLA-DRB1*11:01	65	79	THFVLNFKTNKKSCI	Consensus	0.78	67	0.04	26.90	4.95	2.80	0.78
HLA-DRB1*11:01	66	80	HFVLNFKTNKKSCIS	Consensus	0.78	83	0.05	30.40	5.59	2.80	0.78
HLA-DRB1*11:01	67	81	FVLNFKTNKKSCISA	Consensus	0.78	81	0.05	32.90	6.01	2.80	0.78
HLA-DRB1*11:01	108	122	GTDNCKLVFKKRKRQ	Consensus	0.89	43	0.02	9.20	1.13	2.70	0.89
HLA-DRB1*11:01	113	127	KLVFKKRKRQIAANI	Consensus	0.89	35	0.02	8.70	1.01	2.70	0.89
HLA-DRB1*11:01	114	128	LVFKKRKRQIAANIE	Consensus	0.89	39	0.02	13.50	2.15	2.70	0.89
HLA-DRB1*11:01	124	138	AANIEIYGIPAKKCS	Consensus	1.66	292	0.55	214.70	21.04	2.10	1.66
HLA-DRB1*11:01	125	139	ANIEIYGIPAKKCSF	Consensus	1.66	285	0.53	93.10	13.13	2.10	1.66
HLA-DRB1*11:01	126	140	NIEIYGIPAKKCSFK	Consensus	1.66	275	0.49	42.20	7.44	2.10	1.66
HLA-DRB1*11:01	127	141	IEIYGIPAKKCSFKD	Consensus	1.66	280	0.51	36.80	6.62	2.10	1.66
HLA-DRB1*11:01	128	142	EIYGIPAKKCSFKDR	Consensus	1.66	350	0.75	54.70	9.10	2.10	1.66
HLA-DRB1*11:01	222	236	ILPNEEYKRISFLDV	Consensus	1.66	461	1.16	783.30	37.18	2.10	1.66
HLA-DRB1*11:01	223	237	LPNEEYKRISFLDVY	Consensus	1.66	486	1.27	440.80	29.42	2.10	1.66
HLA-DRB1*11:01	224	238	PNEEYKRISFLDVYW	Consensus	1.66	509	1.36	215.90	21.10	2.10	1.66
HLA-DRB1*11:01	225	239	NEEYKRISFLDVYWF	Consensus	1.66	500	1.32	112	14.70	2.10	1.66
HLA-DRB1*11:01	226	240	EEYKRISFLDVYWFQ	Consensus	1.66	519	1.40	181	19.25	2.10	1.66
HLA-DRB1*11:01	233	247	FLDVYWFQETMRKKP	Consensus	1.66	141	0.16	68.10	10.68	2.10	1.66
HLA-DRB1*11:01	234	248	LDVYWFQETMRKKPK	Consensus	1.66	124	0.13	37.90	6.80	2.10	1.66
HLA-DRB1*11:01	235	249	DVYWFQETMRKKPKY	Consensus	1.66	124	0.13	27.80	5.12	2.10	1.66
HLA-DRB1*11:01	236	250	VYWFQETMRKKPKYP	Consensus	1.66	126	0.13	20.80	3.75	2.10	1.66
HLA-DRB1*11:01	237	251	YWFQETMRKKPKYPY	Consensus	1.66	128	0.13	25.40	4.66	2.10	1.66

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*11:01	11	25	GLISFGFIVAVKCDG	Consensus	2.02	362	0.79	116.70	15.07	1.90	2.02
HLA-DRB1*11:01	12	26	LISFGFIVAVKCDGD	Consensus	2.02	367	0.80	77.20	11.65	1.90	2.02
HLA-DRB1*11:01	13	27	ISFGFIVAVKCDGDE	Consensus	2.02	374	0.83	62.70	10.06	1.90	2.02
HLA-DRB1*11:01	14	28	SFGFIVAVKCDGDEY	Consensus	2.02	507	1.35	41.80	7.38	1.90	2.02
HLA-DRB1*11:01	15	29	FGFIVAVKCDGDEYF	Consensus	2.02	516	1.39	62.10	10	1.90	2.02
HLA-DRB1*11:01	107	121	IGTDNCKLVFKKRKR	Consensus	2.38	157	0.20	14.50	2.38	-3.10	27.23
HLA-DRB1*11:01	9	23	IVGLISFGFIVAVKC	Consensus	2.65	594	1.69	367.60	27.18	1.60	2.65
HLA-DRB1*11:01	129	143	IYGIPAKKCSFKDRY	Consensus	2.71	819	2.71	80.30	11.95	2.10	1.66
HLA-DRB1*11:01	137	151	CSFKDRYIGADPLHV	Consensus	2.88	859	2.88	725.20	36.11	2.60	1.07
HLA-DRB1*11:01	139	153	FKDRYIGADPLHVDS	Consensus	2.90	862	2.90	204.40	20.52	2.60	1.07
HLA-DRB1*11:01	140	154	KDRYIGADPLHVDSY	Consensus	2.93	868	2.93	103.40	14.01	2.60	1.07
HLA-DRB1*11:01	138	152	SFKDRYIGADPLHVD	Consensus	2.94	871	2.94	442.40	29.46	2.60	1.07
HLA-DRB1*11:01	10	24	VGLISFGFIVAVKCD	Consensus	3.02	889	3.02	298	24.68	1.60	2.65
HLA-DRB1*11:01	141	155	DRYIGADPLHVDSYG	Consensus	3.04	894	3.04	177.10	19.04	2.60	1.07
HLA-DRB1*11:01	75	89	KKSCISAIKLTSYPK	Consensus	3.15	412	0.98	18	3.15	-2.50	23.61
HLA-DRB1*11:01	130	144	YGIPAKKCSFKDRYI	Consensus	4	1096	4	146.60	17.18	2.10	1.66
HLA-DRB1*11:01	78	92	CISAIKLTSYPKINQ	Consensus	4.02	422	1.02	47.90	8.25	1.10	4.02
HLA-DRB1*11:01	79	93	ISAIKLTSYPKINQN	Consensus	4.02	469	1.20	105.10	14.15	1.10	4.02
HLA-DRB1*11:01	80	94	SAIKLTSYPKINQNS	Consensus	4.02	528	1.44	101.70	13.86	1.10	4.02
HLA-DRB1*11:01	81	95	AIKLTSYPKINQNSD	Consensus	4.02	719	2.26	103.50	14.02	1.10	4.02
HLA-DRB1*11:01	82	96	IKLTSYPKINQNSDL	Consensus	4.02	746	2.36	186.70	19.60	1.10	4.02
HLA-DRB1*11:01	274	288	LMTYALVKVFTNPES	Consensus	4.30	228	0.37	30	5.51	1.05	4.30
HLA-DRB1*11:01	275	289	MTYALVKVFTNPESD	Consensus	4.30	276	0.50	51.90	8.75	1.05	4.30
HLA-DRB1*11:01	276	290	TYALVKVFTNPESDG	Consensus	4.30	547	1.52	95.20	13.32	1.05	4.30
HLA-DRB1*11:01	277	291	YALVKVFTNPESDGS	Consensus	4.30	604	1.73	233.90	21.97	1.05	4.30
HLA-DRB1*11:01	232	246	SFLDVYWFQETMRKK	Consensus	4.64	1227	4.64	404.10	28.35	2.10	1.66
HLA-DRB1*11:01	7	21	LSIVGLISFGFIVAV	Consensus	4.70	522	1.41	3518	61.88	0.90	4.70
HLA-DRB1*11:01	8	22	SIVGLISFGFIVAVK	Consensus	4.70	1200	4.51	4503.60	66.47	0.90	4.70

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*11:01	76	90	KSCISAIKLTSYPKI	Consensus	4.95	318	0.63	26.90	4.95	-1.25	16.17
HLA-DRB1*11:01	68	82	VLNFKTNKKSCISAI	Consensus	5.07	98	0.08	43	7.56	0.80	5.07
HLA-DRB1*11:01	69	83	LNFKTNKKSCISAIK	Consensus	5.07	102	0.09	66.80	10.53	0.80	5.07
HLA-DRB1*11:01	70	84	NFKTNKKSCISAIKL	Consensus	5.07	328	0.66	148.30	17.29	0.80	5.07
HLA-DRB1*11:01	71	85	FKTNKKSCISAIKLT	Consensus	5.07	332	0.68	211	20.86	0.80	5.07
HLA-DRB1*11:01	123	137	IAANIEIYGIPAKKC	Consensus	5.07	996	3.51	1169.70	43.13	0.80	5.07
HLA-DRB1*11:01	185	199	YHKNGLFNTQITYLA	Consensus	5.07	607	1.74	566	32.68	0.80	5.07
HLA-DRB1*11:01	186	200	HKNGLFNTQITYLAE	Consensus	5.07	617	1.78	414.60	28.66	0.80	5.07
HLA-DRB1*11:01	187	201	KNGLFNTQITYLAEE	Consensus	5.07	615	1.77	267.20	23.43	0.80	5.07
HLA-DRB1*11:01	188	202	NGLFNTQITYLAEED	Consensus	5.07	624	1.81	206.50	20.63	0.80	5.07
HLA-DRB1*11:01	189	203	GLFNTQITYLAEEDS	Consensus	5.07	569	1.60	386.80	27.80	0.80	5.07
HLA-DRB1*11:01	190	204	LFNTQITYLAEEDSF	Consensus	5.07	1282	4.92	848.20	38.32	0.80	5.07
HLA-DRB1*11:01	191	205	FNTQITYLAEEDSFS	Consensus	5.07	1311	5.07	1538.10	47.50	0.80	5.07
HLA-DRB1*11:01	106	120	GIGTDNCKLVFKKRK	Consensus	5.36	475	1.22	29.10	5.36	-3.10	27.23
HLA-DRB1*11:01	271	285	TDELMTYALVKVFTN	Consensus	5.39	232	0.38	114.90	14.93	0.80	5.39
HLA-DRB1*11:01	272	286	DELMTYALVKVFTNP	Consensus	5.39	236	0.39	56.60	9.34	0.80	5.39
HLA-DRB1*11:01	273	287	ELMTYALVKVFTNPE	Consensus	5.39	224	0.36	34.30	6.24	0.80	5.39
HLA-DRB1*11:01	227	241	EYKRISFLDVYWFQE	Consensus	5.72	1438	5.72	260.90	23.18	2.10	1.66
HLA-DRB1*11:01	228	242	YKRISFLDVYWFQET	Consensus	5.74	1443	5.74	370	27.26	2.10	1.66
HLA-DRB1*11:01	238	252	WFQETMRKKPKYPYI	Consensus	5.82	235	0.39	33.10	6.04	0.60	5.82
HLA-DRB1*11:01	239	253	FQETMRKKPKYPYIH	Consensus	5.82	280	0.51	48.90	8.37	0.60	5.82
HLA-DRB1*11:01	240	254	QETMRKKPKYPYIHY	Consensus	5.82	474	1.22	196.10	20.09	0.60	5.82
HLA-DRB1*11:01	241	255	ETMRKKPKYPYIHYN	Consensus	5.82	587	1.66	282.80	24.08	0.60	5.82
HLA-DRB1*11:01	74	88	NKKSCISAIKLTSYP	Consensus	6.52	454	1.14	36.20	6.52	-2.50	23.61
HLA-DRB1*11:01	243	257	MRKKPKYPYIHYNGE	Consensus	6.91	1669	6.91	532.70	31.88	0.60	5.82
HLA-DRB1*11:01	48	62	KRDPCQIVLGYKCSN	Consensus	7.10	1642	6.77	548.70	32.27	0.30	7.10
HLA-DRB1*11:01	49	63	RDPCQIVLGYKCSNN	Consensus	7.10	1653	6.83	300.20	24.77	0.30	7.10
HLA-DRB1*11:01	50	64	DPCQIVLGYKCSNNQ	Consensus	7.10	1630	6.71	178	19.09	0.30	7.10

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*11:01	52	66	CQIVLGYKCSNNQTH	Consensus	7.10	1083	3.94	247.30	22.57	0.30	7.10
HLA-DRB1*11:01	53	67	QIVLGYKCSNNQTHF	Consensus	7.10	1321	5.12	344.40	26.37	0.30	7.10
HLA-DRB1*11:01	54	68	IVLGYKCSNNQTHFV	Consensus	7.10	1329	5.16	669.60	34.98	0.30	7.10
HLA-DRB1*11:01	115	129	VFKKRKRQIAANIEI	Consensus	7.10	131	0.14	59.10	9.66	0.30	7.10
HLA-DRB1*11:01	116	130	FKKRKRQIAANIEIY	Consensus	7.10	152	0.19	121.90	15.47	0.30	7.10
HLA-DRB1*11:01	51	65	PCQIVLGYKCSNNQT	Consensus	7.10	1708	7.10	161.50	18.11	0.30	7.10
HLA-DRB1*11:01	242	256	TMRKKPKYPYIHYNG	Consensus	7.14	1714	7.14	357.10	26.82	0.60	5.82
HLA-DRB1*11:01	77	91	SCISAIKLTSYPKIN	Consensus	7.25	525	1.42	40.90	7.25	-1.25	16.17
HLA-DRB1*11:01	278	292	ALVKVFTNPESDGSR	Consensus	7.27	1739	7.27	1442.20	46.46	1.05	4.30

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*15:01	6	20	FLSIVGLISFGFIVA	Consensus	0.73	69	0.73	139.20	13.04	4.90	0.43
HLA-DRB1*15:01	7	21	LSIVGLISFGFIVAV	Consensus	0.73	69	0.73	140.50	13.13	4.90	0.43
HLA-DRB1*15:01	5	19	NFLSIVGLISFGFIV	Consensus	0.81	72	0.78	121.50	11.69	4.40	0.81
HLA-DRB1*15:01	36	50	KDETLFFASYGLKRD	Consensus	0.84	53	0.48	14.30	0.84	0.90	14.94
HLA-DRB1*15:01	3	17	SINFLSIVGLISFGF	Consensus	0.85	76	0.85	158.20	14.33	4.40	0.81
HLA-DRB1*15:01	4	18	INFLSIVGLISFGFI	Consensus	0.85	76	0.85	156.10	14.19	4.40	0.81
HLA-DRB1*15:01	37	51	DETLFFASYGLKRDP	Consensus	0.90	55	0.51	14.80	0.90	0.90	14.94
HLA-DRB1*15:01	35	49	EKDETLFFASYGLKR	Consensus	1.06	52	0.46	16.20	1.06	0.90	14.94
HLA-DRB1*15:01	275	289	MTYALVKVFTNPESD	Consensus	1.68	484	9.39	21.30	1.68	4.70	0.57
HLA-DRB1*15:01	276	290	TYALVKVFTNPESDG	Consensus	1.92	539	10.54	23.30	1.92	4.70	0.57
HLA-DRB1*15:01	274	288	LMTYALVKVFTNPES	Consensus	2.01	356	6.82	24	2.01	4.70	0.57
HLA-DRB1*15:01	38	52	ETLFFASYGLKRDPC	Consensus	2.05	141	2.05	15.50	0.98	0.90	14.94
HLA-DRB1*15:01	39	53	TLFFASYGLKRDPCQ	Consensus	2.09	143	2.09	21.20	1.67	0.90	14.94

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*15:01	1	15	MNSINFLSIVGLISF	Consensus	2.21	149	2.21	150.60	13.83	3.90	1.40
HLA-DRB1*15:01	8	22	SIVGLISFGFIVAVK	Consensus	2.32	154	2.32	139.80	13.08	4.90	0.43
HLA-DRB1*15:01	9	23	IVGLISFGFIVAVKC	Consensus	2.33	155	2.33	167.40	14.95	4.90	0.43
HLA-DRB1*15:01	277	291	YALVKVFTNPESDGS	Consensus	2.90	541	10.58	31.40	2.90	4.70	0.57
HLA-DRB1*15:01	211	225	TAKDIKKKFSIILPN	Consensus	3.43	256	4.59	32.70	3.06	3	3.43
HLA-DRB1*15:01	212	226	AKDIKKKFSIILPNE	Consensus	3.43	257	4.62	33	3.10	3	3.43
HLA-DRB1*15:01	213	227	KDIKKKFSIILPNEE	Consensus	3.58	211	3.58	37.80	3.67	3	3.43
HLA-DRB1*15:01	210	224	ITAKDIKKKFSIILP	Consensus	3.67	243	4.30	37.80	3.67	3	3.43
HLA-DRB1*15:01	2	16	NSINFLSIVGLISFG	Consensus	3.74	193	3.18	185.80	16.12	2.95	3.74
HLA-DRB1*15:01	278	292	ALVKVFTNPESDGSR	Consensus	3.90	687	13.25	39.80	3.90	4.70	0.57
HLA-DRB1*15:01	62	76	NNQTHFVLNFKTNKK	Consensus	4.04	186	2.99	54	5.52	2.86	4.04
HLA-DRB1*15:01	63	77	NQTHFVLNFKTNKKS	Consensus	4.04	178	2.81	54	5.52	2.86	4.04
HLA-DRB1*15:01	209	223	EITAKDIKKKFSIIL	Consensus	4.15	238	4.19	41.90	4.15	3	3.43
HLA-DRB1*15:01	273	287	ELMTYALVKVFTNPE	Consensus	4.23	506	9.85	42.60	4.23	4.40	0.81
HLA-DRB1*15:01	65	79	THFVLNFKTNKKSCI	Consensus	4.35	245	4.35	66.90	6.86	2.86	4.04
HLA-DRB1*15:01	64	78	QTHFVLNFKTNKKSC	Consensus	4.72	261	4.72	58.20	5.97	2.86	4.04
HLA-DRB1*15:01	214	228	DIKKKFSIILPNEEY	Consensus	4.86	454	8.78	48	4.86	3	3.43
HLA-DRB1*15:01	271	285	TDELMTYALVKVFTN	Consensus	5.16	280	5.16	367	24.65	4.40	0.81
HLA-DRB1*15:01	269	283	FDTDELMTYALVKVF	Consensus	5.29	286	5.29	492.50	28.83	4.40	0.81
HLA-DRB1*15:01	215	229	IKKKFSIILPNEEYK	Consensus	5.33	437	8.45	52.30	5.33	3	3.43
HLA-DRB1*15:01	76	90	KSCISAIKLTSYPKI	Consensus	5.35	289	5.35	87	8.79	3.20	2.88
HLA-DRB1*15:01	77	91	SCISAIKLTSYPKIN	Consensus	5.44	293	5.44	77.80	7.93	3.20	2.88
HLA-DRB1*15:01	268	282	VFDTDELMTYALVKV	Consensus	5.56	299	5.56	650.60	33.13	4.40	0.81
HLA-DRB1*15:01	272	286	DELMTYALVKVFTNP	Consensus	5.56	299	5.56	497.90	28.99	4.40	0.81
HLA-DRB1*15:01	270	284	DTDELMTYALVKVFT	Consensus	5.59	300	5.59	402.30	25.92	4.40	0.81
HLA-DRB1*15:01	66	80	HFVLNFKTNKKSCIS	Consensus	5.98	317	5.98	125.10	11.98	2.86	4.04
HLA-DRB1*15:01	79	93	ISAIKLTSYPKINQN	Consensus	6.24	329	6.24	80	8.14	3.20	2.88

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*15:01	78	92	CISAIKLTSYPKINQ	Consensus	6.27	330	6.27	73	7.48	3.20	2.88
HLA-DRB1*15:01	279	293	LVKVFTNPESDGSRL	Consensus	6.88	1623	26.60	67	6.88	4.70	0.57

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB5*01:01	3	17	SINFLSIVGLISFGF	Consensus	0.55	60	0.33	337.20	27.99	4.10	0.55
HLA-DRB5*01:01	4	18	INFLSIVGLISFGFI	Consensus	0.55	60	0.33	283.60	25.94	4.10	0.55
HLA-DRB5*01:01	5	19	NFLSIVGLISFGFIV	Consensus	0.67	99	0.67	230.20	23.53	4.10	0.55
HLA-DRB5*01:01	6	20	FLSIVGLISFGFIVA	Consensus	0.67	99	0.67	180.80	20.93	4.10	0.55
HLA-DRB5*01:01	109	123	TDNCKLVFKKRKRQI	Consensus	0.69	101	0.69	4.40	0.66	0.50	10.53
HLA-DRB5*01:01	108	122	GTDNCKLVFKKRKRQ	Consensus	0.78	107	0.75	4.80	0.78	0.50	10.53
HLA-DRB5*01:01	7	21	LSIVGLISFGFIVAV	Consensus	0.85	116	0.85	292.10	26.28	4.10	0.55
HLA-DRB5*01:01	107	121	IGTDNCKLVFKKRKR	Consensus	0.94	127	0.94	4.80	0.78	-1.70	23.94
HLA-DRB5*01:01	1	15	MNSINFLSIVGLISF	Consensus	1.05	74	0.46	1346.30	48.05	3.50	1.05
HLA-DRB5*01:01	2	16	NSINFLSIVGLISFG	Consensus	1.05	76	0.48	1278.60	47.21	3.50	1.05
HLA-DRB5*01:01	110	124	DNCKLVFKKRKRQIA	Consensus	1.07	102	0.70	5.70	1.07	0.50	10.53
HLA-DRB5*01:01	9	23	IVGLISFGFIVAVKC	Consensus	1.11	149	1.11	318.70	27.32	4.10	0.55
HLA-DRB5*01:01	232	246	SFLDVYWFQETMRKK	Consensus	1.13	85	0.54	5.90	1.13	2.30	3.19
HLA-DRB5*01:01	36	50	KDETLFFASYGLKRD	Consensus	1.15	24	0.08	6	1.15	3.10	1.54
HLA-DRB5*01:01	35	49	EKDETLFFASYGLKR	Consensus	1.24	23	0.07	6.30	1.24	3.10	1.54
HLA-DRB5*01:01	37	51	DETLFFASYGLKRDP	Consensus	1.31	25	0.09	6.50	1.31	3.10	1.54
HLA-DRB5*01:01	38	52	ETLFFASYGLKRDPC	Consensus	1.37	25	0.09	6.70	1.37	3.10	1.54
HLA-DRB5*01:01	106	120	GIGTDNCKLVFKKRK	Consensus	1.37	148	1.11	6.70	1.37	-1.70	23.94
HLA-DRB5*01:01	233	247	FLDVYWFQETMRKKP	Consensus	1.50	81	0.51	7.10	1.50	2.30	3.19
HLA-DRB5*01:01	234	248	LDVYWFQETMRKKPK	Consensus	1.50	77	0.48	7.10	1.50	2.30	3.19

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB5*01:01	39	53	TLFFASYGLKRDPCQ	Consensus	1.54	25	0.09	8.80	2.02	3.10	1.54
HLA-DRB5*01:01	40	54	LFFASYGLKRDPCQI	Consensus	1.54	76	0.48	13.50	3.32	3.10	1.54
HLA-DRB5*01:01	41	55	FFASYGLKRDPCQIV	Consensus	1.54	80	0.50	24.50	5.84	3.10	1.54
HLA-DRB5*01:01	231	245	ISFLDVYWFQETMRK	Consensus	1.55	97	0.64	7.30	1.55	2.30	3.19
HLA-DRB5*01:01	111	125	NCKLVFKKRKRQIAA	Consensus	1.59	154	1.16	7.40	1.59	0.50	10.53
HLA-DRB5*01:01	61	75	SNNQTHFVLNFKTNK	Consensus	1.87	136	1.01	46.90	9.56	2.90	1.87
HLA-DRB5*01:01	62	76	NNQTHFVLNFKTNKK	Consensus	1.87	107	0.75	26.60	6.25	2.90	1.87
HLA-DRB5*01:01	63	77	NQTHFVLNFKTNKKS	Consensus	1.87	97	0.64	29.20	6.72	2.90	1.87
HLA-DRB5*01:01	64	78	QTHFVLNFKTNKKSC	Consensus	1.87	100	0.68	32.30	7.27	2.90	1.87
HLA-DRB5*01:01	65	79	THFVLNFKTNKKSCI	Consensus	1.87	97	0.64	22.90	5.51	2.90	1.87
HLA-DRB5*01:01	66	80	HFVLNFKTNKKSCIS	Consensus	1.87	151	1.13	28.60	6.61	2.90	1.87
HLA-DRB5*01:01	67	81	FVLNFKTNKKSCISA	Consensus	1.87	201	1.59	35.20	7.79	2.90	1.87
HLA-DRB5*01:01	235	249	DVYWFQETMRKKPKY	Consensus	2.28	82	0.52	9.70	2.28	2.30	3.19
HLA-DRB5*01:01	213	227	KDIKKKFSIILPNEE	Consensus	2.44	194	1.54	1075.90	44.39	2.60	2.44
HLA-DRB5*01:01	215	229	IKKKFSIILPNEEYK	Consensus	2.44	236	1.90	379.30	29.44	2.60	2.44
HLA-DRB5*01:01	216	230	KKKFSIILPNEEYKR	Consensus	2.44	230	1.85	187.30	21.29	2.60	2.44
HLA-DRB5*01:01	217	231	KKFSIILPNEEYKRI	Consensus	2.44	232	1.87	161.40	19.76	2.60	2.44
HLA-DRB5*01:01	112	126	CKLVFKKRKRQIAAN	Consensus	2.49	202	1.60	10.40	2.49	0.50	10.53
HLA-DRB5*01:01	214	228	DIKKKFSIILPNEEY	Consensus	2.56	310	2.56	1041.50	43.87	2.60	2.44
HLA-DRB5*01:01	24	38	DGDEYFIGKYKEKDE	Consensus	2.67	321	2.67	176	20.64	3.30	1.27
HLA-DRB5*01:01	23	37	CDGDEYFIGKYKEKD	Consensus	2.68	322	2.68	124.20	17.22	3.30	1.27
HLA-DRB5*01:01	8	22	SIVGLISFGFIVAVK	Consensus	3	358	3	411.20	30.47	4.10	0.55
HLA-DRB5*01:01	10	24	VGLISFGFIVAVKCD	Consensus	3.19	214	1.72	599.10	35.55	2.30	3.19
HLA-DRB5*01:01	11	25	GLISFGFIVAVKCDG	Consensus	3.19	203	1.61	579.30	35.10	2.30	3.19
HLA-DRB5*01:01	12	26	LISFGFIVAVKCDGD	Consensus	3.19	207	1.65	591.30	35.37	2.30	3.19
HLA-DRB5*01:01	13	27	ISFGFIVAVKCDGDE	Consensus	3.19	218	1.76	814.90	40.06	2.30	3.19
HLA-DRB5*01:01	68	82	VLNFKTNKKSCISAI	Consensus	3.19	329	2.74	37.40	8.14	2.30	3.19
HLA-DRB5*01:01	123	137	IAANIEIYGIPAKKC	Consensus	3.19	220	1.78	52.30	10.29	2.30	3.19

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB5*01:01	124	138	AANIEIYGIPAKKCS	Consensus	3.19	219	1.77	40.70	8.66	2.30	3.19
HLA-DRB5*01:01	125	139	ANIEIYGIPAKKCSF	Consensus	3.19	212	1.71	27.10	6.34	2.30	3.19
HLA-DRB5*01:01	126	140	NIEIYGIPAKKCSFK	Consensus	3.19	196	1.55	25.80	6.10	2.30	3.19
HLA-DRB5*01:01	127	141	IEIYGIPAKKCSFKD	Consensus	3.19	318	2.64	41.70	8.82	2.30	3.19
HLA-DRB5*01:01	236	250	VYWFQETMRKKPKYP	Consensus	3.19	162	1.23	13.20	3.24	2.30	3.19
HLA-DRB5*01:01	237	251	YWFQETMRKKPKYPY	Consensus	3.19	195	1.55	22.90	5.51	2.30	3.19
HLA-DRB5*01:01	271	285	TDELMTYALVKVFTN	Consensus	3.19	279	2.27	89.20	14.34	2.30	3.19
HLA-DRB5*01:01	272	286	DELMTYALVKVFTNP	Consensus	3.19	343	2.86	123.60	17.18	2.30	3.19
HLA-DRB5*01:01	273	287	ELMTYALVKVFTNPE	Consensus	3.19	331	2.75	119.10	16.83	2.30	3.19
HLA-DRB5*01:01	78	92	CISAIKLTSYPKINQ	Consensus	3.21	160	1.21	13.10	3.21	1	7.95
HLA-DRB5*01:01	218	232	KFSIILPNEEYKRIS	Consensus	3.22	383	3.22	155.30	19.38	2.60	2.44
HLA-DRB5*01:01	219	233	FSIILPNEEYKRISF	Consensus	3.30	394	3.30	276.50	25.65	2.60	2.44
HLA-DRB5*01:01	274	288	LMTYALVKVFTNPES	Consensus	3.39	403	3.39	136.10	18.09	2.30	3.19
HLA-DRB5*01:01	275	289	MTYALVKVFTNPESD	Consensus	3.56	422	3.56	219.20	22.99	2.30	3.19
HLA-DRB5*01:01	26	40	DEYFIGKYKEKDETL	Consensus	3.65	431	3.65	281.70	25.86	3.30	1.27
HLA-DRB5*01:01	25	39	GDEYFIGKYKEKDET	Consensus	3.76	445	3.76	207.20	22.36	3.30	1.27
HLA-DRB5*01:01	77	91	SCISAIKLTSYPKIN	Consensus	4.19	160	1.21	17	4.19	1	7.95
HLA-DRB5*01:01	27	41	EYFIGKYKEKDETLF	Consensus	4.23	500	4.23	761.80	39.06	3.30	1.27
HLA-DRB5*01:01	238	252	WFQETMRKKPKYPYI	Consensus	4.24	501	4.24	179	20.82	2	4.01
HLA-DRB5*01:01	137	151	CSFKDRYIGADPLHV	Consensus	4.34	64	0.38	127.70	17.49	1.90	4.34
HLA-DRB5*01:01	138	152	SFKDRYIGADPLHVD	Consensus	4.34	65	0.39	98.70	15.18	1.90	4.34
HLA-DRB5*01:01	139	153	FKDRYIGADPLHVDS	Consensus	4.34	65	0.39	68.80	12.28	1.90	4.34
HLA-DRB5*01:01	140	154	KDRYIGADPLHVDSY	Consensus	4.34	64	0.38	44.30	9.20	1.90	4.34
HLA-DRB5*01:01	141	155	DRYIGADPLHVDSYG	Consensus	4.34	66	0.40	73.50	12.78	1.90	4.34
HLA-DRB5*01:01	142	156	RYIGADPLHVDSYGL	Consensus	4.34	191	1.51	84.90	13.92	1.90	4.34
HLA-DRB5*01:01	143	157	YIGADPLHVDSYGLP	Consensus	4.34	199	1.58	168.10	20.17	1.90	4.34
HLA-DRB5*01:01	128	142	EIYGIPAKKCSFKDR	Consensus	4.49	528	4.49	60.80	11.35	2.30	3.19
HLA-DRB5*01:01	79	93	ISAIKLTSYPKINQN	Consensus	4.62	156	1.18	18.90	4.62	1	7.95

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB5*01:01	14	28	SFGFIVAVKCDGDEY	Consensus	4.74	560	4.74	1019.50	43.54	2.30	3.19
HLA-DRB5*01:01	15	29	FGFIVAVKCDGDEYF	Consensus	4.82	571	4.82	1384.10	48.51	2.30	3.19
HLA-DRB5*01:01	76	90	KSCISAIKLTSYPKI	Consensus	5.42	161	1.22	22.50	5.42	1	7.95
HLA-DRB5*01:01	22	36	KCDGDEYFIGKYKEK	Consensus	5.53	656	5.53	150.20	19.06	3.30	1.27
HLA-DRB5*01:01	34	48	KEKDETLFFASYGLK	Consensus	5.97	708	5.97	153.30	19.26	2.90	1.87
HLA-DRB5*01:01	55	69	VLGYKCSNNQTHFVL	Consensus	6.50	618	5.22	28	6.50	1	7.95
HLA-DRB5*01:01	80	94	SAIKLTSYPKINQNS	Consensus	6.97	446	3.76	30.60	6.97	1	7.95
HLA-DRB5*01:01	165	179	GWNVERYNIFKDTRF	Consensus	7.46	338	2.81	170.90	20.35	1.20	7.46
HLA-DRB5*01:01	166	180	WNVERYNIFKDTRFS	Consensus	7.46	340	2.83	168.90	20.22	1.20	7.46
HLA-DRB5*01:01	167	181	NVERYNIFKDTRFST	Consensus	7.46	336	2.79	209.10	22.47	1.20	7.46
HLA-DRB5*01:01	168	182	VERYNIFKDTRFSTE	Consensus	7.46	337	2.80	223.10	23.19	1.20	7.46
HLA-DRB5*01:01	169	183	ERYNIFKDTRFSTEV	Consensus	7.46	351	2.93	328.10	27.66	1.20	7.46

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB4*01:01	4	18	INFLSIVGLISFGFI	Consensus	0.84	0.01	0.01	155	0.84	316.10	20.19
HLA-DRB4*01:01	3	17	SINFLSIVGLISFGF	Consensus	0.89	0.01	0.01	161	0.89	286.60	18.85
HLA-DRB4*01:01	5	19	NFLSIVGLISFGFIV	Consensus	1.17	0.01	0.01	194	1.17	912.40	38.71
HLA-DRB4*01:01	213	227	KDIKKKFSIILPNEE	Consensus	3.60	0.01	0.01	456	3.60	282.50	18.64
HLA-DRB4*01:01	6	20	FLSIVGLISFGFIVA	Consensus	3.86	0.01	0.01	484	3.86	995.70	40.46
HLA-DRB4*01:01	7	21	LSIVGLISFGFIVAV	Consensus	4.09	0.01	0.01	507	4.09	1357.50	47.08
HLA-DRB4*01:01	113	127	KLVFKKRKRQIAANI	Consensus	4.73	0.01	0.01	572	4.73	583.70	30.15
HLA-DRB4*01:01	212	226	AKDIKKKFSIILPNE	Consensus	5.01	0.01	0.01	601	5.01	333.20	20.96
HLA-DRB4*01:01	211	225	TAKDIKKKFSIILPN	Consensus	5.06	0.01	0.01	607	5.06	371.80	22.59

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB4*01:01	214	228	DIKKKFSIILPNEEY	Consensus	5.26	0.01	0.01	626	5.26	302.10	19.57
HLA-DRB4*01:01	112	126	CKLVFKKRKRQIAAN	Consensus	5.49	0.01	0.01	650	5.49	189	13.66
HLA-DRB4*01:01	111	125	NCKLVFKKRKRQIAA	Consensus	5.55	0.08	4.86	657	5.55	229.10	15.92
HLA-DRB4*01:01	210	224	ITAKDIKKKFSIILP	Consensus	5.59	0.01	0.01	661	5.59	504.20	27.56
HLA-DRB4*01:01	110	124	DNCKLVFKKRKRQIA	Consensus	5.77	0.08	4.86	679	5.77	344.70	21.46
HLA-DRB4*01:01	215	229	IKKKFSIILPNEEYK	Consensus	6.11	0.01	0.01	716	6.11	279.90	18.52
HLA-DRB4*01:01	114	128	LVFKKRKRQIAANIE	Consensus	6.37	0.01	0.01	744	6.37	590.60	30.37
HLA-DRB4*01:01	225	239	NEEYKRISFLDVYWF	Consensus	6.51	554.81	40.27	245	1.60	83.50	6.51
HLA-DRB4*01:01	226	240	EEYKRISFLDVYWFQ	Consensus	6.76	554.81	40.27	246	1.61	86.50	6.76

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	NetMHCIIpan IC50 (nM)	NetMHCIIpan rank
HLA-DRB3*02:02	36	50	KDETLFFASYGLKRD	NetMHCIIpan	2.23	-	-	-	-	109.86	2.23
HLA-DRB3*02:02	37	51	DETLFFASYGLKRDP	NetMHCIIpan	2.25	-	-	-	-	110.19	2.25
HLA-DRB3*02:02	38	52	ETLFFASYGLKRDPC	NetMHCIIpan	2.43	-	-	-	-	114.94	2.43
HLA-DRB3*02:02	39	53	TLFFASYGLKRDPCQ	NetMHCIIpan	2.80	-	-	-	-	125.77	2.80
HLA-DRB3*02:02	140	154	KDRYIGADPLHVDSY	NetMHCIIpan	3.50	-	-	-	-	143.57	3.50
HLA-DRB3*02:02	35	49	EKDETLFFASYGLKR	NetMHCIIpan	3.95	-	-	-	-	154.13	3.95
HLA-DRB3*02:02	66	80	HFVLNFKTNKKSCIS	NetMHCIIpan	4.03	-	-	-	-	156.39	4.03
HLA-DRB3*02:02	139	153	FKDRYIGADPLHVDS	NetMHCIIpan	4.28	-	-	-	-	161.10	4.28
HLA-DRB3*02:02	65	79	THFVLNFKTNKKSCI	NetMHCIIpan	4.32	-	-	-	-	162.16	4.32
HLA-DRB3*02:02	40	54	LFFASYGLKRDPCQI	NetMHCIIpan	5.15	-	-	-	-	186.71	5.15
HLA-DRB3*02:02	67	81	FVLNFKTNKKSCISA	NetMHCIIpan	5.56	-	-	-	-	197.07	5.56

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	NetMHCIIpan IC50 (nM)	NetMHCIIpan rank
HLA-DRB3*02:02	234	248	LDVYWFQETMRKKPK	NetMHCIIpan	6.07	-	-	-	-	209.56	6.07
HLA-DRB3*02:02	138	152	SFKDRYIGADPLHVD	NetMHCIIpan	6.40	-	-	-	-	216.27	6.40
HLA-DRB3*02:02	141	155	DRYIGADPLHVDSYG	NetMHCIIpan	6.59	-	-	-	-	219.23	6.59
HLA-DRB3*02:02	64	78	QTHFVLNFKTNKKSC	NetMHCIIpan	6.70	-	-	-	-	221.06	6.70
HLA-DRB3*02:02	233	247	FLDVYWFQETMRKKP	NetMHCIIpan	6.94	-	-	-	-	225.49	6.94
HLA-DRB3*02:02	78	92	CISAIKLTSYPKINQ	NetMHCIIpan	7.02	-	-	-	-	227.43	7.02
HLA-DRB3*02:02	232	246	SFLDVYWFQETMRKK	NetMHCIIpan	7.12	-	-	-	-	230.21	7.12
HLA-DRB3*02:02	63	77	NQTHFVLNFKTNKKS	NetMHCIIpan	7.24	-	-	-	-	234.09	7.24
HLA-DRB3*02:02	170	184	RYNIFKDTRFSTEVF	NetMHCIIpan	7.42	-	-	-	-	238.57	7.42

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB3*01:01	266	280	ELVFDTDELMTYALV	Consensus	0.24	7.89	16.45	200	0.01	6.60	0.24
HLA-DRB3*01:01	265	279	CELVFDTDELMTYAL	Consensus	0.27	4443.24	45.36	146	0.01	7.10	0.27
HLA-DRB3*01:01	264	278	TCELVFDTDELMTYA	Consensus	0.33	4443.24	45.36	147	0.01	7.80	0.33
HLA-DRB3*01:01	263	277	KTCELVFDTDELMTY	Consensus	0.40	4443.24	45.36	146	0.01	8.60	0.40
HLA-DRB3*01:01	267	281	LVFDTDELMTYALVK	Consensus	0.48	7.89	16.45	202	0.01	9.60	0.48
HLA-DRB3*01:01	268	282	VFDTDELMTYALVKV	Consensus	0.85	7.89	16.45	667	0.05	14.40	0.85
HLA-DRB3*01:01	154	168	YGLPYQFDQEHGWNV	Consensus	1.30	3262.50	43.63	147	0.01	21	1.30
HLA-DRB3*01:01	155	169	GLPYQFDQEHGWNVE	Consensus	1.32	3262.50	43.63	147	0.01	21.30	1.32
HLA-DRB3*01:01	269	283	FDTDELMTYALVKVF	Consensus	1.49	7.89	16.45	587	0.04	23.90	1.49
HLA-DRB3*01:01	153	167	SYGLPYQFDQEHGWN	Consensus	1.54	3262.50	43.63	151	0.01	24.80	1.54
HLA-DRB3*01:01	152	166	DSYGLPYQFDQEHGW	Consensus	1.64	3262.50	43.63	150	0.01	26.50	1.64

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB3*01:01	16	30	GFIVAVKCDGDEYFI	Consensus	1.72	2156.82	41.34	82	0.01	27.90	1.72
HLA-DRB3*01:01	17	31	FIVAVKCDGDEYFIG	Consensus	1.80	8687.51	49.16	84	0.01	29.50	1.80
HLA-DRB3*01:01	18	32	IVAVKCDGDEYFIGK	Consensus	1.92	8687.51	49.16	85	0.01	31.70	1.92
HLA-DRB3*01:01	15	29	FGFIVAVKCDGDEYF	Consensus	1.98	2156.82	41.34	85	0.01	32.80	1.98
HLA-DRB3*01:01	156	170	LPYQFDQEHGWNVER	Consensus	2.24	3262.50	43.63	156	0.01	37.70	2.24
HLA-DRB3*01:01	42	56	FASYGLKRDPCQIVL	Consensus	2.53	123.47	26.99	235	0.01	43.90	2.53
HLA-DRB3*01:01	44	58	SYGLKRDPCQIVLGY	Consensus	2.71	123.47	26.99	237	0.01	48.10	2.71
HLA-DRB3*01:01	43	57	ASYGLKRDPCQIVLG	Consensus	2.72	123.47	26.99	240	0.01	48.40	2.72
HLA-DRB3*01:01	41	55	FFASYGLKRDPCQIV	Consensus	2.91	123.47	26.99	241	0.01	53	2.91
HLA-DRB3*01:01	19	33	VAVKCDGDEYFIGKY	Consensus	3.07	8687.51	49.16	88	0.01	57	3.07
HLA-DRB3*01:01	157	171	PYQFDQEHGWNVERY	Consensus	3.26	3262.50	43.63	450	0.02	62	3.26
HLA-DRB3*01:01	261	275	ENKTCELVFDTDELM	Consensus	4.06	125476.82	64.37	219	0.01	85.70	4.06
HLA-DRB3*01:01	262	276	NKTCELVFDTDELMT	Consensus	4.36	125476.82	64.37	219	0.01	95.80	4.36
HLA-DRB3*01:01	20	34	AVKCDGDEYFIGKYK	Consensus	4.60	8687.51	49.16	267	0.01	104.40	4.60
HLA-DRB3*01:01	45	59	YGLKRDPCQIVLGYK	Consensus	4.64	123.47	26.99	243	0.01	105.40	4.64
HLA-DRB3*01:01	138	152	SFKDRYIGADPLHVD	Consensus	4.71	61.71	24	940	0.12	108.10	4.71
HLA-DRB3*01:01	140	154	KDRYIGADPLHVDSY	Consensus	4.80	281333.08	68.76	933	0.12	111.60	4.80
HLA-DRB3*01:01	139	153	FKDRYIGADPLHVDS	Consensus	4.83	61.71	24	946	0.12	112.70	4.83
HLA-DRB3*01:01	167	181	NVERYNIFKDTRFST	Consensus	4.98	0.04	4.98	1333	0.27	1283	20.81
HLA-DRB3*01:01	168	182	VERYNIFKDTRFSTE	Consensus	4.98	0.04	4.98	1336	0.27	1224	20.19
HLA-DRB3*01:01	169	183	ERYNIFKDTRFSTEV	Consensus	4.98	0.04	4.98	1337	0.27	1173.60	19.65
HLA-DRB3*01:01	170	184	RYNIFKDTRFSTEVF	Consensus	4.98	0.04	4.98	1772	0.48	1097.70	18.86
HLA-DRB3*01:01	171	185	YNIFKDTRFSTEVFY	Consensus	4.98	0.04	4.98	1651	0.43	1109.20	18.98
HLA-DRB3*01:01	172	186	NIFKDTRFSTEVFYH	Consensus	4.98	0.04	4.98	4001	1.98	1166	19.57
HLA-DRB3*01:01	173	187	IFKDTRFSTEVFYHK	Consensus	4.98	0.04	4.98	3355	1.49	1028.30	18.15
HLA-DRB3*01:01	28	42	YFIGKYKEKDETLFF	Consensus	5.32	0.05	5.32	561	0.03	186.60	6.59
HLA-DRB3*01:01	29	43	FIGKYKEKDETLFFA	Consensus	5.32	0.05	5.32	562	0.03	175.10	6.34
HLA-DRB3*01:01	30	44	IGKYKEKDETLFFAS	Consensus	5.32	0.05	5.32	566	0.03	173.30	6.30

Supplementary data 11 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB3*01:01	31	45	GKYKEKDETLFFASY	Consensus	5.32	0.05	5.32	564	0.03	211.30	7.08
HLA-DRB3*01:01	32	46	KYKEKDETLFFASYG	Consensus	5.32	0.05	5.32	671	0.05	393.80	10.21
HLA-DRB3*01:01	33	47	YKEKDETLFFASYGL	Consensus	5.32	0.05	5.32	1799	0.50	701.80	14.37
HLA-DRB3*01:01	34	48	KEKDETLFFASYGLK	Consensus	5.32	0.05	5.32	2005	0.60	1139.10	19.29
HLA-DRB3*01:01	158	172	YQFDQEHGWNVERYN	Consensus	5.33	3262.50	43.63	454	0.02	132	5.33
HLA-DRB3*01:01	162	176	QEHGWNVERYNIFKD	Consensus	5.43	4.64	14.81	416	0.02	135.90	5.43
HLA-DRB3*01:01	163	177	EHGWNVERYNIFKDT	Consensus	5.53	4.64	14.81	416	0.02	140.40	5.53
HLA-DRB3*01:01	161	175	DQEHGWNVERYNIFK	Consensus	6.04	8825.38	49.25	428	0.02	162	6.04
HLA-DRB3*01:01	160	174	FDQEHGWNVERYNIF	Consensus	6.21	8825.38	49.25	422	0.02	169.70	6.21
HLA-DRB3*01:01	137	151	CSFKDRYIGADPLHV	Consensus	6.55	61.71	24	1303	0.26	184.90	6.55
HLA-DRB3*01:01	141	155	DRYIGADPLHVDSYG	Consensus	6.86	281333.08	68.76	952	0.12	199.70	6.86
HLA-DRB3*01:01	194	208	QITYLAEEDSFSEAR	Consensus	7.23	4146	44.97	2056	0.63	218.60	7.23
HLA-DRB3*01:01	21	35	VKCDGDEYFIGKYKE	Consensus	7.30	8687.51	49.16	279	0.01	221.70	7.30
HLA-DRB3*01:01	193	207	TQITYLAEEDSFSEA	Consensus	7.31	4146	44.97	2041	0.62	222.20	7.31

Notes: The MHCII binding predictions were made on 02/13/2017 using the IEDB analysis resource Consensus tool. In terms of population coverage, the reference sets for class II should provide >99%. Results are presented by allele, and from lowest to highest percentile rank. Peptides predicted as good binders are all of those with a percentile ranking lower or equal to 10.

Supplementary data 12. PpeSP06 in silico analysis: MHC-II restricted-epitopes prediction - Human (99% population coverage)

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*01/DPB1*04:01	40	54	DNTDEELLYSTFDFI	Consensus	0.03	0.01	0.01	87	0.03	34.60	2.24
HLA-DPA1*01/DPB1*04:01	41	55	NTDEELLYSTFDFIK	Consensus	0.03	0.01	0.01	81	0.03	18.40	1.17
HLA-DPA1*01/DPB1*04:01	42	56	TDEELLYSTFDFIKN	Consensus	0.03	0.01	0.01	76	0.03	16.20	1.02
HLA-DPA1*01/DPB1*04:01	43	57	DEELLYSTFDFIKNT	Consensus	0.03	0.01	0.01	76	0.03	15	0.93
HLA-DPA1*01/DPB1*04:01	44	58	EELLYSTFDFIKNTC	Consensus	0.03	0.01	0.01	79	0.03	14.90	0.92
HLA-DPA1*01/DPB1*04:01	45	59	ELLYSTFDFIKNTCA	Consensus	0.19	0.01	0.01	182	0.19	24.10	1.58
HLA-DPA1*01/DPB1*04:01	46	60	LLYSTFDFIKNTCAN	Consensus	0.24	0.01	0.01	202	0.24	39.80	2.54
HLA-DPA1*01/DPB1*04:01	238	252	YRISFLDVYWFQESV	Consensus	1.62	0.35	9.25	453	1.34	24.70	1.62
HLA-DPA1*01/DPB1*04:01	237	251	QYRISFLDVYWFQES	Consensus	2.02	0.35	9.25	380	0.95	31.20	2.02
HLA-DPA1*01/DPB1*04:01	239	253	RISFLDVYWFQESVK	Consensus	2.18	0.88	11.85	449	1.32	33.70	2.18
HLA-DPA1*01/DPB1*04:01	236	250	GQYRISFLDVYWFQE	Consensus	2.29	0.35	9.25	438	1.26	35.50	2.29
HLA-DPA1*01/DPB1*04:01	1	15	MTYFKISTCCLVLIS	Consensus	2.80	1.05	12.39	514	1.71	44.60	2.80
HLA-DPA1*01/DPB1*04:01	2	16	TYFKISTCCLVLISL	Consensus	2.89	1.05	12.39	561	2.02	46.40	2.89
HLA-DPA1*01/DPB1*04:01	240	254	ISFLDVYWFQESVKH	Consensus	3.12	4.30	17.58	490	1.57	50.90	3.12
HLA-DPA1*01/DPB1*04:01	235	249	YGQYRISFLDVYWFQ	Consensus	3.75	0.35	9.25	433	1.23	63.80	3.75
HLA-DPA1*01/DPB1*04:01	241	255	SFLDVYWFQESVKHK	Consensus	3.81	4.30	17.58	696	3	65.10	3.81
HLA-DPA1*01/DPB1*04:01	3	17	YFKISTCCLVLISLI	Consensus	4.01	1.05	12.39	820	4.01	55.90	3.38
HLA-DPA1*01/DPB1*04:01	115	129	VGGIGQSYCLLVFKK	Consensus	6.04	513.17	43.59	1047	6.04	120.40	6.04
HLA-DPA1*01/DPB1*04:01	234	248	NYGQYRISFLDVYWF	Consensus	6.24	0.35	9.25	459	1.38	126.40	6.24
HLA-DPA1*01/DPB1*04:01	233	247	SNYGQYRISFLDVYW	Consensus	6.76	0.35	9.25	451	1.33	141.50	6.76
HLA-DPA1*01/DPB1*04:01	114	128	QVGGIGQSYCLLVFK	Consensus	7	513.17	43.59	1069	6.25	148.50	7
HLA-DPA1*01/DPB1*04:01	232	246	FSNYGQYRISFLDVY	Consensus	7.04	0.35	9.25	443	1.29	149.80	7.04

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*01:03/DPB1*02:01	40	54	DNTDEELLYSTFDFI	Consensus	0.01	0.01	0.01	11	0.01	5.70	0.52
HLA-DPA1*01:03/DPB1*02:01	41	55	NTDEELLYSTFDFIK	Consensus	0.01	0.01	0.01	11	0.01	5.10	0.43
HLA-DPA1*01:03/DPB1*02:01	42	56	TDEELLYSTFDFIKN	Consensus	0.01	0.01	0.01	11	0.01	5.20	0.44
HLA-DPA1*01:03/DPB1*02:01	43	57	DEELLYSTFDFIKNT	Consensus	0.01	0.01	0.01	11	0.01	5.60	0.50
HLA-DPA1*01:03/DPB1*02:01	44	58	EELLYSTFDFIKNTC	Consensus	0.01	0.01	0.01	11	0.01	6.30	0.60
HLA-DPA1*01:03/DPB1*02:01	45	59	ELLYSTFDFIKNTCA	Consensus	0.01	0.01	0.01	32	0.01	11.50	1.35
HLA-DPA1*01:03/DPB1*02:01	46	60	LLYSTFDFIKNTCAN	Consensus	0.01	0.01	0.01	33	0.01	27.90	3.22
HLA-DPA1*01:03/DPB1*02:01	237	251	QYRISFLDVYWFQES	Consensus	0.08	0.01	0.01	96	0.08	9.30	1.04
HLA-DPA1*01:03/DPB1*02:01	239	253	RISFLDVYWFQESVK	Consensus	0.08	0.01	0.01	98	0.08	7.80	0.83
HLA-DPA1*01:03/DPB1*02:01	238	252	YRISFLDVYWFQESV	Consensus	0.09	0.01	0.01	99	0.09	7.80	0.83
HLA-DPA1*01:03/DPB1*02:01	240	254	ISFLDVYWFQESVKH	Consensus	0.10	0.01	0.01	108	0.10	9.20	1.03
HLA-DPA1*01:03/DPB1*02:01	236	250	GQYRISFLDVYWFQE	Consensus	0.23	0.01	0.01	164	0.23	10.50	1.21
HLA-DPA1*01:03/DPB1*02:01	241	255	SFLDVYWFQESVKHK	Consensus	0.23	0.01	0.01	161	0.23	11.30	1.32
HLA-DPA1*01:03/DPB1*02:01	235	249	YGQYRISFLDVYWFQ	Consensus	0.34	0.01	0.01	207	0.34	50.70	5.19
HLA-DPA1*01:03/DPB1*02:01	242	256	FLDVYWFQESVKHKP	Consensus	2.80	111.05	30	295	0.58	23.80	2.80
HLA-DPA1*01:03/DPB1*02:01	1	15	MTYFKISTCCLVLIS	Consensus	7.38	372.40	37.35	821	3.56	82.80	7.38

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*02:01/DPB1*01:01	43	57	DEELLYSTFDFIKNT	Consensus	0.53	55.62	17.43	46	0.03	11	0.53
HLA-DPA1*02:01/DPB1*01:01	42	56	TDEELLYSTFDFIKN	Consensus	0.55	55.62	17.43	47	0.03	11.10	0.55
HLA-DPA1*02:01/DPB1*01:01	44	58	EELLYSTFDFIKNTC	Consensus	0.76	55.62	17.43	47	0.03	12.90	0.76

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*02:01/DPB1*01:01	41	55	NTDEELLYSTFDFIK	Consensus	0.95	55.62	17.43	54	0.03	14.70	0.95
HLA-DPA1*02:01/DPB1*01:01	238	252	YRISFLDVYWFQESV	Consensus	1.20	48.93	16.38	335	1.15	16.80	1.20
HLA-DPA1*02:01/DPB1*01:01	239	253	RISFLDVYWFQESVK	Consensus	1.28	48.93	16.38	263	0.72	17.50	1.28
HLA-DPA1*02:01/DPB1*01:01	240	254	ISFLDVYWFQESVKH	Consensus	1.66	48.93	16.38	282	0.82	20.60	1.66
HLA-DPA1*02:01/DPB1*01:01	45	59	ELLYSTFDFIKNTCA	Consensus	1.99	55.62	17.43	105	0.13	23.20	1.99
HLA-DPA1*02:01/DPB1*01:01	241	255	SFLDVYWFQESVKHK	Consensus	1.99	48.93	16.38	299	0.92	23.20	1.99
HLA-DPA1*02:01/DPB1*01:01	237	251	QYRISFLDVYWFQES	Consensus	2.14	48.93	16.38	467	2.14	21.10	1.72
HLA-DPA1*02:01/DPB1*01:01	40	54	DNTDEELLYSTFDFI	Consensus	2.19	408.73	33.62	63	0.05	24.80	2.19
HLA-DPA1*02:01/DPB1*01:01	242	256	FLDVYWFQESVKHKP	Consensus	2.60	71.31	18.89	340	1.18	28	2.60
HLA-DPA1*02:01/DPB1*01:01	236	250	GQYRISFLDVYWFQE	Consensus	2.65	48.93	16.38	529	2.65	25	2.21
HLA-DPA1*02:01/DPB1*01:01	46	60	LLYSTFDFIKNTCAN	Consensus	3.52	55.62	17.43	117	0.16	35.30	3.52
HLA-DPA1*02:01/DPB1*01:01	4	18	FKISTCCLVLISLIL	Consensus	4.66	3.31	4.66	407	1.66	392.90	26.96
HLA-DPA1*02:01/DPB1*01:01	5	19	KISTCCLVLISLILP	Consensus	4.66	3.31	4.66	126	0.19	523.10	31.46
HLA-DPA1*02:01/DPB1*01:01	6	20	ISTCCLVLISLILPI	Consensus	4.66	3.31	4.66	94	0.11	247.80	20.46
HLA-DPA1*02:01/DPB1*01:01	7	21	STCCLVLISLILPII	Consensus	4.66	3.31	4.66	86	0.09	146.20	14.12
HLA-DPA1*02:01/DPB1*01:01	8	22	TCCLVLISLILPIIC	Consensus	4.66	3.31	4.66	89	0.09	140.50	13.71
HLA-DPA1*02:01/DPB1*01:01	9	23	CCLVLISLILPIICI	Consensus	4.66	3.31	4.66	88	0.09	168.60	15.71
HLA-DPA1*02:01/DPB1*01:01	10	24	CLVLISLILPIICIK	Consensus	4.66	3.31	4.66	153	0.27	211.90	18.45
HLA-DPA1*02:01/DPB1*01:01	232	246	FSNYGQYRISFLDVY	Consensus	5.38	2098.45	50.37	374	1.41	50.80	5.38
HLA-DPA1*02:01/DPB1*01:01	233	247	SNYGQYRISFLDVYW	Consensus	5.76	2098.45	50.37	406	1.65	54.10	5.76
HLA-DPA1*02:01/DPB1*01:01	234	248	NYGQYRISFLDVYWF	Consensus	5.94	898.98	41.52	464	2.12	55.70	5.94
HLA-DPA1*02:01/DPB1*01:01	243	257	LDVYWFQESVKHKPK	Consensus	5.96	71.31	18.89	521	2.58	55.90	5.96
HLA-DPA1*02:01/DPB1*01:01	235	249	YGQYRISFLDVYWFQ	Consensus	6.22	48.93	16.38	472	2.18	58.30	6.22
HLA-DPA1*02:01/DPB1*01:01	1	15	MTYFKISTCCLVLIS	Consensus	6.24	12.94	9.28	245	0.63	58.40	6.24

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*02:01/DPB1*05:01	44	58	EELLYSTFDFIKNTC	Consensus	2.84	17.15	10.62	219	0.64	133.70	2.84
HLA-DPA1*02:01/DPB1*05:01	201	215	NHQIRYLSKFDKYTV	Consensus	2.99	858.91	36.11	561	2.99	63.20	1.08
HLA-DPA1*02:01/DPB1*05:01	202	216	HQIRYLSKFDKYTVT	Consensus	3.03	858.91	36.11	565	3.03	62.60	1.06
HLA-DPA1*02:01/DPB1*05:01	43	57	DEELLYSTFDFIKNT	Consensus	3.25	17.15	10.62	216	0.63	150.50	3.25
HLA-DPA1*02:01/DPB1*05:01	42	56	TDEELLYSTFDFIKN	Consensus	3.45	17.15	10.62	218	0.64	158.60	3.45
HLA-DPA1*02:01/DPB1*05:01	119	133	GQSYCLLVFKKKERR	Consensus	3.55	3299.68	47.96	556	2.95	162.90	3.55
HLA-DPA1*02:01/DPB1*05:01	120	134	QSYCLLVFKKKERRE	Consensus	3.95	3299.68	47.96	674	3.95	153.70	3.33
HLA-DPA1*02:01/DPB1*05:01	45	59	ELLYSTFDFIKNTCA	Consensus	3.96	17.15	10.62	286	1.02	180.50	3.96
HLA-DPA1*02:01/DPB1*05:01	118	132	IGQSYCLLVFKKKER	Consensus	4.26	3299.68	47.96	480	2.35	193.20	4.26
HLA-DPA1*02:01/DPB1*05:01	199	213	LMNHQIRYLSKFDKY	Consensus	4.47	858.91	36.11	735	4.47	144.10	3.10
HLA-DPA1*02:01/DPB1*05:01	41	55	NTDEELLYSTFDFIK	Consensus	5.34	17.15	10.62	271	0.93	241	5.34
HLA-DPA1*02:01/DPB1*05:01	46	60	LLYSTFDFIKNTCAN	Consensus	5.36	17.15	10.62	447	2.11	242.20	5.36
HLA-DPA1*02:01/DPB1*05:01	203	217	QIRYLSKFDKYTVTR	Consensus	5.60	858.91	36.11	863	5.60	92.40	1.81
HLA-DPA1*02:01/DPB1*05:01	281	295	DEPITYAFVKVFSNP	Consensus	5.76	3.85	5.76	820	5.22	558.50	11.38
HLA-DPA1*02:01/DPB1*05:01	282	296	EPITYAFVKVFSNPD	Consensus	5.76	3.85	5.76	856	5.54	476.90	9.96
HLA-DPA1*02:01/DPB1*05:01	283	297	PITYAFVKVFSNPDH	Consensus	5.91	3.85	5.76	897	5.91	679.10	13.32
HLA-DPA1*02:01/DPB1*05:01	117	131	GIGQSYCLLVFKKKE	Consensus	6.28	3299.68	47.96	556	2.95	285	6.28
HLA-DPA1*02:01/DPB1*05:01	204	218	IRYLSKFDKYTVTRE	Consensus	6.37	858.91	36.11	948	6.37	164.20	3.58
HLA-DPA1*02:01/DPB1*05:01	200	214	MNHQIRYLSKFDKYT	Consensus	6.74	858.91	36.11	987	6.74	102.40	2.06
HLA-DPA1*02:01/DPB1*05:01	235	249	YGQYRISFLDVYWFQ	Consensus	6.89	5.81	6.89	1571	12.20	230.30	5.10
HLA-DPA1*02:01/DPB1*05:01	237	251	QYRISFLDVYWFQES	Consensus	6.89	5.81	6.89	1811	14.45	295.20	6.49
HLA-DPA1*02:01/DPB1*05:01	238	252	YRISFLDVYWFQESV	Consensus	6.89	5.81	6.89	1843	14.74	283.50	6.25
HLA-DPA1*02:01/DPB1*05:01	224	238	RAKKFTMDFSNYGQY	Consensus	7.04	0.51	2.18	2526	20.85	321.70	7.04
HLA-DPA1*02:01/DPB1*05:01	223	237	HRAKKFTMDFSNYGQ	Consensus	7.36	0.51	2.18	1939	15.62	337.60	7.36
HLA-DPA1*02:01/DPB1*05:01	121	135	SYCLLVFKKKERRED	Consensus	7.37	3299.68	47.96	1056	7.37	259.80	5.75

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	NetMHCIIpan IC50 (nM)	NetMHCIIpan rank
HLA-DPA1*02:01/DPB1*14:01	237	251	QYRISFLDVYWFQES	NetMHCIIpan	1.88	-	-	-	-	17.43	1.88
HLA-DPA1*02:01/DPB1*14:01	236	250	GQYRISFLDVYWFQE	NetMHCIIpan	2.02	-	-	-	-	18.35	2.02
HLA-DPA1*02:01/DPB1*14:01	238	252	YRISFLDVYWFQESV	NetMHCIIpan	2.09	-	-	-	-	18.58	2.09
HLA-DPA1*02:01/DPB1*14:01	1	15	MTYFKISTCCLVLIS	NetMHCIIpan	2.12	-	-	-	-	18.69	2.12
HLA-DPA1*02:01/DPB1*14:01	239	253	RISFLDVYWFQESVK	NetMHCIIpan	2.79	-	-	-	-	21.09	2.79
HLA-DPA1*02:01/DPB1*14:01	235	249	YGQYRISFLDVYWFQ	NetMHCIIpan	2.85	-	-	-	-	21.32	2.85
HLA-DPA1*02:01/DPB1*14:01	240	254	ISFLDVYWFQESVKH	NetMHCIIpan	3.13	-	-	-	-	22.45	3.13
HLA-DPA1*02:01/DPB1*14:01	241	255	SFLDVYWFQESVKHK	NetMHCIIpan	3.38	-	-	-	-	23.50	3.38
HLA-DPA1*02:01/DPB1*14:01	2	16	TYFKISTCCLVLISL	NetMHCIIpan	4.11	-	-	-	-	26.77	4.11
HLA-DPA1*02:01/DPB1*14:01	234	248	NYGQYRISFLDVYWF	NetMHCIIpan	4.44	-	-	-	-	28.23	4.44
HLA-DPA1*02:01/DPB1*14:01	43	57	DEELLYSTFDFIKNT	NetMHCIIpan	4.60	-	-	-	-	29.04	4.60
HLA-DPA1*02:01/DPB1*14:01	44	58	EELLYSTFDFIKNTC	NetMHCIIpan	5.02	-	-	-	-	30.81	5.02
HLA-DPA1*02:01/DPB1*14:01	42	56	TDEELLYSTFDFIKN	NetMHCIIpan	5.19	-	-	-	-	31.53	5.19
HLA-DPA1*02:01/DPB1*14:01	233	247	SNYGQYRISFLDVYW	NetMHCIIpan	6.23	-	-	-	-	35.42	6.23
HLA-DPA1*02:01/DPB1*14:01	41	55	NTDEELLYSTFDFIK	NetMHCIIpan	6.88	-	-	-	-	38.83	6.88
HLA-DPA1*02:01/DPB1*14:01	45	59	ELLYSTFDFIKNTCA	NetMHCIIpan	6.89	-	-	-	-	39.05	6.89
HLA-DPA1*02:01/DPB1*14:01	232	246	FSNYGQYRISFLDVY	NetMHCIIpan	6.96	-	-	-	-	39.41	6.96
HLA-DPA1*02:01/DPB1*14:01	283	297	PITYAFVKVFSNPDH	NetMHCIIpan	6.99	-	-	-	-	39.62	6.99
HLA-DPA1*02:01/DPB1*14:01	242	256	FLDVYWFQESVKHKP	NetMHCIIpan	7.07	-	-	-	-	39.93	7.07
HLA-DPA1*02:01/DPB1*14:01	282	296	EPITYAFVKVFSNPD	NetMHCIIpan	7.07	-	-	-	-	39.83	7.07
HLA-DPA1*02:01/DPB1*14:01	118	132	IGQSYCLLVFKKKER	NetMHCIIpan	7.27	-	-	-	-	40.83	7.27
HLA-DPA1*02:01/DPB1*14:01	117	131	GIGQSYCLLVFKKKE	NetMHCIIpan	7.42	-	-	-	-	41.23	7.42

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*03:01/DPB1*04:02	8	22	TCCLVLISLILPIIC	Consensus	1.60	0.15	3.72	55	0.02	15.10	1.60
HLA-DPA1*03:01/DPB1*04:02	9	23	CCLVLISLILPIICI	Consensus	1.60	0.15	3.72	54	0.02	15.10	1.60
HLA-DPA1*03:01/DPB1*04:02	7	21	STCCLVLISLILPII	Consensus	1.78	0.15	3.72	55	0.02	16.40	1.78
HLA-DPA1*03:01/DPB1*04:02	10	24	CLVLISLILPIICIK	Consensus	2.08	0.20	4.20	68	0.03	18.50	2.08
HLA-DPA1*03:01/DPB1*04:02	6	20	ISTCCLVLISLILPI	Consensus	3.15	0.15	3.72	59	0.02	26.40	3.15
HLA-DPA1*03:01/DPB1*04:02	44	58	EELLYSTFDFIKNTC	Consensus	3.51	77.69	27.05	157	0.18	29.40	3.51
HLA-DPA1*03:01/DPB1*04:02	11	25	LVLISLILPIICIKV	Consensus	3.53	0.20	4.20	126	0.12	29.60	3.53
HLA-DPA1*03:01/DPB1*04:02	3	17	YFKISTCCLVLISLI	Consensus	3.72	0.15	3.72	262	0.44	48.30	5.59
HLA-DPA1*03:01/DPB1*04:02	4	18	FKISTCCLVLISLIL	Consensus	3.72	0.15	3.72	271	0.46	61.60	6.81
HLA-DPA1*03:01/DPB1*04:02	5	19	KISTCCLVLISLILP	Consensus	3.72	0.15	3.72	133	0.13	88.10	8.88
HLA-DPA1*03:01/DPB1*04:02	43	57	DEELLYSTFDFIKNT	Consensus	3.89	77.69	27.05	157	0.18	32.60	3.89
HLA-DPA1*03:01/DPB1*04:02	1	15	MTYFKISTCCLVLIS	Consensus	4.17	1842.27	51.63	248	0.40	35.10	4.17
HLA-DPA1*03:01/DPB1*04:02	12	26	VLISLILPIICIKVI	Consensus	4.20	0.20	4.20	208	0.30	85.10	8.66
HLA-DPA1*03:01/DPB1*04:02	13	27	LISLILPIICIKVIR	Consensus	4.20	0.20	4.20	535	1.67	220.40	15.61
HLA-DPA1*03:01/DPB1*04:02	14	28	ISLILPIICIKVIRF	Consensus	4.20	0.20	4.20	593	2.01	143	12.14
HLA-DPA1*03:01/DPB1*04:02	45	59	ELLYSTFDFIKNTCA	Consensus	4.52	77.69	27.05	377	0.85	38.20	4.52
HLA-DPA1*03:01/DPB1*04:02	2	16	TYFKISTCCLVLISL	Consensus	4.80	0.38	5.21	243	0.39	40.70	4.80
HLA-DPA1*03:01/DPB1*04:02	42	56	TDEELLYSTFDFIKN	Consensus	5.09	77.69	27.05	159	0.18	43.40	5.09
HLA-DPA1*03:01/DPB1*04:02	238	252	YRISFLDVYWFQESV	Consensus	5.21	120.26	29.97	632	2.25	44.60	5.21
HLA-DPA1*03:01/DPB1*04:02	239	253	RISFLDVYWFQESVK	Consensus	5.23	120.26	29.97	631	2.24	44.80	5.23
HLA-DPA1*03:01/DPB1*04:02	237	251	QYRISFLDVYWFQES	Consensus	5.81	120.26	29.97	596	2.03	50.50	5.81
HLA-DPA1*03:01/DPB1*04:02	240	254	ISFLDVYWFQESVKH	Consensus	6	120.26	29.97	637	2.27	52.40	6
HLA-DPA1*03:01/DPB1*04:02	46	60	LLYSTFDFIKNTCAN	Consensus	6.04	77.69	27.05	404	0.98	52.90	6.04
HLA-DPA1*03:01/DPB1*04:02	236	250	GQYRISFLDVYWFQE	Consensus	6.69	120.26	29.97	658	2.40	60.20	6.69

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DPA1*03:01/DPB1*04:02	241	255	SFLDVYWFQESVKHK	Consensus	7.10	120.26	29.97	807	3.32	65.10	7.10
HLA-DPA1*03:01/DPB1*04:02	41	55	NTDEELLYSTFDFIK	Consensus	7.23	77.69	27.05	165	0.20	66.60	7.23

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*01:01/DQB1*05:01	238	252	YRISFLDVYWFQESV	Consensus	0.03	0.01	0.01	302	0.03	16.60	0.18
HLA-DQA1*01:01/DQB1*05:01	237	251	QYRISFLDVYWFQES	Consensus	0.10	0.01	0.01	544	0.10	43.50	0.81
HLA-DQA1*01:01/DQB1*05:01	236	250	GQYRISFLDVYWFQE	Consensus	0.12	0.01	0.01	588	0.12	53.10	1.06
HLA-DQA1*01:01/DQB1*05:01	240	254	ISFLDVYWFQESVKH	Consensus	0.15	0.15	2.41	337	0.03	15	0.15
HLA-DQA1*01:01/DQB1*05:01	235	249	YGQYRISFLDVYWFQ	Consensus	0.16	0.01	0.01	671	0.16	49.20	0.96
HLA-DQA1*01:01/DQB1*05:01	239	253	RISFLDVYWFQESVK	Consensus	0.16	0.15	2.41	309	0.03	15.70	0.16
HLA-DQA1*01:01/DQB1*05:01	233	247	SNYGQYRISFLDVYW	Consensus	0.17	0.01	0.01	684	0.17	148.50	3.28
HLA-DQA1*01:01/DQB1*05:01	234	248	NYGQYRISFLDVYWF	Consensus	0.17	0.01	0.01	681	0.17	58.20	1.19
HLA-DQA1*01:01/DQB1*05:01	241	255	SFLDVYWFQESVKHK	Consensus	0.19	0.15	2.41	360	0.04	17.30	0.19
HLA-DQA1*01:01/DQB1*05:01	242	256	FLDVYWFQESVKHKP	Consensus	0.29	0.15	2.41	451	0.06	22.10	0.29
HLA-DQA1*01:01/DQB1*05:01	243	257	LDVYWFQESVKHKPK	Consensus	0.66	0.15	2.41	994	0.39	37.40	0.66
HLA-DQA1*01:01/DQB1*05:01	9	23	CCLVLISLILPIICI	Consensus	0.81	0.01	0.01	1417	0.81	195.70	4.24
HLA-DQA1*01:01/DQB1*05:01	8	22	TCCLVLISLILPIIC	Consensus	0.82	0.01	0.01	1432	0.82	255.50	5.34
HLA-DQA1*01:01/DQB1*05:01	10	24	CLVLISLILPIICIK	Consensus	0.94	0.01	0.01	1527	0.94	235.90	4.98
HLA-DQA1*01:01/DQB1*05:01	232	246	FSNYGQYRISFLDVY	Consensus	1.03	0.01	0.01	1594	1.03	295.10	6.02
HLA-DQA1*01:01/DQB1*05:01	67	81	NNATHFVLDFSDPKK	Consensus	1.07	0.09	1.91	410	0.05	53.40	1.07

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*01:01/DQB1*05:01	66	80	TNNATHFVLDFSDPK	Consensus	1.20	0.09	1.91	433	0.06	58.50	1.20
HLA-DQA1*01:01/DQB1*05:01	68	82	NATHFVLDFSDPKKR	Consensus	1.25	0.09	1.91	716	0.19	60.50	1.25
HLA-DQA1*01:01/DQB1*05:01	244	258	DVYWFQESVKHKPKL	Consensus	1.44	0.15	2.41	1767	1.27	67.90	1.44
HLA-DQA1*01:01/DQB1*05:01	270	284	SNKTCQLVFDADEPI	Consensus	1.45	0.05	1.45	798	0.24	179.40	3.93
HLA-DQA1*01:01/DQB1*05:01	271	285	NKTCQLVFDADEPIT	Consensus	1.45	0.05	1.45	782	0.23	128.10	2.83
HLA-DQA1*01:01/DQB1*05:01	272	286	KTCQLVFDADEPITY	Consensus	1.45	0.05	1.45	758	0.21	107.80	2.38
HLA-DQA1*01:01/DQB1*05:01	273	287	TCQLVFDADEPITYA	Consensus	1.45	0.05	1.45	792	0.24	95.90	2.10
HLA-DQA1*01:01/DQB1*05:01	274	288	CQLVFDADEPITYAF	Consensus	1.45	0.05	1.45	945	0.35	114.10	2.52
HLA-DQA1*01:01/DQB1*05:01	275	289	QLVFDADEPITYAFV	Consensus	1.55	0.05	1.45	1960	1.55	168.70	3.72
HLA-DQA1*01:01/DQB1*05:01	69	83	ATHFVLDFSDPKKRC	Consensus	1.60	0.09	1.91	723	0.19	74.50	1.60
HLA-DQA1*01:01/DQB1*05:01	7	21	STCCLVLISLILPII	Consensus	1.67	0.07	1.67	1501	0.91	477.20	8.74
HLA-DQA1*01:01/DQB1*05:01	11	25	LVLISLILPIICIKV	Consensus	1.71	0.01	0.01	2057	1.71	271.50	5.62
HLA-DQA1*01:01/DQB1*05:01	65	79	CTNNATHFVLDFSDP	Consensus	1.75	14.92	13.48	696	0.18	80.70	1.75
HLA-DQA1*01:01/DQB1*05:01	70	84	THFVLDFSDPKKRCI	Consensus	1.91	0.09	1.91	1199	0.57	155.50	3.44
HLA-DQA1*01:01/DQB1*05:01	71	85	HFVLDFSDPKKRCIS	Consensus	1.91	0.09	1.91	1929	1.51	326.20	6.53
HLA-DQA1*01:01/DQB1*05:01	6	20	ISTCCLVLISLILPI	Consensus	1.93	0.07	1.67	2187	1.93	974.90	14.60
HLA-DQA1*01:01/DQB1*05:01	276	290	LVFDADEPITYAFVK	Consensus	2.24	0.05	1.45	2364	2.24	235.50	4.98
HLA-DQA1*01:01/DQB1*05:01	64	78	KCTNNATHFVLDFSD	Consensus	2.77	14.92	13.48	705	0.18	125.90	2.77
HLA-DQA1*01:01/DQB1*05:01	12	26	VLISLILPIICIKVI	Consensus	3.30	0.01	0.01	2918	3.30	345.90	6.83
HLA-DQA1*01:01/DQB1*05:01	20	34	IICIKVIRFDDRDEY	Consensus	3.32	0.32	3.32	1912	1.48	971.60	14.57
HLA-DQA1*01:01/DQB1*05:01	21	35	ICIKVIRFDDRDEYL	Consensus	3.32	0.32	3.32	1530	0.94	693.50	11.50
HLA-DQA1*01:01/DQB1*05:01	22	36	CIKVIRFDDRDEYLL	Consensus	3.32	0.32	3.32	1520	0.93	600.20	10.35
HLA-DQA1*01:01/DQB1*05:01	23	37	IKVIRFDDRDEYLLG	Consensus	3.32	0.32	3.32	1590	1.02	683.70	11.38
HLA-DQA1*01:01/DQB1*05:01	24	38	KVIRFDDRDEYLLGK	Consensus	3.32	0.32	3.32	2726	2.92	850.90	13.28

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*01:01/DQB1*05:01	41	55	NTDEELLYSTFDFIK	Consensus	3.87	0.47	3.87	2500	2.48	714.50	11.73
HLA-DQA1*01:01/DQB1*05:01	42	56	TDEELLYSTFDFIKN	Consensus	3.87	0.47	3.87	2127	1.83	637.30	10.80
HLA-DQA1*01:01/DQB1*05:01	43	57	DEELLYSTFDFIKNT	Consensus	3.87	0.47	3.87	2062	1.72	559.60	9.83
HLA-DQA1*01:01/DQB1*05:01	44	58	EELLYSTFDFIKNTC	Consensus	3.87	0.47	3.87	2264	2.06	312	6.29
HLA-DQA1*01:01/DQB1*05:01	45	59	ELLYSTFDFIKNTCA	Consensus	3.87	0.47	3.87	3036	3.54	293.60	5.99
HLA-DQA1*01:01/DQB1*05:01	25	39	VIRFDDRDEYLLGKP	Consensus	4.08	0.32	3.32	3279	4.08	1128	16.09
HLA-DQA1*01:01/DQB1*05:01	72	86	FVLDFSDPKKRCISS	Consensus	4.46	0.09	1.91	3455	4.46	587.60	10.19
HLA-DQA1*01:01/DQB1*05:01	13	27	LISLILPIICIKVIR	Consensus	5.08	0.01	0.01	3720	5.08	1379.70	18.35
HLA-DQA1*01:01/DQB1*05:01	46	60	LLYSTFDFIKNTCAN	Consensus	5.21	0.47	3.87	3774	5.21	393.70	7.55
HLA-DQA1*01:01/DQB1*05:01	113	127	CQVGGIGQSYCLLVF	Consensus	5.26	1.01	5.26	3063	3.60	4433.80	36.72
HLA-DQA1*01:01/DQB1*05:01	114	128	QVGGIGQSYCLLVFK	Consensus	5.26	1.01	5.26	3032	3.54	4397.40	36.55
HLA-DQA1*01:01/DQB1*05:01	115	129	VGGIGQSYCLLVFKK	Consensus	5.26	1.01	5.26	3112	3.71	4877.60	38.67
HLA-DQA1*01:01/DQB1*05:01	116	130	GGIGQSYCLLVFKKK	Consensus	5.26	1.01	5.26	3055	3.58	5940.30	42.97
HLA-DQA1*01:01/DQB1*05:01	117	131	GIGQSYCLLVFKKKE	Consensus	5.26	1.01	5.26	3440	4.43	5906	42.84
HLA-DQA1*01:01/DQB1*05:01	5	19	KISTCCLVLISLILP	Consensus	5.69	0.07	1.67	3972	5.69	2485.20	26.33
HLA-DQA1*01:01/DQB1*05:01	63	77	MKCTNNATHFVLDFS	Consensus	7.15	14.92	13.48	716	0.19	366.90	7.15

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*01:02/DQB1*06:02	4	18	NTDEELLYSTFDFIK	Consensus	6.84	0.05	3.84	308	6.84	155.80	11.22

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*03:01/DQB1*03:02	238	252	YRISFLDVYWFQESV	Consensus	6.99	0.38	15.34	1270	4.27	413.30	6.99

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*04:01/DQB1*04:02	88	102	HVFSTPDGPVNLEEE	Consensus	4.79	66.39	31.49	792	0.13	327.90	4.79
HLA-DQA1*04:01/DQB1*04:02	89	103	VFSTPDGPVNLEEEN	Consensus	5.11	66.39	31.49	794	0.14	345.80	5.11
HLA-DQA1*04:01/DQB1*04:02	65	79	CTNNATHFVLDFSDP	Consensus	5.62	137.31	34.25	2871	3.02	375.10	5.62
HLA-DQA1*04:01/DQB1*04:02	240	254	ISFLDVYWFQESVKH	Consensus	6.31	1.47	18.42	2965	3.23	413.60	6.31
HLA-DQA1*04:01/DQB1*04:02	237	251	QYRISFLDVYWFQES	Consensus	6.55	1.47	18.42	2650	2.54	427.70	6.55
HLA-DQA1*04:01/DQB1*04:02	238	252	YRISFLDVYWFQESV	Consensus	7.30	1.47	18.42	2484	2.21	470.50	7.30

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*05:01/DQB1*03:01	NO PREDICTED BINDERS										

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DQA1*05:01/DQB1*02:01	272	286	KTCQLVFDADEPITY	Consensus	0.53	874.44	38.81	284	0.48	48.60	0.53
HLA-DQA1*05:01/DQB1*02:01	271	285	NKTCQLVFDADEPIT	Consensus	0.64	874.44	38.81	317	0.64	49.30	0.54
HLA-DQA1*05:01/DQB1*02:01	270	284	SNKTCQLVFDADEPI	Consensus	0.66	874.44	38.81	322	0.66	46.10	0.48
HLA-DQA1*05:01/DQB1*02:01	273	287	TCQLVFDADEPITYA	Consensus	0.68	874.44	38.81	289	0.50	55.60	0.68
HLA-DQA1*05:01/DQB1*02:01	274	288	CQLVFDADEPITYAF	Consensus	2.05	874.44	38.81	519	2.05	65.50	0.91
HLA-DQA1*05:01/DQB1*02:01	234	248	NYGQYRISFLDVYWF	Consensus	2.89	0.12	6.10	480	1.73	144.40	2.89
HLA-DQA1*05:01/DQB1*02:01	275	289	QLVFDADEPITYAFV	Consensus	3.22	874.44	38.81	644	3.22	82.50	1.31
HLA-DQA1*05:01/DQB1*02:01	235	249	YGQYRISFLDVYWFQ	Consensus	3.31	0.12	6.10	478	1.71	160.20	3.31
HLA-DQA1*05:01/DQB1*02:01	233	247	SNYGQYRISFLDVYW	Consensus	3.48	0.12	6.10	526	2.12	166.90	3.48
HLA-DQA1*05:01/DQB1*02:01	236	250	GQYRISFLDVYWFQE	Consensus	3.90	0.12	6.10	501	1.90	183.80	3.90
HLA-DQA1*05:01/DQB1*02:01	232	246	FSNYGQYRISFLDVY	Consensus	4.18	0.12	6.10	562	2.44	195.20	4.18
HLA-DQA1*05:01/DQB1*02:01	238	252	YRISFLDVYWFQESV	Consensus	5.54	0.12	6.10	858	5.54	204.40	4.42
HLA-DQA1*05:01/DQB1*02:01	237	251	QYRISFLDVYWFQES	Consensus	6.10	0.12	6.10	973	6.89	199.50	4.29
HLA-DQA1*05:01/DQB1*02:01	21	35	ICIKVIRFDDRDEYL	Consensus	6.42	0.22	7.09	599	2.78	286.10	6.42

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*01:01	7	21	STCCLVLISLILPII	Consensus	6.65	0.02	6.34	34	6.65	118.70	32.64
HLA-DRB1*01:01	8	22	TCCLVLISLILPIIC	Consensus	6.65	0.02	6.34	34	6.65	128.30	33.79
HLA-DRB1*01:01	135	149	DALVDIRGLKTCSLK	Consensus	7.28	18.71	33.80	33	6.42	12.90	7.28

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*03:01	20	34	IICIKVIRFDDRDEY	Consensus	0.04	81	0.01	18.90	1.07	6	0.04
HLA-DRB1*03:01	21	35	ICIKVIRFDDRDEYL	Consensus	0.04	76	0.01	10	0.42	6	0.04
HLA-DRB1*03:01	22	36	CIKVIRFDDRDEYLL	Consensus	0.04	72	0.01	6.10	0.16	6	0.04
HLA-DRB1*03:01	23	37	IKVIRFDDRDEYLLG	Consensus	0.04	76	0.01	5.50	0.11	6	0.04
HLA-DRB1*03:01	24	38	KVIRFDDRDEYLLGK	Consensus	0.04	78	0.01	6.90	0.22	6	0.04
HLA-DRB1*03:01	69	83	ATHFVLDFSDPKKRC	Consensus	0.05	69	0.01	4.20	0.05	3.50	1.59
HLA-DRB1*03:01	275	289	QLVFDADEPITYAFV	Consensus	0.06	135	0.03	4.40	0.06	3.20	2.22
HLA-DRB1*03:01	70	84	THFVLDFSDPKKRCI	Consensus	0.07	69	0.01	4.70	0.07	3.50	1.59
HLA-DRB1*03:01	274	288	CQLVFDADEPITYAF	Consensus	0.08	126	0.03	4.80	0.08	3.20	2.22
HLA-DRB1*03:01	25	39	VIRFDDRDEYLLGKP	Consensus	0.11	222	0.11	9.50	0.37	6	0.04
HLA-DRB1*03:01	26	40	IRFDDRDEYLLGKPD	Consensus	0.12	240	0.12	14.90	0.78	6	0.04
HLA-DRB1*03:01	71	85	HFVLDFSDPKKRCIS	Consensus	0.12	192	0.09	5.60	0.12	3.50	1.59
HLA-DRB1*03:01	68	82	NATHFVLDFSDPKKR	Consensus	0.15	69	0.01	6	0.15	3.50	1.59
HLA-DRB1*03:01	273	287	TCQLVFDADEPITYA	Consensus	0.15	128	0.03	6	0.15	3.20	2.22
HLA-DRB1*03:01	276	290	LVFDADEPITYAFVK	Consensus	0.23	136	0.03	7.10	0.23	3.20	2.22
HLA-DRB1*03:01	272	286	KTCQLVFDADEPITY	Consensus	0.26	129	0.03	7.70	0.26	3.20	2.22
HLA-DRB1*03:01	72	86	FVLDFSDPKKRCISS	Consensus	0.34	191	0.09	8.90	0.34	3.50	1.59
HLA-DRB1*03:01	6	20	ISTCCLVLISLILPI	Consensus	0.36	178	0.06	1757.10	27.56	4.70	0.36
HLA-DRB1*03:01	7	21	STCCLVLISLILPII	Consensus	0.36	170	0.05	1341	24.09	4.70	0.36
HLA-DRB1*03:01	8	22	TCCLVLISLILPIIC	Consensus	0.36	170	0.05	1002.30	20.68	4.70	0.36
HLA-DRB1*03:01	9	23	CCLVLISLILPIICI	Consensus	0.36	163	0.05	730.80	17.39	4.70	0.36
HLA-DRB1*03:01	10	24	CLVLISLILPIICIK	Consensus	0.36	159	0.04	1330.30	23.99	4.70	0.36
HLA-DRB1*03:01	11	25	LVLISLILPIICIKV	Consensus	0.45	454	0.45	1906.50	28.64	4.70	0.36
HLA-DRB1*03:01	67	81	NNATHFVLDFSDPKK	Consensus	0.45	71	0.01	10.50	0.45	3.50	1.59

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*03:01	12	26	VLISLILPIICIKVI	Consensus	0.48	480	0.48	3047.10	35.87	4.70	0.36
HLA-DRB1*03:01	277	291	VFDADEPITYAFVKV	Consensus	0.75	429	0.41	14.60	0.75	1.20	11.59
HLA-DRB1*03:01	66	80	TNNATHFVLDFSDPK	Consensus	1.26	72	0.01	21.60	1.26	3.50	1.59
HLA-DRB1*03:01	133	147	REDALVDIRGLKTCS	Consensus	1.33	243	0.13	22.90	1.33	0.30	18.08
HLA-DRB1*03:01	119	133	GQSYCLLVFKKKERR	Consensus	1.59	824	1.03	277.90	9.87	3.50	1.59
HLA-DRB1*03:01	120	134	QSYCLLVFKKKERRE	Consensus	1.59	820	1.03	166.10	7.13	3.50	1.59
HLA-DRB1*03:01	121	135	SYCLLVFKKKERRED	Consensus	1.59	818	1.02	150	6.65	3.50	1.59
HLA-DRB1*03:01	122	136	YCLLVFKKKERREDA	Consensus	1.59	815	1.02	204.50	8.14	3.50	1.59
HLA-DRB1*03:01	123	137	CLLVFKKKERREDAL	Consensus	1.59	830	1.04	399.20	12.34	3.50	1.59
HLA-DRB1*03:01	225	239	AKKFTMDFSNYGQYR	Consensus	1.96	520	0.53	33.10	1.96	2.91	2.99
HLA-DRB1*03:01	270	284	SNKTCQLVFDADEPI	Consensus	2.22	362	0.26	1904.50	28.63	3.20	2.22
HLA-DRB1*03:01	271	285	NKTCQLVFDADEPIT	Consensus	2.22	358	0.25	741.80	17.53	3.20	2.22
HLA-DRB1*03:01	132	146	RREDALVDIRGLKTC	Consensus	2.27	243	0.13	38.20	2.27	0.30	18.08
HLA-DRB1*03:01	278	292	FDADEPITYAFVKVF	Consensus	2.29	329	0.21	38.60	2.29	2	6.71
HLA-DRB1*03:01	134	148	EDALVDIRGLKTCSL	Consensus	2.37	250	0.13	40.20	2.37	3.16	2.42
HLA-DRB1*03:01	135	149	DALVDIRGLKTCSLK	Consensus	2.42	744	0.90	74	3.96	3.16	2.42
HLA-DRB1*03:01	136	150	ALVDIRGLKTCSLKE	Consensus	2.42	743	0.90	142.20	6.43	3.16	2.42
HLA-DRB1*03:01	194	208	YRKNGLMNHQIRYLS	Consensus	2.69	1618	2.54	583.10	15.32	3.05	2.69
HLA-DRB1*03:01	195	209	RKNGLMNHQIRYLSK	Consensus	2.69	1587	2.48	390.20	12.18	3.05	2.69
HLA-DRB1*03:01	196	210	KNGLMNHQIRYLSKF	Consensus	2.69	1618	2.54	575.10	15.20	3.05	2.69
HLA-DRB1*03:01	235	249	YGQYRISFLDVYWFQ	Consensus	2.70	1694	2.70	821.90	18.51	3.30	1.99
HLA-DRB1*03:01	236	250	GQYRISFLDVYWFQE	Consensus	2.71	1700	2.71	1524.40	25.71	3.30	1.99
HLA-DRB1*03:01	232	246	FSNYGQYRISFLDVY	Consensus	2.81	1752	2.81	1691.60	27.08	3.30	1.99
HLA-DRB1*03:01	233	247	SNYGQYRISFLDVYW	Consensus	2.89	1802	2.89	1644.90	26.71	3.30	1.99

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*03:01	234	248	NYGQYRISFLDVYWF	Consensus	2.90	1805	2.90	1146.80	22.17	3.30	1.99
HLA-DRB1*03:01	226	240	KKFTMDFSNYGQYRI	Consensus	2.91	521	0.54	50.80	2.91	2.91	2.99
HLA-DRB1*03:01	222	236	KHRAKKFTMDFSNYG	Consensus	2.99	542	0.57	460.90	13.41	2.91	2.99
HLA-DRB1*03:01	223	237	HRAKKFTMDFSNYGQ	Consensus	2.99	538	0.56	180.80	7.52	2.91	2.99
HLA-DRB1*03:01	224	238	RAKKFTMDFSNYGQY	Consensus	2.99	530	0.55	63.80	3.50	2.91	2.99
HLA-DRB1*03:01	227	241	KFTMDFSNYGQYRIS	Consensus	2.99	1558	2.42	103.90	5.15	2.91	2.99
HLA-DRB1*03:01	228	242	FTMDFSNYGQYRISF	Consensus	2.99	1587	2.48	268.80	9.67	2.91	2.99
HLA-DRB1*03:01	17	31	ILPIICIKVIRFDDR	Consensus	3.43	2071	3.43	742.30	17.53	4.10	0.78
HLA-DRB1*03:01	14	28	ISLILPIICIKVIRF	Consensus	3.45	2084	3.45	1568.20	26.08	4.10	0.78
HLA-DRB1*03:01	15	29	SLILPIICIKVIRFD	Consensus	3.50	2101	3.50	1327.90	23.96	4.10	0.78
HLA-DRB1*03:01	16	30	LILPIICIKVIRFDD	Consensus	3.63	2160	3.63	896.30	19.46	4.10	0.78
HLA-DRB1*03:01	124	138	LLVFKKKERREDALV	Consensus	3.96	2319	3.96	736.70	17.47	3.50	1.59
HLA-DRB1*03:01	18	32	LPIICIKVIRFDDRD	Consensus	4.16	2413	4.16	767.20	17.84	4.10	0.78
HLA-DRB1*03:01	125	139	LVFKKKERREDALVD	Consensus	4.27	2465	4.27	3623.60	39.05	3.50	1.59
HLA-DRB1*03:01	1	15	MTYFKISTCCLVLIS	Consensus	4.29	1770	2.84	1085.80	21.56	2.60	4.29
HLA-DRB1*03:01	2	16	TYFKISTCCLVLISL	Consensus	4.29	1813	2.91	2090.70	29.92	2.60	4.29
HLA-DRB1*03:01	131	145	ERREDALVDIRGLKT	Consensus	4.45	242	0.13	86.30	4.45	0.30	18.08
HLA-DRB1*03:01	213	227	YTVTREMVVKHRAKK	Consensus	4.77	1662	2.63	571.60	15.15	2.40	4.77
HLA-DRB1*03:01	214	228	TVTREMVVKHRAKKF	Consensus	4.77	251	0.13	474.80	13.67	2.40	4.77
HLA-DRB1*03:01	215	229	VTREMVVKHRAKKFT	Consensus	4.77	248	0.13	327.70	10.92	2.40	4.77
HLA-DRB1*03:01	216	230	TREMVVKHRAKKFTM	Consensus	4.77	241	0.13	185.70	7.66	2.40	4.77
HLA-DRB1*03:01	217	231	REMVVKHRAKKFTMD	Consensus	4.77	240	0.12	183.60	7.60	2.40	4.77
HLA-DRB1*03:01	218	232	EMVVKHRAKKFTMDF	Consensus	4.77	251	0.13	364.30	11.65	2.40	4.77
HLA-DRB1*03:01	219	233	MVVKHRAKKFTMDFS	Consensus	4.77	738	0.89	701.70	16.99	2.40	4.77

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*03:01	192	206	IFYRKNGLMNHQIRY	Consensus	5.69	1842	2.97	1791.80	27.82	2.20	5.69
HLA-DRB1*03:01	193	207	FYRKNGLMNHQIRYL	Consensus	5.69	1709	2.74	1108.40	21.78	2.20	5.69
HLA-DRB1*03:01	86	100	SIHVFSTPDGPVNLE	Consensus	5.73	3168	5.73	10117.80	61.18	2.20	5.69
HLA-DRB1*03:01	87	101	IHVFSTPDGPVNLEE	Consensus	5.75	3174	5.75	10853.60	62.92	2.20	5.69
HLA-DRB1*03:01	85	99	SSIHVFSTPDGPVNL	Consensus	5.77	3183	5.77	13234.90	68.04	2.20	5.69
HLA-DRB1*03:01	220	234	VVKHRAKKFTMDFSN	Consensus	6.18	770	0.95	1811.70	27.95	2.10	6.18
HLA-DRB1*03:01	246	260	YWFQESVKHKPKLPY	Consensus	6.18	831	1.04	3484.40	38.31	2.10	6.18
HLA-DRB1*03:01	247	261	WFQESVKHKPKLPYI	Consensus	6.18	1029	1.40	2585.90	33.14	2.10	6.18
HLA-DRB1*03:01	248	262	FQESVKHKPKLPYIY	Consensus	6.18	1012	1.38	1964.10	29.04	2.10	6.18
HLA-DRB1*03:01	249	263	QESVKHKPKLPYIYY	Consensus	6.18	1040	1.42	1532.20	25.78	2.10	6.18
HLA-DRB1*03:01	250	264	ESVKHKPKLPYIYYN	Consensus	6.18	1058	1.45	2993.50	35.54	2.10	6.18
HLA-DRB1*03:01	251	265	SVKHKPKLPYIYYNG	Consensus	6.18	3126	5.65	5177.80	46.03	2.10	6.18
HLA-DRB1*03:01	252	266	VKHKPKLPYIYYNGE	Consensus	6.18	3181	5.76	8218.80	56.26	2.10	6.18
HLA-DRB1*03:01	197	211	NGLMNHQIRYLSKFD	Consensus	6.23	3402	6.23	1038.60	21.07	3.05	2.69
HLA-DRB1*03:01	279	293	DADEPITYAFVKVFS	Consensus	6.71	741	0.90	1337.60	24.06	2	6.71
HLA-DRB1*03:01	280	294	ADEPITYAFVKVFSN	Consensus	6.71	739	0.89	1028.70	20.96	2	6.71
HLA-DRB1*03:01	281	295	DEPITYAFVKVFSNP	Consensus	6.71	774	0.96	1067.10	21.37	2	6.71
HLA-DRB1*03:01	282	296	EPITYAFVKVFSNPD	Consensus	6.71	769	0.95	1844.60	28.17	2	6.71
HLA-DRB1*03:01	283	297	PITYAFVKVFSNPDH	Consensus	6.71	2127	3.56	2379	31.78	2	6.71
HLA-DRB1*03:01	284	298	ITYAFVKVFSNPDHN	Consensus	6.71	2085	3.46	2259.90	31.06	2	6.71
HLA-DRB1*03:01	13	27	LISLILPIICIKVIR	Consensus	6.98	3761	6.98	4214.70	41.94	2.80	3.31
HLA-DRB1*03:01	138	152	VDIRGLKTCSLKERY	Consensus	7.07	3802	7.07	9289.60	59.10	3.16	2.42
HLA-DRB1*03:01	163	177	YGMAYKFDKNDNWSI	Consensus	7.12	3824	7.12	1323.50	23.92	2.50	4.37
HLA-DRB1*03:01	140	154	IRGLKTCSLKERYTS	Consensus	7.14	3835	7.14	6565.30	51.07	3.16	2.42

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*03:01	198	212	GLMNHQIRYLSKFDK	Consensus	7.14	3834	7.14	2188.40	30.59	4.60	0.41
HLA-DRB1*03:01	139	153	DIRGLKTCSLKERYT	Consensus	7.17	3848	7.17	8906.70	58.17	3.16	2.42

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:01	69	83	ATHFVLDFSDPKKRC	Consensus	0.92	293	3.07	19.40	0.92	4.90	0.06
HLA-DRB1*04:01	241	255	SFLDVYWFQESVKHK	Consensus	1.20	740	8.06	18.80	0.86	2.66	1.20
HLA-DRB1*04:01	242	256	FLDVYWFQESVKHKP	Consensus	1.20	585	6.39	15.30	0.57	2.66	1.20
HLA-DRB1*04:01	243	257	LDVYWFQESVKHKPK	Consensus	1.20	569	6.22	12.50	0.36	2.66	1.20
HLA-DRB1*04:01	244	258	DVYWFQESVKHKPKL	Consensus	1.20	613	6.68	14.50	0.51	2.66	1.20
HLA-DRB1*04:01	245	259	VYWFQESVKHKPKLP	Consensus	1.20	1052	11.25	20.60	1.02	2.66	1.20
HLA-DRB1*04:01	284	298	ITYAFVKVFSNPDHN	Consensus	1.26	133	1.26	20	0.97	1.10	4.47
HLA-DRB1*04:01	283	297	PITYAFVKVFSNPDH	Consensus	1.30	134	1.27	23.60	1.30	1.10	4.47
HLA-DRB1*04:01	285	299	TYAFVKVFSNPDHNE	Consensus	1.41	147	1.41	17.50	0.76	1.10	4.47
HLA-DRB1*04:01	70	84	THFVLDFSDPKKRCI	Consensus	1.43	304	3.20	25	1.43	4.90	0.06
HLA-DRB1*04:01	68	82	NATHFVLDFSDPKKR	Consensus	1.47	286	3	25.50	1.47	4.90	0.06
HLA-DRB1*04:01	286	300	YAFVKVFSNPDHNEP	Consensus	1.50	151	1.45	25.80	1.50	1.10	4.47
HLA-DRB1*04:01	282	296	EPITYAFVKVFSNPD	Consensus	2.01	148	1.42	31.10	2.01	1.10	4.47
HLA-DRB1*04:01	225	239	AKKFTMDFSNYGQYR	Consensus	2.09	324	3.42	32	2.09	3.40	0.54
HLA-DRB1*04:01	226	240	KKFTMDFSNYGQYRI	Consensus	2.44	337	3.57	35.50	2.44	3.40	0.54
HLA-DRB1*04:01	71	85	HFVLDFSDPKKRCIS	Consensus	2.46	735	8.02	35.70	2.46	4.90	0.06

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:01	246	260	YWFQESVKHKPKLPY	Consensus	2.52	1431	14.81	36.50	2.52	2.66	1.20
HLA-DRB1*04:01	224	238	RAKKFTMDFSNYGQY	Consensus	2.59	333	3.52	37.20	2.59	3.40	0.54
HLA-DRB1*04:01	67	81	NNATHFVLDFSDPKK	Consensus	2.73	298	3.13	38.70	2.73	4.90	0.06
HLA-DRB1*04:01	48	62	YSTFDFIKNTCANPK	Consensus	3.26	300	3.15	63	5.01	1.60	3.26
HLA-DRB1*04:01	49	63	STFDFIKNTCANPKM	Consensus	3.26	298	3.13	64.90	5.18	1.60	3.26
HLA-DRB1*04:01	240	254	ISFLDVYWFQESVKH	Consensus	3.39	763	8.29	45.70	3.39	2.66	1.20
HLA-DRB1*04:01	66	80	TNNATHFVLDFSDPK	Consensus	3.45	327	3.45	64.60	5.15	4.90	0.06
HLA-DRB1*04:01	223	237	HRAKKFTMDFSNYGQ	Consensus	3.51	332	3.51	50.10	3.82	3.40	0.54
HLA-DRB1*04:01	287	301	AFVKVFSNPDHNEPR	Consensus	3.52	333	3.52	36.80	2.55	1.10	4.47
HLA-DRB1*04:01	222	236	KHRAKKFTMDFSNYG	Consensus	4.06	377	4.06	96.40	7.82	3.40	0.54
HLA-DRB1*04:01	274	288	CQLVFDADEPITYAF	Consensus	4.10	380	4.10	136.70	10.86	2.10	1.98
HLA-DRB1*04:01	273	287	TCQLVFDADEPITYA	Consensus	4.11	381	4.11	128.20	10.26	2.10	1.98
HLA-DRB1*04:01	227	241	KFTMDFSNYGQYRIS	Consensus	4.23	723	7.89	54.50	4.23	3.40	0.54
HLA-DRB1*04:01	272	286	KTCQLVFDADEPITY	Consensus	4.26	394	4.26	189.70	14.36	2.10	1.98
HLA-DRB1*04:01	50	64	TFDFIKNTCANPKMK	Consensus	4.42	408	4.42	64.60	5.15	1.60	3.26
HLA-DRB1*04:01	7	21	STCCLVLISLILPII	Consensus	4.54	418	4.54	945.90	41.71	2.60	1.33
HLA-DRB1*04:01	8	22	TCCLVLISLILPIIC	Consensus	4.54	418	4.54	1074.40	44.62	2.60	1.33
HLA-DRB1*04:01	51	65	FDFIKNTCANPKMKC	Consensus	4.54	418	4.54	91.30	7.41	1.60	3.26
HLA-DRB1*04:01	9	23	CCLVLISLILPIICI	Consensus	4.56	420	4.56	1079.10	44.72	2.60	1.33
HLA-DRB1*04:01	45	59	ELLYSTFDFIKNTCA	Consensus	4.81	441	4.81	147.70	11.63	1.50	3.30
HLA-DRB1*04:01	275	289	QLVFDADEPITYAFV	Consensus	4.81	441	4.81	130.70	10.44	2.10	1.98
HLA-DRB1*04:01	276	290	LVFDADEPITYAFVK	Consensus	5.04	462	5.04	218.90	16.10	2.10	1.98
HLA-DRB1*04:01	47	61	LYSTFDFIKNTCANP	Consensus	5.08	465	5.08	74.70	6.03	1.50	3.30
HLA-DRB1*04:01	46	60	LLYSTFDFIKNTCAN	Consensus	5.12	469	5.12	136.40	10.84	1.50	3.30

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:01	60	74	NPKMKCTNNATHFVL	Consensus	5.28	483	5.28	147.10	11.59	2.18	1.95
HLA-DRB1*04:01	288	302	FVKVFSNPDHNEPRL	Consensus	5.32	492	5.39	66.50	5.32	1.10	4.47
HLA-DRB1*04:01	72	86	FVLDFSDPKKRCISS	Consensus	5.37	883	9.56	67	5.37	4.90	0.06
HLA-DRB1*04:01	59	73	ANPKMKCTNNATHFV	Consensus	5.38	491	5.38	212.30	15.72	2.18	1.95
HLA-DRB1*04:01	58	72	CANPKMKCTNNATHF	Consensus	5.56	508	5.56	310	20.90	2.18	1.95
HLA-DRB1*04:01	10	24	CLVLISLILPIICIK	Consensus	5.61	512	5.61	1053.90	44.17	2.60	1.33
HLA-DRB1*04:01	57	71	TCANPKMKCTNNATH	Consensus	5.64	515	5.64	482.10	28.16	2.18	1.95
HLA-DRB1*04:01	164	178	GMAYKFDKNDNWSIK	Consensus	5.68	518	5.68	213.70	15.80	3.30	0.55
HLA-DRB1*04:01	11	25	LVLISLILPIICIKV	Consensus	5.73	523	5.73	1271.70	48.59	2.60	1.33
HLA-DRB1*04:01	163	177	YGMAYKFDKNDNWSI	Consensus	5.83	533	5.83	270.50	18.91	3.30	0.55
HLA-DRB1*04:01	165	179	MAYKFDKNDNWSIKR	Consensus	6	549	6	238	17.16	3.30	0.55
HLA-DRB1*04:01	6	20	ISTCCLVLISLILPI	Consensus	6.07	555	6.07	1783.30	56.81	2.60	1.33
HLA-DRB1*04:01	61	75	PKMKCTNNATHFVLD	Consensus	6.15	562	6.15	215.90	15.92	2.18	1.95
HLA-DRB1*04:01	258	272	LPYIYYNGECLPSNK	Consensus	6.55	600	6.55	137.60	10.93	1.20	4.42
HLA-DRB1*04:01	52	66	DFIKNTCANPKMKCT	Consensus	6.58	603	6.58	205.70	15.34	1.60	3.26
HLA-DRB1*04:01	134	148	EDALVDIRGLKTCSL	Consensus	6.64	609	6.64	1141.70	46.03	3.10	0.77
HLA-DRB1*04:01	135	149	DALVDIRGLKTCSLK	Consensus	6.64	609	6.64	1412.40	51.14	3.10	0.77
HLA-DRB1*04:01	257	271	KLPYIYYNGECLPSN	Consensus	6.65	610	6.65	153.50	12.03	1.20	4.42
HLA-DRB1*04:01	259	273	PYIYYNGECLPSNKT	Consensus	7.23	665	7.23	109.60	8.86	1.20	4.42
HLA-DRB1*04:01	228	242	FTMDFSNYGQYRISF	Consensus	7.24	955	10.29	89.20	7.24	3.40	0.54
HLA-DRB1*04:01	162	176	AYGMAYKFDKNDNWS	Consensus	7.29	671	7.29	462.10	27.41	3.30	0.55
HLA-DRB1*04:01	260	274	YIYYNGECLPSNKTC	Consensus	7.31	673	7.31	127.10	10.17	1.20	4.42
HLA-DRB1*04:01	161	175	DAYGMAYKFDKNDNW	Consensus	7.39	680	7.39	560.80	30.95	3.30	0.55

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:05	284	298	ITYAFVKVFSNPDHN	Consensus	0.04	7	0.04	3.40	0.01	5.30	0.08
HLA-DRB1*04:05	285	299	TYAFVKVFSNPDHNE	Consensus	0.04	7	0.04	3.40	0.01	5.30	0.08
HLA-DRB1*04:05	286	300	YAFVKVFSNPDHNEP	Consensus	0.04	7	0.04	3.80	0.01	5.30	0.08
HLA-DRB1*04:05	282	296	EPITYAFVKVFSNPD	Consensus	0.05	8	0.05	4	0.01	5.30	0.08
HLA-DRB1*04:05	283	297	PITYAFVKVFSNPDH	Consensus	0.05	8	0.05	3.60	0.01	5.30	0.08
HLA-DRB1*04:05	287	301	AFVKVFSNPDHNEPR	Consensus	0.08	21	0.22	4.40	0.01	5.30	0.08
HLA-DRB1*04:05	288	302	FVKVFSNPDHNEPRL	Consensus	0.18	23	0.24	8.70	0.18	5.30	0.08
HLA-DRB1*04:05	81	95	KRCISSIHVFSTPDG	Consensus	2.01	124	1.69	32.40	2.79	2.50	2.01
HLA-DRB1*04:05	82	96	RCISSIHVFSTPDGP	Consensus	2.01	129	1.78	33.80	2.97	2.50	2.01
HLA-DRB1*04:05	83	97	CISSIHVFSTPDGPV	Consensus	2.01	128	1.77	30.20	2.50	2.50	2.01
HLA-DRB1*04:05	84	98	ISSIHVFSTPDGPVN	Consensus	2.01	125	1.72	35	3.11	2.50	2.01
HLA-DRB1*04:05	80	94	KKRCISSIHVFSTPD	Consensus	3.30	123	1.68	36.60	3.30	0.55	8.46
HLA-DRB1*04:05	207	221	LSKFDKYTVTREMVV	Consensus	3.32	226	3.32	21.30	1.43	0.90	7.09
HLA-DRB1*04:05	208	222	SKFDKYTVTREMVVK	Consensus	3.48	236	3.48	28	2.24	0.90	7.09
HLA-DRB1*04:05	206	220	YLSKFDKYTVTREMV	Consensus	3.51	238	3.51	22.30	1.55	0.90	7.09
HLA-DRB1*04:05	205	219	RYLSKFDKYTVTREM	Consensus	3.62	246	3.62	25.20	1.91	0.90	7.09
HLA-DRB1*04:05	204	218	IRYLSKFDKYTVTRE	Consensus	3.66	249	3.66	31.70	2.70	0.90	7.09
HLA-DRB1*04:05	67	81	NNATHFVLDFSDPKK	Consensus	4.02	275	4.02	31.20	2.63	0.80	7.14
HLA-DRB1*04:05	66	80	TNNATHFVLDFSDPK	Consensus	4.05	277	4.05	40.90	3.82	0.80	7.14
HLA-DRB1*04:05	68	82	NATHFVLDFSDPKKR	Consensus	4.07	278	4.07	30.40	2.53	0.80	7.14
HLA-DRB1*04:05	85	99	SSIHVFSTPDGPVNL	Consensus	4.11	348	5.13	43.40	4.11	2.50	2.01
HLA-DRB1*04:05	49	63	STFDFIKNTCANPKM	Consensus	4.75	323	4.75	178.50	15.27	2.30	2.39
HLA-DRB1*04:05	69	83	ATHFVLDFSDPKKRC	Consensus	4.75	323	4.75	29.30	2.39	0.80	7.14
HLA-DRB1*04:05	48	62	YSTFDFIKNTCANPK	Consensus	4.86	330	4.86	152.60	13.56	2.30	2.39
HLA-DRB1*04:05	233	247	SNYGQYRISFLDVYW	Consensus	4.89	312	4.58	50.30	4.89	0.80	7.52

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*04:05	70	84	THFVLDFSDPKKRCI	Consensus	4.91	334	4.91	39.70	3.68	0.80	7.14
HLA-DRB1*04:05	4	18	FKISTCCLVLISLIL	Consensus	5.13	348	5.13	279.50	20.78	1.50	4.49
HLA-DRB1*04:05	231	245	DFSNYGQYRISFLDV	Consensus	5.34	362	5.34	42.50	4	0.50	8.49
HLA-DRB1*04:05	232	246	FSNYGQYRISFLDVY	Consensus	5.35	363	5.35	38.80	3.58	0.80	7.52
HLA-DRB1*04:05	230	244	MDFSNYGQYRISFLD	Consensus	5.44	369	5.44	41.90	3.94	0.50	8.49
HLA-DRB1*04:05	5	19	KISTCCLVLISLILP	Consensus	5.49	284	4.15	318.50	22.57	1.28	5.49
HLA-DRB1*04:05	6	20	ISTCCLVLISLILPI	Consensus	5.49	175	2.52	249.80	19.28	1.28	5.49
HLA-DRB1*04:05	7	21	STCCLVLISLILPII	Consensus	5.49	110	1.46	163.80	14.32	1.28	5.49
HLA-DRB1*04:05	8	22	TCCLVLISLILPIIC	Consensus	5.49	96	1.23	180.70	15.41	1.28	5.49
HLA-DRB1*04:05	9	23	CCLVLISLILPIICI	Consensus	5.49	91	1.16	198.50	16.47	1.28	5.49
HLA-DRB1*04:05	10	24	CLVLISLILPIICIK	Consensus	5.49	90	1.15	325	22.85	1.28	5.49
HLA-DRB1*04:05	11	25	LVLISLILPIICIKV	Consensus	5.49	99	1.28	388.20	25.40	1.28	5.49
HLA-DRB1*04:05	86	100	SIHVFSTPDGPVNLE	Consensus	5.67	384	5.67	87.80	8.58	2.50	2.01
HLA-DRB1*04:05	229	243	TMDFSNYGQYRISFL	Consensus	5.96	405	5.96	43.20	4.08	0.50	8.49
HLA-DRB1*04:05	12	26	VLISLILPIICIKVI	Consensus	6.26	190	2.74	603.30	32.33	1.08	6.26
HLA-DRB1*04:05	13	27	LISLILPIICIKVIR	Consensus	6.26	312	4.58	912.40	39.67	1.08	6.26
HLA-DRB1*04:05	50	64	TFDFIKNTCANPKMK	Consensus	6.51	443	6.51	215.90	17.47	2.30	2.39
HLA-DRB1*04:05	45	59	ELLYSTFDFIKNTCA	Consensus	6.60	449	6.60	77.30	7.60	2.30	2.39
HLA-DRB1*04:05	51	65	FDFIKNTCANPKMKC	Consensus	6.70	456	6.70	249.60	19.27	2.30	2.39
HLA-DRB1*04:05	14	28	ISLILPIICIKVIRF	Consensus	7.04	480	7.04	2214.80	57.47	2.08	3.04
HLA-DRB1*04:05	209	223	KFDKYTVTREMVVKH	Consensus	7.09	652	9.31	46.40	4.46	0.90	7.09
HLA-DRB1*04:05	46	60	LLYSTFDFIKNTCAN	Consensus	7.39	525	7.65	75.10	7.39	2.30	2.39

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*07:01	207	221	LSKFDKYTVTREMVV	Consensus	2.51	42.80	28.30	133	1.29	13.70	2.51
HLA-DRB1*07:01	134	148	EDALVDIRGLKTCSL	Consensus	2.55	0.04	2.55	123	1.17	48.90	8.55
HLA-DRB1*07:01	135	149	DALVDIRGLKTCSLK	Consensus	2.55	0.04	2.55	124	1.18	61.70	10.15
HLA-DRB1*07:01	78	92	DPKKRCISSIHVFST	Consensus	2.63	0.66	8.27	237	2.63	13.10	2.36
HLA-DRB1*07:01	1	15	MTYFKISTCCLVLIS	Consensus	2.88	0.70	8.50	114	1.06	15.30	2.88
HLA-DRB1*07:01	79	93	PKKRCISSIHVFSTP	Consensus	2.97	0.66	8.27	240	2.68	15.80	2.97
HLA-DRB1*07:01	136	150	ALVDIRGLKTCSLKE	Consensus	3.02	0.04	2.55	267	3.02	83.10	12.51
HLA-DRB1*07:01	137	151	LVDIRGLKTCSLKER	Consensus	3.08	0.04	2.55	272	3.08	116.30	15.62
HLA-DRB1*07:01	282	296	EPITYAFVKVFSNPD	Consensus	3.08	0.62	8.04	107	0.98	16.40	3.08
HLA-DRB1*07:01	283	297	PITYAFVKVFSNPDH	Consensus	3.46	0.62	8.04	120	1.13	18.30	3.46
HLA-DRB1*07:01	76	90	FSDPKKRCISSIHVF	Consensus	3.53	34.74	26.91	308	3.53	12.70	2.27
HLA-DRB1*07:01	208	222	SKFDKYTVTREMVVK	Consensus	3.63	42.80	28.30	136	1.33	19.10	3.63
HLA-DRB1*07:01	77	91	SDPKKRCISSIHVFS	Consensus	3.82	34.74	26.91	330	3.82	13.80	2.53
HLA-DRB1*07:01	2	16	TYFKISTCCLVLISL	Consensus	3.86	0.70	8.50	152	1.53	20.10	3.86
HLA-DRB1*07:01	75	89	DFSDPKKRCISSIHV	Consensus	3.98	34.74	26.91	342	3.98	15.80	2.97
HLA-DRB1*07:01	284	298	ITYAFVKVFSNPDHN	Consensus	4.01	0.62	8.04	121	1.14	20.80	4.01
HLA-DRB1*07:01	80	94	KKRCISSIHVFSTPD	Consensus	4.41	0.66	8.27	376	4.41	19.10	3.63
HLA-DRB1*07:01	179	193	REGVKQWKRSGNEIF	Consensus	4.52	276.49	41.40	236	2.61	23.40	4.52
HLA-DRB1*07:01	216	230	TREMVVKHRAKKFTM	Consensus	4.83	13.41	21.28	153	1.54	25	4.83
HLA-DRB1*07:01	285	299	TYAFVKVFSNPDHNE	Consensus	5.05	0.62	8.04	133	1.29	26.20	5.05
HLA-DRB1*07:01	3	17	YFKISTCCLVLISLI	Consensus	5.13	0.70	8.50	221	2.42	26.70	5.13
HLA-DRB1*07:01	214	228	TVTREMVVKHRAKKF	Consensus	5.23	13.41	21.28	163	1.65	27.30	5.23
HLA-DRB1*07:01	229	243	TMDFSNYGQYRISFL	Consensus	5.43	395.37	43.51	378	4.43	28.40	5.43
HLA-DRB1*07:01	180	194	EGVKQWKRSGNEIFY	Consensus	5.45	276.49	41.40	231	2.55	28.50	5.45
HLA-DRB1*07:01	209	223	KFDKYTVTREMVVKH	Consensus	5.72	42.80	28.30	136	1.33	30.10	5.72

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*07:01	81	95	KRCISSIHVFSTPDG	Consensus	5.73	0.66	8.27	419	4.95	30.20	5.73
HLA-DRB1*07:01	215	229	VTREMVVKHRAKKFT	Consensus	5.75	13.41	21.28	162	1.64	30.30	5.75
HLA-DRB1*07:01	138	152	VDIRGLKTCSLKERY	Consensus	6.03	0.04	2.55	505	6.03	237.80	23.39
HLA-DRB1*07:01	286	300	YAFVKVFSNPDHNEP	Consensus	6.21	0.62	8.04	135	1.32	33.10	6.21
HLA-DRB1*07:01	226	240	KKFTMDFSNYGQYRI	Consensus	6.93	395.37	43.51	521	6.23	38.10	6.93
HLA-DRB1*07:01	217	231	REMVVKHRAKKFTMD	Consensus	6.94	13.41	21.28	157	1.59	38.20	6.94
HLA-DRB1*07:01	48	62	YSTFDFIKNTCANPK	Consensus	7.09	2419.41	56.82	144	1.43	39.30	7.09

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*08:02	135	149	DALVDIRGLKTCSLK	Consensus	1.67	802	1.67	555.30	13.49	1.90	1.57
HLA-DRB1*08:02	134	148	EDALVDIRGLKTCSL	Consensus	1.69	811	1.69	723.50	17.13	1.90	1.57
HLA-DRB1*08:02	198	212	GLMNHQIRYLSKFDK	Consensus	2.28	986	2.28	317.20	7.54	2.80	0.60
HLA-DRB1*08:02	137	151	LVDIRGLKTCSLKER	Consensus	2.70	1113	2.70	507.60	12.35	1.90	1.57
HLA-DRB1*08:02	136	150	ALVDIRGLKTCSLKE	Consensus	2.84	1157	2.84	503.80	12.26	1.90	1.57
HLA-DRB1*08:02	215	229	VTREMVVKHRAKKFT	Consensus	2.84	1156	2.84	1339.70	27.78	4.60	0.03
HLA-DRB1*08:02	194	208	YRKNGLMNHQIRYLS	Consensus	2.91	1178	2.91	1897.50	35.03	2.18	1.29
HLA-DRB1*08:02	216	230	TREMVVKHRAKKFTM	Consensus	2.94	1185	2.94	1171.10	25.20	4.60	0.03
HLA-DRB1*08:02	15	29	SLILPIICIKVIRFD	Consensus	2.97	1044	2.48	1422	28.97	1.20	2.97
HLA-DRB1*08:02	217	231	REMVVKHRAKKFTMD	Consensus	3.07	1225	3.07	1120.30	24.37	4.60	0.03
HLA-DRB1*08:02	17	31	ILPIICIKVIRFDDR	Consensus	3.22	1267	3.22	944.80	21.46	1.20	2.97
HLA-DRB1*08:02	218	232	EMVVKHRAKKFTMDF	Consensus	3.49	1348	3.49	1217.80	25.93	4.60	0.03
HLA-DRB1*08:02	204	218	IRYLSKFDKYTVTRE	Consensus	3.66	1393	3.66	493.60	11.97	2.80	0.60

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*08:02	16	30	LILPIICIKVIRFDD	Consensus	3.76	1423	3.76	1229.10	26.12	1.20	2.97
HLA-DRB1*08:02	172	186	NDNWSIKREGVKQWK	Consensus	3.86	1449	3.86	936.20	21.31	1.30	2.73
HLA-DRB1*08:02	18	32	LPIICIKVIRFDDRD	Consensus	3.97	1479	3.97	1336.70	27.75	1.20	2.97
HLA-DRB1*08:02	171	185	KNDNWSIKREGVKQW	Consensus	3.98	1484	3.98	1464.90	29.59	1.30	2.73
HLA-DRB1*08:02	173	187	DNWSIKREGVKQWKR	Consensus	4	1488	4	667.60	15.93	1.30	2.73
HLA-DRB1*08:02	199	213	LMNHQIRYLSKFDKY	Consensus	4.08	1510	4.08	361.80	8.70	2.80	0.60
HLA-DRB1*08:02	132	146	RREDALVDIRGLKTC	Consensus	4.10	1398	3.67	3367	48.69	0.90	4.10
HLA-DRB1*08:02	133	147	REDALVDIRGLKTCS	Consensus	4.10	1314	3.38	1981	36.01	0.90	4.10
HLA-DRB1*08:02	174	188	NWSIKREGVKQWKRS	Consensus	4.25	1562	4.25	591.50	14.29	1.30	2.73
HLA-DRB1*08:02	219	233	MVVKHRAKKFTMDFS	Consensus	4.47	1626	4.47	1705.70	32.76	4.60	0.03
HLA-DRB1*08:02	189	203	GNEIFYRKNGLMNHQ	Consensus	4.51	1889	5.39	204.80	4.51	2.18	1.29
HLA-DRB1*08:02	175	189	WSIKREGVKQWKRSG	Consensus	4.67	1686	4.67	815.10	18.96	1.30	2.73
HLA-DRB1*08:02	138	152	VDIRGLKTCSLKERY	Consensus	4.73	1706	4.73	728.40	17.25	1.90	1.57
HLA-DRB1*08:02	200	214	MNHQIRYLSKFDKYT	Consensus	4.84	1736	4.84	358.90	8.62	2.80	0.60
HLA-DRB1*08:02	201	215	NHQIRYLSKFDKYTV	Consensus	4.86	1743	4.86	295.70	6.97	2.80	0.60
HLA-DRB1*08:02	247	261	WFQESVKHKPKLPYI	Consensus	4.94	1764	4.94	5653.70	62.17	2.60	0.76
HLA-DRB1*08:02	246	260	YWFQESVKHKPKLPY	Consensus	5.05	1793	5.05	5482.90	61.32	2.60	0.76
HLA-DRB1*08:02	7	21	STCCLVLISLILPII	Consensus	5.11	1243	3.13	8221.30	72.05	0.60	5.11
HLA-DRB1*08:02	8	22	TCCLVLISLILPIIC	Consensus	5.11	921	2.05	7265.30	68.78	0.60	5.11
HLA-DRB1*08:02	9	23	CCLVLISLILPIICI	Consensus	5.11	912	2.02	6704.90	66.70	0.60	5.11
HLA-DRB1*08:02	10	24	CLVLISLILPIICIK	Consensus	5.11	897	1.97	6726.20	66.78	0.60	5.11
HLA-DRB1*08:02	11	25	LVLISLILPIICIKV	Consensus	5.11	719	1.41	2472.40	41.13	0.60	5.11
HLA-DRB1*08:02	12	26	VLISLILPIICIKVI	Consensus	5.11	893	1.95	2168.60	38.09	0.60	5.11
HLA-DRB1*08:02	13	27	LISLILPIICIKVIR	Consensus	5.13	1181	2.92	1731.60	33.07	0.50	5.13
HLA-DRB1*08:02	14	28	ISLILPIICIKVIRF	Consensus	5.13	1018	2.39	1460.40	29.53	0.50	5.13

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*08:02	285	299	TYAFVKVFSNPDHNE	Consensus	5.16	512	0.84	228.40	5.16	0	7.32
HLA-DRB1*08:02	286	300	YAFVKVFSNPDHNEP	Consensus	5.21	516	0.85	230.70	5.21	0	7.32
HLA-DRB1*08:02	248	262	FQESVKHKPKLPYIY	Consensus	5.44	1903	5.44	5194	59.87	2.60	0.76
HLA-DRB1*08:02	190	204	NEIFYRKNGLMNHQI	Consensus	5.48	1913	5.48	300.70	7.11	2.18	1.29
HLA-DRB1*08:02	249	263	QESVKHKPKLPYIYY	Consensus	5.54	1926	5.54	4182.30	54.18	2.60	0.76
HLA-DRB1*08:02	188	202	SGNEIFYRKNGLMNH	Consensus	5.59	1939	5.59	289.40	6.78	2.18	1.29
HLA-DRB1*08:02	214	228	TVTREMVVKHRAKKF	Consensus	5.72	1976	5.72	1617.10	31.62	4.60	0.03
HLA-DRB1*08:02	202	216	HQIRYLSKFDKYTVT	Consensus	5.78	1992	5.78	309.80	7.35	2.80	0.60
HLA-DRB1*08:02	284	298	ITYAFVKVFSNPDHN	Consensus	5.78	493	0.80	251.20	5.78	0	7.32
HLA-DRB1*08:02	19	33	PIICIKVIRFDDRDE	Consensus	5.84	2011	5.84	986.10	22.20	1.20	2.97
HLA-DRB1*08:02	20	34	IICIKVIRFDDRDEY	Consensus	6.36	2151	6.36	805.40	18.78	1.20	2.97
HLA-DRB1*08:02	250	264	ESVKHKPKLPYIYYN	Consensus	6.79	2268	6.79	4557.80	56.42	2.60	0.76
HLA-DRB1*08:02	6	20	ISTCCLVLISLILPI	Consensus	6.89	2294	6.89	8842.80	73.98	0.60	5.11
HLA-DRB1*08:02	1	15	MTYFKISTCCLVLIS	Consensus	7.14	2363	7.14	1112.60	24.22	0.90	3.83
HLA-DRB1*08:02	283	297	PITYAFVKVFSNPDH	Consensus	7.21	497	0.80	304.40	7.21	0	7.32
HLA-DRB1*08:02	280	294	ADEPITYAFVKVFSN	Consensus	7.32	2287	6.87	952.20	21.61	0	7.32
HLA-DRB1*08:02	281	295	DEPITYAFVKVFSNP	Consensus	7.32	2302	6.92	692.40	16.44	0	7.32
HLA-DRB1*08:02	282	296	EPITYAFVKVFSNPD	Consensus	7.32	902	1.98	415.90	10.08	0	7.32
HLA-DRB1*08:02	84	98	ISSIHVFSTPDGPVN	Consensus	7.40	1113	2.70	311.80	7.40	-0.60	10.24

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*09:01	11	25	LVLISLILPIICIKV	Consensus	5.02	0.01	0.01	567	5.02	1529.40	48.81

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*09:01	9	23	CCLVLISLILPIICI	Consensus	5.05	0.01	0.01	570	5.05	1427.10	47.13
HLA-DRB1*09:01	10	24	CLVLISLILPIICIK	Consensus	5.34	0.01	0.01	597	5.34	1516.60	48.62
HLA-DRB1*09:01	222	236	KHRAKKFTMDFSNYG	Consensus	6.11	0.01	0.01	1052	10.47	88	6.11
HLA-DRB1*09:01	179	193	REGVKQWKRSGNEIF	Consensus	6.66	0.01	0.01	704	6.66	1446.10	47.46

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*11:01	198	212	GLMNHQIRYLSKFDK	Consensus	0.26	67	0.04	24.80	4.55	3.70	0.26
HLA-DRB1*11:01	199	213	LMNHQIRYLSKFDKY	Consensus	0.26	82	0.05	21.10	3.80	3.70	0.26
HLA-DRB1*11:01	200	214	MNHQIRYLSKFDKYT	Consensus	0.26	81	0.05	16.80	2.88	3.70	0.26
HLA-DRB1*11:01	201	215	NHQIRYLSKFDKYTV	Consensus	0.26	78	0.05	12.80	1.98	3.70	0.26
HLA-DRB1*11:01	202	216	HQIRYLSKFDKYTVT	Consensus	0.26	79	0.05	17.60	3.06	3.70	0.26
HLA-DRB1*11:01	203	217	QIRYLSKFDKYTVTR	Consensus	0.28	187	0.28	24.50	4.49	3.70	0.26
HLA-DRB1*11:01	204	218	IRYLSKFDKYTVTRE	Consensus	0.28	188	0.28	34.90	6.33	3.70	0.26
HLA-DRB1*11:01	194	208	YRKNGLMNHQIRYLS	Consensus	0.34	212	0.34	127.90	15.90	3.78	0.25
HLA-DRB1*11:01	8	22	TCCLVLISLILPIIC	Consensus	0.96	409	0.96	3874.60	63.66	2.70	0.89
HLA-DRB1*11:01	7	21	STCCLVLISLILPII	Consensus	1.08	439	1.08	4508.30	66.49	2.70	0.89
HLA-DRB1*11:01	188	202	SGNEIFYRKNGLMNH	Consensus	1.08	439	1.08	158.80	17.94	3.78	0.25
HLA-DRB1*11:01	189	203	GNEIFYRKNGLMNHQ	Consensus	1.28	490	1.28	112.50	14.74	3.78	0.25
HLA-DRB1*11:01	9	23	CCLVLISLILPIICI	Consensus	1.31	499	1.31	2329.90	54.52	2.70	0.89
HLA-DRB1*11:01	134	148	EDALVDIRGLKTCSL	Consensus	1.33	258	0.45	75.70	11.49	2.30	1.33
HLA-DRB1*11:01	135	149	DALVDIRGLKTCSLK	Consensus	1.33	256	0.45	84.20	12.33	2.30	1.33
HLA-DRB1*11:01	137	151	LVDIRGLKTCSLKER	Consensus	1.33	404	0.94	123.20	15.56	2.30	1.33

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*11:01	6	20	ISTCCLVLISLILPI	Consensus	1.35	507	1.35	6654.90	73.94	2.70	0.89
HLA-DRB1*11:01	190	204	NEIFYRKNGLMNHQI	Consensus	1.35	507	1.35	75.30	11.45	3.78	0.25
HLA-DRB1*11:01	136	150	ALVDIRGLKTCSLKE	Consensus	1.38	513	1.38	84.60	12.37	2.30	1.33
HLA-DRB1*11:01	10	24	CLVLISLILPIICIK	Consensus	1.53	549	1.53	1289.30	44.65	2.70	0.89
HLA-DRB1*11:01	138	152	VDIRGLKTCSLKERY	Consensus	1.57	561	1.57	280.70	23.99	2.30	1.33
HLA-DRB1*11:01	191	205	EIFYRKNGLMNHQIR	Consensus	1.60	570	1.60	81	12.02	3.78	0.25
HLA-DRB1*11:01	11	25	LVLISLILPIICIKV	Consensus	1.63	580	1.63	961.40	40.18	2.70	0.89
HLA-DRB1*11:01	5	19	KISTCCLVLISLILP	Consensus	1.69	593	1.69	5568.90	70.51	2.70	0.89
HLA-DRB1*11:01	14	28	ISLILPIICIKVIRF	Consensus	1.82	409	0.96	534.30	31.92	2	1.82
HLA-DRB1*11:01	15	29	SLILPIICIKVIRFD	Consensus	1.82	445	1.10	447.90	29.62	2	1.82
HLA-DRB1*11:01	16	30	LILPIICIKVIRFDD	Consensus	1.82	512	1.37	364.50	27.08	2	1.82
HLA-DRB1*11:01	17	31	ILPIICIKVIRFDDR	Consensus	1.82	575	1.62	297.10	24.65	2	1.82
HLA-DRB1*11:01	119	133	GQSYCLLVFKKKERR	Consensus	1.86	107	0.09	12.30	1.86	1	4.33
HLA-DRB1*11:01	13	27	LISLILPIICIKVIR	Consensus	2.02	625	1.81	890.50	39.03	1.90	2.02
HLA-DRB1*11:01	120	134	QSYCLLVFKKKERRE	Consensus	2.05	108	0.10	13.10	2.05	1	4.33
HLA-DRB1*11:01	12	26	VLISLILPIICIKVI	Consensus	2.10	684	2.10	1018.70	41.03	2.70	0.89
HLA-DRB1*11:01	18	32	LPIICIKVIRFDDRD	Consensus	2.12	691	2.12	355.20	26.75	2	1.82
HLA-DRB1*11:01	161	175	DAYGMAYKFDKNDNW	Consensus	2.16	699	2.16	91.20	12.97	2.50	1.08
HLA-DRB1*11:01	213	227	YTVTREMVVKHRAKK	Consensus	2.21	305	0.59	113.50	14.82	1.80	2.21
HLA-DRB1*11:01	214	228	TVTREMVVKHRAKKF	Consensus	2.21	239	0.40	71.70	11.07	1.80	2.21
HLA-DRB1*11:01	215	229	VTREMVVKHRAKKFT	Consensus	2.21	179	0.26	50.90	8.62	1.80	2.21
HLA-DRB1*11:01	216	230	TREMVVKHRAKKFTM	Consensus	2.21	174	0.25	39.40	7.02	1.80	2.21
HLA-DRB1*11:01	217	231	REMVVKHRAKKFTMD	Consensus	2.21	191	0.29	46.90	8.11	1.80	2.21
HLA-DRB1*11:01	218	232	EMVVKHRAKKFTMDF	Consensus	2.21	234	0.39	59.20	9.67	1.80	2.21
HLA-DRB1*11:01	219	233	MVVKHRAKKFTMDFS	Consensus	2.21	299	0.57	97.90	13.55	1.80	2.21

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*11:01	140	154	IRGLKTCSLKERYTS	Consensus	2.26	718	2.26	481.20	30.55	2.30	1.33
HLA-DRB1*11:01	139	153	DIRGLKTCSLKERYT	Consensus	2.33	736	2.33	314.10	25.30	2.30	1.33
HLA-DRB1*11:01	246	260	YWFQESVKHKPKLPY	Consensus	2.65	313	0.62	170.50	18.65	1.60	2.65
HLA-DRB1*11:01	247	261	WFQESVKHKPKLPYI	Consensus	2.65	370	0.82	249.90	22.69	1.60	2.65
HLA-DRB1*11:01	248	262	FQESVKHKPKLPYIY	Consensus	2.65	388	0.88	305.80	24.98	1.60	2.65
HLA-DRB1*11:01	249	263	QESVKHKPKLPYIYY	Consensus	2.65	406	0.95	284.10	24.13	1.60	2.65
HLA-DRB1*11:01	250	264	ESVKHKPKLPYIYYN	Consensus	2.65	413	0.98	459.40	29.94	1.60	2.65
HLA-DRB1*11:01	121	135	SYCLLVFKKKERRED	Consensus	2.75	96	0.07	16.20	2.75	1	4.33
HLA-DRB1*11:01	157	171	PKKTDAYGMAYKFDK	Consensus	2.84	850	2.84	194.50	20.02	2.50	1.08
HLA-DRB1*11:01	158	172	KKTDAYGMAYKFDKN	Consensus	2.88	858	2.88	112.70	14.76	2.50	1.08
HLA-DRB1*11:01	1	15	MTYFKISTCCLVLIS	Consensus	2.90	839	2.79	377.40	27.49	1.50	2.90
HLA-DRB1*11:01	159	173	KTDAYGMAYKFDKND	Consensus	2.94	872	2.94	86.70	12.57	2.50	1.08
HLA-DRB1*11:01	160	174	TDAYGMAYKFDKNDN	Consensus	3.01	887	3.01	63.10	10.11	2.50	1.08
HLA-DRB1*11:01	163	177	YGMAYKFDKNDNWSI	Consensus	3.14	915	3.14	169.50	18.59	2.50	1.08
HLA-DRB1*11:01	118	132	IGQSYCLLVFKKKER	Consensus	3.27	119	0.12	18.50	3.27	1	4.33
HLA-DRB1*11:01	4	18	FKISTCCLVLISLIL	Consensus	3.72	860	2.89	2516	55.84	1.20	3.72
HLA-DRB1*11:01	122	136	YCLLVFKKKERREDA	Consensus	4.09	98	0.08	22.50	4.09	1	4.33
HLA-DRB1*11:01	48	62	YSTFDFIKNTCANPK	Consensus	4.13	184	0.27	22.70	4.13	-0.20	9.53
HLA-DRB1*11:01	123	137	CLLVFKKKERREDAL	Consensus	4.33	143	0.17	47.10	8.14	1	4.33
HLA-DRB1*11:01	124	138	LLVFKKKERREDALV	Consensus	4.33	170	0.24	105.70	14.20	1	4.33
HLA-DRB1*11:01	193	207	FYRKNGLMNHQIRYL	Consensus	4.38	1173	4.38	157.30	17.85	3.78	0.25
HLA-DRB1*11:01	251	265	SVKHKPKLPYIYYNG	Consensus	4.41	1181	4.41	625.50	34.01	1.60	2.65
HLA-DRB1*11:01	252	266	VKHKPKLPYIYYNGE	Consensus	4.52	1201	4.52	978.10	40.43	1.60	2.65
HLA-DRB1*11:01	192	206	IFYRKNGLMNHQIRY	Consensus	4.61	1220	4.61	108.60	14.43	3.78	0.25
HLA-DRB1*11:01	195	209	RKNGLMNHQIRYLSK	Consensus	4.70	202	0.32	99.20	13.66	0.90	4.70

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*11:01	196	210	KNGLMNHQIRYLSKF	Consensus	4.70	197	0.31	62.30	10.02	0.90	4.70
HLA-DRB1*11:01	197	211	NGLMNHQIRYLSKFD	Consensus	4.70	201	0.31	48.30	8.30	0.90	4.70
HLA-DRB1*11:01	2	16	TYFKISTCCLVLISL	Consensus	4.90	1278	4.90	603.30	33.52	1.50	2.90
HLA-DRB1*11:01	162	176	AYGMAYKFDKNDNWS	Consensus	5.48	1390	5.48	122.60	15.52	2.50	1.08
HLA-DRB1*11:01	283	297	PITYAFVKVFSNPDH	Consensus	5.69	225	0.37	31	5.69	0.60	5.82
HLA-DRB1*11:01	280	294	ADEPITYAFVKVFSN	Consensus	5.82	312	0.61	97.60	13.53	0.60	5.82
HLA-DRB1*11:01	281	295	DEPITYAFVKVFSNP	Consensus	5.82	317	0.63	54.80	9.11	0.60	5.82
HLA-DRB1*11:01	282	296	EPITYAFVKVFSNPD	Consensus	5.82	222	0.36	40.20	7.15	0.60	5.82
HLA-DRB1*11:01	284	298	ITYAFVKVFSNPDHN	Consensus	5.82	235	0.39	48.70	8.34	0.60	5.82
HLA-DRB1*11:01	285	299	TYAFVKVFSNPDHNE	Consensus	5.82	406	0.95	69.10	10.79	0.60	5.82
HLA-DRB1*11:01	286	300	YAFVKVFSNPDHNEP	Consensus	5.82	416	1	123.70	15.60	0.60	5.82
HLA-DRB1*11:01	19	33	PIICIKVIRFDDRDE	Consensus	5.93	1481	5.93	316.10	25.37	2	1.82
HLA-DRB1*11:01	47	61	LYSTFDFIKNTCANP	Consensus	6.09	200	0.31	33.40	6.09	-0.20	9.53
HLA-DRB1*11:01	117	131	GIGQSYCLLVFKKKE	Consensus	6.11	285	0.53	33.50	6.11	0.20	7.59
HLA-DRB1*11:01	49	63	STFDFIKNTCANPKM	Consensus	6.14	183	0.27	33.70	6.14	-0.20	9.53
HLA-DRB1*11:01	187	201	RSGNEIFYRKNGLMN	Consensus	6.23	449	1.12	206.40	20.62	0.50	6.23
HLA-DRB1*11:01	81	95	KRCISSIHVFSTPDG	Consensus	6.64	1025	3.64	216.50	21.13	0.45	6.64
HLA-DRB1*11:01	82	96	RCISSIHVFSTPDGP	Consensus	6.64	1291	4.96	448.80	29.65	0.45	6.64
HLA-DRB1*11:01	125	139	LVFKKKERREDALVD	Consensus	6.64	217	0.35	346.90	26.45	0.40	6.64
HLA-DRB1*11:01	287	301	AFVKVFSNPDHNEPR	Consensus	6.64	1450	5.78	626.40	34.03	0.43	6.64
HLA-DRB1*11:01	20	34	IICIKVIRFDDRDEY	Consensus	6.91	1668	6.91	445.80	29.56	2	1.82
HLA-DRB1*11:01	131	145	ERREDALVDIRGLKT	Consensus	7.10	328	0.66	522.20	31.63	0.30	7.10
HLA-DRB1*11:01	132	146	RREDALVDIRGLKTC	Consensus	7.10	337	0.70	306.50	25.01	0.30	7.10
HLA-DRB1*11:01	133	147	REDALVDIRGLKTCS	Consensus	7.10	331	0.67	145	17.07	0.30	7.10
HLA-DRB1*11:01	288	302	FVKVFSNPDHNEPRL	Consensus	7.45	1773	7.45	926	39.61	0.43	6.64

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB1*12:01	11	25	LVLISLILPIICIKV	smm	0.24	-	-	97	0.24	-	-
HLA-DRB1*12:01	12	26	VLISLILPIICIKVI	smm	0.34	-	-	111	0.34	-	-
HLA-DRB1*12:01	10	24	CLVLISLILPIICIK	smm	0.50	-	-	132	0.50	-	-
HLA-DRB1*12:01	13	27	LISLILPIICIKVIR	smm	0.74	-	-	160	0.74	-	-
HLA-DRB1*12:01	14	28	ISLILPIICIKVIRF	smm	0.89	-	-	175	0.89	-	-
HLA-DRB1*12:01	9	23	CCLVLISLILPIICI	smm	1.10	-	-	194	1.10	-	-
HLA-DRB1*12:01	8	22	TCCLVLISLILPIIC	smm	1.13	-	-	197	1.13	-	-
HLA-DRB1*12:01	7	21	STCCLVLISLILPII	smm	1.19	-	-	203	1.19	-	-
HLA-DRB1*12:01	15	29	SLILPIICIKVIRFD	smm	1.21	-	-	205	1.21	-	-
HLA-DRB1*12:01	216	230	TREMVVKHRAKKFTM	smm	2.32	-	-	297	2.32	-	-
HLA-DRB1*12:01	215	229	VTREMVVKHRAKKFT	smm	2.35	-	-	299	2.35	-	-
HLA-DRB1*12:01	217	231	REMVVKHRAKKFTMD	smm	2.45	-	-	307	2.45	-	-
HLA-DRB1*12:01	218	232	EMVVKHRAKKFTMDF	smm	2.47	-	-	309	2.47	-	-
HLA-DRB1*12:01	6	20	ISTCCLVLISLILPI	smm	2.67	-	-	324	2.67	-	-
HLA-DRB1*12:01	16	30	LILPIICIKVIRFDD	smm	3.03	-	-	350	3.03	-	-
HLA-DRB1*12:01	246	260	YWFQESVKHKPKLPY	smm	4.96	-	-	493	4.96	-	-
HLA-DRB1*12:01	247	261	WFQESVKHKPKLPYI	smm	5.95	-	-	565	5.95	-	-
HLA-DRB1*12:01	214	228	TVTREMVVKHRAKKF	smm	6.19	-	-	582	6.19	-	-
HLA-DRB1*12:01	194	208	YRKNGLMNHQIRYLS	smm	6.57	-	-	610	6.57	-	-
HLA-DRB1*12:01	196	210	KNGLMNHQIRYLSKF	smm	6.58	-	-	611	6.58	-	-
HLA-DRB1*12:01	219	233	MVVKHRAKKFTMDFS	smm	6.68	-	-	618	6.68	-	-
HLA-DRB1*12:01	197	211	NGLMNHQIRYLSKFD	smm	7.11	-	-	649	7.11	-	-
HLA-DRB1*12:01	48	62	YSTFDFIKNTCANPK	smm	7.13	-	-	650	7.13	-	-
HLA-DRB1*12:01	17	31	ILPIICIKVIRFDDR	smm	7.24	-	-	658	7.24	-	-

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*13:02	163	177	YGMAYKFDKNDNWSI	Consensus	0.69	39	0.69	487.50	17.14	3.60	0.53
HLA-DRB1*13:02	9	23	CCLVLISLILPIICI	Consensus	1.86	73	1.80	217.90	10.38	2.60	1.86
HLA-DRB1*13:02	10	24	CLVLISLILPIICIK	Consensus	1.86	72	1.77	300.20	12.73	2.60	1.86
HLA-DRB1*13:02	8	22	TCCLVLISLILPIIC	Consensus	1.88	76	1.88	278	12.13	2.60	1.86
HLA-DRB1*13:02	7	21	STCCLVLISLILPII	Consensus	1.96	78	1.96	337.40	13.71	2.60	1.86
HLA-DRB1*13:02	6	20	ISTCCLVLISLILPI	Consensus	2.08	82	2.08	452.10	16.39	2.60	1.86
HLA-DRB1*13:02	273	287	TCQLVFDADEPITYA	Consensus	3.67	133	3.67	9.90	0.58	-2.30	28.44
HLA-DRB1*13:02	272	286	KTCQLVFDADEPITY	Consensus	3.74	135	3.74	10.20	0.61	-2.30	28.44
HLA-DRB1*13:02	274	288	CQLVFDADEPITYAF	Consensus	3.74	135	3.74	12.50	0.82	-2.30	28.44
HLA-DRB1*13:02	271	285	NKTCQLVFDADEPIT	Consensus	3.88	139	3.88	13.30	0.89	-2.30	28.44
HLA-DRB1*13:02	270	284	SNKTCQLVFDADEPI	Consensus	3.91	140	3.91	15.40	1.06	-2.30	28.44
HLA-DRB1*13:02	11	25	LVLISLILPIICIKV	Consensus	4.34	154	4.34	253.50	11.44	2.60	1.86
HLA-DRB1*13:02	12	26	VLISLILPIICIKVI	Consensus	4.38	155	4.38	284.60	12.31	2.60	1.86
HLA-DRB1*13:02	217	231	REMVVKHRAKKFTMD	Consensus	4.93	193	5.53	75.50	4.93	3.50	0.61
HLA-DRB1*13:02	15	29	SLILPIICIKVIRFD	Consensus	5.04	270	7.62	77.50	5.04	2.60	1.86
HLA-DRB1*13:02	216	230	TREMVVKHRAKKFTM	Consensus	5.33	194	5.56	83.40	5.33	3.50	0.61
HLA-DRB1*13:02	16	30	LILPIICIKVIRFDD	Consensus	5.42	364	9.92	85.40	5.42	2.60	1.86
HLA-DRB1*13:02	17	31	ILPIICIKVIRFDDR	Consensus	5.58	371	10.07	88.90	5.58	2.60	1.86
HLA-DRB1*13:02	218	232	EMVVKHRAKKFTMDF	Consensus	5.59	195	5.59	104.20	6.25	3.50	0.61
HLA-DRB1*13:02	214	228	TVTREMVVKHRAKKF	Consensus	5.67	198	5.67	143.10	7.83	3.50	0.61
HLA-DRB1*13:02	215	229	VTREMVVKHRAKKFT	Consensus	5.67	198	5.67	113.30	6.64	3.50	0.61
HLA-DRB1*13:02	50	64	TFDFIKNTCANPKMK	Consensus	6.49	227	6.49	48.50	3.38	0.90	8.25
HLA-DRB1*13:02	49	63	STFDFIKNTCANPKM	Consensus	6.51	228	6.51	59.50	4.06	0.90	8.25
HLA-DRB1*13:02	51	65	FDFIKNTCANPKMKC	Consensus	6.51	228	6.51	52.20	3.62	0.90	8.25
HLA-DRB1*13:02	48	62	YSTFDFIKNTCANPK	Consensus	6.65	233	6.65	80.60	5.19	0.90	8.25

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*15:01	284	298	ITYAFVKVFSNPDHN	Consensus	1.56	331	6.29	20.30	1.56	3.90	1.40
HLA-DRB1*15:01	283	297	PITYAFVKVFSNPDH	Consensus	1.58	328	6.22	20.50	1.58	3.90	1.40
HLA-DRB1*15:01	285	299	TYAFVKVFSNPDHNE	Consensus	1.59	353	6.76	20.60	1.59	3.90	1.40
HLA-DRB1*15:01	11	25	LVLISLILPIICIKV	Consensus	1.83	130	1.83	116.20	11.27	4.85	0.50
HLA-DRB1*15:01	8	22	TCCLVLISLILPIIC	Consensus	2.09	143	2.09	432.40	26.94	4.85	0.50
HLA-DRB1*15:01	7	21	STCCLVLISLILPII	Consensus	2.38	157	2.38	523.30	29.74	4.85	0.50
HLA-DRB1*15:01	12	26	VLISLILPIICIKVI	Consensus	2.40	158	2.40	99.30	9.87	4.85	0.50
HLA-DRB1*15:01	286	300	YAFVKVFSNPDHNEP	Consensus	2.44	361	6.93	27.60	2.44	3.90	1.40
HLA-DRB1*15:01	10	24	CLVLISLILPIICIK	Consensus	2.56	166	2.56	318.50	22.71	4.85	0.50
HLA-DRB1*15:01	9	23	CCLVLISLILPIICI	Consensus	2.58	167	2.58	336	23.44	4.85	0.50
HLA-DRB1*15:01	14	28	ISLILPIICIKVIRF	Consensus	2.83	179	2.83	66.60	6.83	3.70	1.92
HLA-DRB1*15:01	15	29	SLILPIICIKVIRFD	Consensus	3.07	189	3.07	73.40	7.52	3.70	1.92
HLA-DRB1*15:01	13	27	LISLILPIICIKVIR	Consensus	3.31	198	3.31	116.90	11.33	3.70	1.92
HLA-DRB1*15:01	287	301	AFVKVFSNPDHNEPR	Consensus	3.32	576	11.23	34.90	3.32	3.90	1.40
HLA-DRB1*15:01	6	20	ISTCCLVLISLILPI	Consensus	3.66	214	3.66	1212.20	43.43	4.85	0.50
HLA-DRB1*15:01	134	148	EDALVDIRGLKTCSL	Consensus	3.98	229	3.98	317.80	22.68	3.60	2.13
HLA-DRB1*15:01	135	149	DALVDIRGLKTCSLK	Consensus	3.98	229	3.98	368.80	24.72	3.60	2.13
HLA-DRB1*15:01	198	212	GLMNHQIRYLSKFDK	Consensus	4.02	284	5.24	17.40	1.20	2.90	4.02
HLA-DRB1*15:01	199	213	LMNHQIRYLSKFDKY	Consensus	4.02	291	5.39	15	0.93	2.90	4.02
HLA-DRB1*15:01	200	214	MNHQIRYLSKFDKYT	Consensus	4.02	340	6.50	14	0.81	2.90	4.02
HLA-DRB1*15:01	201	215	NHQIRYLSKFDKYTV	Consensus	4.02	257	4.62	12.10	0.62	2.90	4.02
HLA-DRB1*15:01	202	216	HQIRYLSKFDKYTVT	Consensus	4.02	310	5.82	13.90	0.80	2.90	4.02
HLA-DRB1*15:01	203	217	QIRYLSKFDKYTVTR	Consensus	4.02	410	7.90	24.10	2.02	2.90	4.02
HLA-DRB1*15:01	197	211	NGLMNHQIRYLSKFD	Consensus	4.40	313	5.88	23.70	1.97	2.75	4.40
HLA-DRB1*15:01	5	19	KISTCCLVLISLILP	Consensus	4.98	272	4.98	2021.40	52.59	4.30	0.90

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB1*15:01	216	230	TREMVVKHRAKKFTM	Consensus	5.18	558	10.90	40.50	3.98	2.50	5.18
HLA-DRB1*15:01	217	231	REMVVKHRAKKFTMD	Consensus	5.18	597	11.62	46.10	4.64	2.50	5.18
HLA-DRB1*15:01	131	145	ERREDALVDIRGLKT	Consensus	5.48	295	5.48	585.10	31.45	3.60	2.13
HLA-DRB1*15:01	133	147	REDALVDIRGLKTCS	Consensus	5.59	300	5.59	476.40	28.35	3.60	2.13
HLA-DRB1*15:01	132	146	RREDALVDIRGLKTC	Consensus	5.61	301	5.61	543.90	30.33	3.60	2.13
HLA-DRB1*15:01	226	240	KKFTMDFSNYGQYRI	Consensus	6.07	374	7.21	44.40	4.45	2.30	6.07
HLA-DRB1*15:01	227	241	KFTMDFSNYGQYRIS	Consensus	6.07	542	10.60	55.20	5.65	2.30	6.07
HLA-DRB1*15:01	228	242	FTMDFSNYGQYRISF	Consensus	6.07	584	11.37	50.20	5.11	2.30	6.07
HLA-DRB1*15:01	215	229	VTREMVVKHRAKKFT	Consensus	6.12	587	11.43	59.50	6.12	2.50	5.18
HLA-DRB1*15:01	235	249	YGQYRISFLDVYWFQ	Consensus	6.13	808	15.38	59.60	6.13	2.85	4.06
HLA-DRB1*15:01	218	232	EMVVKHRAKKFTMDF	Consensus	6.20	609	11.84	60.30	6.20	2.50	5.18
HLA-DRB1*15:01	288	302	FVKVFSNPDHNEPRL	Consensus	6.23	1091	19.86	60.50	6.23	3.90	1.40
HLA-DRB1*15:01	234	248	NYGQYRISFLDVYWF	Consensus	6.42	798	15.19	62.40	6.42	2.85	4.06
HLA-DRB1*15:01	16	30	LILPIICIKVIRFDD	Consensus	6.52	341	6.52	81.90	8.32	3.70	1.92
HLA-DRB1*15:01	254	268	HKPKLPYIYYNGECL	Consensus	6.64	347	6.64	119.10	11.51	3.70	1.76
HLA-DRB1*15:01	187	201	RSGNEIFYRKNGLMN	Consensus	6.70	350	6.70	218.10	17.96	2.58	5.12
HLA-DRB1*15:01	17	31	ILPIICIKVIRFDDR	Consensus	6.72	396	7.63	65.40	6.72	3.70	1.92
HLA-DRB1*15:01	188	202	SGNEIFYRKNGLMNH	Consensus	6.74	352	6.74	157.40	14.27	2.58	5.12
HLA-DRB1*15:01	255	269	KPKLPYIYYNGECLP	Consensus	6.80	355	6.80	119.40	11.53	3.70	1.76
HLA-DRB1*15:01	256	270	PKLPYIYYNGECLPS	Consensus	6.89	359	6.89	115.80	11.24	3.70	1.76
HLA-DRB1*15:01	186	200	KRSGNEIFYRKNGLM	Consensus	7.21	374	7.21	321.90	22.86	2.58	5.12
HLA-DRB1*15:01	257	271	KLPYIYYNGECLPSN	Consensus	7.22	375	7.22	102.60	10.14	3.70	1.76
HLA-DRB1*15:01	189	203	GNEIFYRKNGLMNHQ	Consensus	7.24	376	7.24	159.90	14.45	2.58	5.12

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB3*01:01	20	34	IICIKVIRFDDRDEY	Consensus	0.01	0.01	0.01	184	0.01	81.10	3.91
HLA-DRB3*01:01	21	35	ICIKVIRFDDRDEYL	Consensus	0.01	0.01	0.01	178	0.01	65.80	3.40
HLA-DRB3*01:01	22	36	CIKVIRFDDRDEYLL	Consensus	0.01	0.01	0.01	174	0.01	52.40	2.89
HLA-DRB3*01:01	23	37	IKVIRFDDRDEYLLG	Consensus	0.01	0.01	0.01	178	0.01	52.10	2.88
HLA-DRB3*01:01	24	38	KVIRFDDRDEYLLGK	Consensus	0.01	0.01	0.01	183	0.01	86.60	4.09
HLA-DRB3*01:01	163	177	YGMAYKFDKNDNWSI	Consensus	0.01	0.01	0.01	115	0.01	15.60	0.94
HLA-DRB3*01:01	164	178	GMAYKFDKNDNWSIK	Consensus	0.01	0.01	0.01	117	0.01	15.30	0.92
HLA-DRB3*01:01	165	179	MAYKFDKNDNWSIKR	Consensus	0.01	0.01	0.01	124	0.01	22.20	1.38
HLA-DRB3*01:01	166	180	AYKFDKNDNWSIKRE	Consensus	0.01	0.01	0.01	306	0.01	32	1.94
HLA-DRB3*01:01	167	181	YKFDKNDNWSIKREG	Consensus	0.01	0.01	0.01	320	0.01	70.90	3.58
HLA-DRB3*01:01	270	284	SNKTCQLVFDADEPI	Consensus	0.01	0.01	0.01	154	0.01	16.80	1.02
HLA-DRB3*01:01	271	285	NKTCQLVFDADEPIT	Consensus	0.01	0.01	0.01	155	0.01	18.70	1.15
HLA-DRB3*01:01	272	286	KTCQLVFDADEPITY	Consensus	0.01	0.01	0.01	81	0.01	4.80	0.10
HLA-DRB3*01:01	273	287	TCQLVFDADEPITYA	Consensus	0.01	0.01	0.01	81	0.01	4.60	0.09
HLA-DRB3*01:01	274	288	CQLVFDADEPITYAF	Consensus	0.01	0.01	0.01	80	0.01	4.40	0.08
HLA-DRB3*01:01	275	289	QLVFDADEPITYAFV	Consensus	0.01	0.01	0.01	97	0.01	4.30	0.07
HLA-DRB3*01:01	276	290	LVFDADEPITYAFVK	Consensus	0.01	0.01	0.01	97	0.01	5.80	0.18
HLA-DRB3*01:01	224	238	RAKKFTMDFSNYGQY	Consensus	0.26	1.42	11.58	210	0.01	6.90	0.26
HLA-DRB3*01:01	223	237	HRAKKFTMDFSNYGQ	Consensus	0.28	1.42	11.58	213	0.01	7.20	0.28
HLA-DRB3*01:01	225	239	AKKFTMDFSNYGQYR	Consensus	0.28	1.42	11.58	212	0.01	7.20	0.28
HLA-DRB3*01:01	222	236	KHRAKKFTMDFSNYG	Consensus	0.31	1.42	11.58	211	0.01	7.60	0.31
HLA-DRB3*01:01	69	83	ATHFVLDFSDPKKRC	Consensus	0.35	3.47	13.96	115	0.01	8.10	0.35
HLA-DRB3*01:01	68	82	NATHFVLDFSDPKKR	Consensus	0.38	3.47	13.96	115	0.01	8.40	0.38
HLA-DRB3*01:01	277	291	VFDADEPITYAFVKV	Consensus	0.39	8131.91	48.78	314	0.01	8.50	0.39
HLA-DRB3*01:01	67	81	NNATHFVLDFSDPKK	Consensus	0.43	3.47	13.96	114	0.01	9	0.43

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB3*01:01	66	80	TNNATHFVLDFSDPK	Consensus	0.50	3.47	13.96	113	0.01	9.90	0.50
HLA-DRB3*01:01	226	240	KKFTMDFSNYGQYRI	Consensus	0.60	0.19	7.45	217	0.01	11	0.60
HLA-DRB3*01:01	168	182	KFDKNDNWSIKREGV	Consensus	0.66	0.01	0.01	2104	0.66	453.30	11.09
HLA-DRB3*01:01	18	32	LPIICIKVIRFDDRD	Consensus	0.70	0.01	0.01	2163	0.70	2784.60	33.88
HLA-DRB3*01:01	70	84	THFVLDFSDPKKRCI	Consensus	0.72	3.47	13.96	121	0.01	12.50	0.72
HLA-DRB3*01:01	169	183	FDKNDNWSIKREGVK	Consensus	0.78	0.01	0.01	2296	0.78	941.80	17.19
HLA-DRB3*01:01	278	292	FDADEPITYAFVKVF	Consensus	0.82	8131.91	48.78	292	0.01	13.90	0.82
HLA-DRB3*01:01	227	241	KFTMDFSNYGQYRIS	Consensus	1.21	0.19	7.45	594	0.04	19.60	1.21
HLA-DRB3*01:01	71	85	HFVLDFSDPKKRCIS	Consensus	1.35	3.47	13.96	361	0.02	21.80	1.35
HLA-DRB3*01:01	162	176	AYGMAYKFDKNDNWS	Consensus	1.64	23.56	20.24	125	0.01	26.60	1.64
HLA-DRB3*01:01	161	175	DAYGMAYKFDKNDNW	Consensus	1.78	23.56	20.24	124	0.01	29.20	1.78
HLA-DRB3*01:01	228	242	FTMDFSNYGQYRISF	Consensus	2.32	0.19	7.45	604	0.04	39.40	2.32
HLA-DRB3*01:01	72	86	FVLDFSDPKKRCISS	Consensus	2.55	3.47	13.96	354	0.01	44.50	2.55
HLA-DRB3*01:01	1	15	MTYFKISTCCLVLIS	Consensus	3.36	29573.01	56.19	735	0.07	64.80	3.36
HLA-DRB3*01:01	19	33	PIICIKVIRFDDRDE	Consensus	3.98	0.01	0.01	6473	3.98	4054.60	42.66
HLA-DRB3*01:01	235	249	YGQYRISFLDVYWFQ	Consensus	4.52	6.69	15.93	242	0.01	101.40	4.52
HLA-DRB3*01:01	234	248	NYGQYRISFLDVYWF	Consensus	4.57	6.69	15.93	241	0.01	103.30	4.57
HLA-DRB3*01:01	233	247	SNYGQYRISFLDVYW	Consensus	5.34	2869.30	42.92	247	0.01	132.50	5.34
HLA-DRB3*01:01	2	16	TYFKISTCCLVLISL	Consensus	5.39	29573.01	56.19	750	0.07	134.30	5.39
HLA-DRB3*01:01	232	246	FSNYGQYRISFLDVY	Consensus	5.50	0.19	7.45	249	0.01	139.20	5.50
HLA-DRB3*01:01	236	250	GQYRISFLDVYWFQE	Consensus	5.89	6.69	15.93	241	0.01	155.70	5.89
HLA-DRB3*01:01	25	39	VIRFDDRDEYLLGKP	Consensus	5.95	205.76	29.33	523	0.03	158.20	5.95
HLA-DRB3*01:01	237	251	QYRISFLDVYWFQES	Consensus	7.43	6.69	15.93	698	0.06	229.40	7.43
HLA-DRB3*01:01	229	243	TMDFSNYGQYRISFL	Consensus	7.45	0.19	7.45	1807	0.50	827.20	15.88
HLA-DRB3*01:01	230	244	MDFSNYGQYRISFLD	Consensus	7.45	0.19	7.45	1832	0.51	1466.60	22.61

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	NetMHCIIpan IC50 (nM)	NetMHCIIpan rank
HLA-DRB3*02:02	225	239	AKKFTMDFSNYGQYR	NetMHCIIpan	2.07	-	-	-	-	104.45	2.07
HLA-DRB3*02:02	226	240	KKFTMDFSNYGQYRI	NetMHCIIpan	2.46	-	-	-	-	115.71	2.46
HLA-DRB3*02:02	224	238	RAKKFTMDFSNYGQY	NetMHCIIpan	2.62	-	-	-	-	120.64	2.62
HLA-DRB3*02:02	191	205	EIFYRKNGLMNHQIR	NetMHCIIpan	3.29	-	-	-	-	136.69	3.29
HLA-DRB3*02:02	190	204	NEIFYRKNGLMNHQI	NetMHCIIpan	3.35	-	-	-	-	138.14	3.35
HLA-DRB3*02:02	192	206	IFYRKNGLMNHQIRY	NetMHCIIpan	4.12	-	-	-	-	157.98	4.12
HLA-DRB3*02:02	223	237	HRAKKFTMDFSNYGQ	NetMHCIIpan	4.86	-	-	-	-	180.45	4.86
HLA-DRB3*02:02	189	203	GNEIFYRKNGLMNHQ	NetMHCIIpan	4.90	-	-	-	-	181.10	4.90
HLA-DRB3*02:02	227	241	KFTMDFSNYGQYRIS	NetMHCIIpan	5.23	-	-	-	-	189.40	5.23
HLA-DRB3*02:02	69	83	ATHFVLDFSDPKKRC	NetMHCIIpan	6.23	-	-	-	-	213.04	6.23
HLA-DRB3*02:02	70	84	THFVLDFSDPKKRCI	NetMHCIIpan	6.25	-	-	-	-	213.55	6.25
HLA-DRB3*02:02	193	207	FYRKNGLMNHQIRYL	NetMHCIIpan	6.47	-	-	-	-	217.24	6.47
HLA-DRB3*02:02	244	258	DVYWFQESVKHKPKL	NetMHCIIpan	6.78	-	-	-	-	222.41	6.78
HLA-DRB3*02:02	1	15	MTYFKISTCCLVLIS	NetMHCIIpan	7	-	-	-	-	226.49	7
HLA-DRB3*02:02	243	257	LDVYWFQESVKHKPK	NetMHCIIpan	7	-	-	-	-	226.25	7
HLA-DRB3*02:02	50	64	TFDFIKNTCANPKMK	NetMHCIIpan	7.39	-	-	-	-	237.88	7.39
HLA-DRB3*02:02	245	259	VYWFQESVKHKPKLP	NetMHCIIpan	7.43	-	-	-	-	239.52	7.43

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	Comblib. score	Comblib. Rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank
HLA-DRB4*01:01	17	31	ILPIICIKVIRFDDR	Consensus	0.42	0.01	0.01	94	0.42	23.40	1.34
HLA-DRB4*01:01	18	32	LPIICIKVIRFDDRD	Consensus	0.43	0.01	0.01	95	0.43	23.50	1.35
HLA-DRB4*01:01	15	29	SLILPIICIKVIRFD	Consensus	0.57	0.01	0.01	118	0.57	53.80	4.05
HLA-DRB4*01:01	19	33	PIICIKVIRFDDRDE	Consensus	0.64	0.01	0.01	128	0.64	52.30	3.91
HLA-DRB4*01:01	20	34	IICIKVIRFDDRDEY	Consensus	0.64	0.01	0.01	127	0.64	81	6.31
HLA-DRB4*01:01	16	30	LILPIICIKVIRFDD	Consensus	0.70	0.01	0.01	135	0.70	41.80	2.97
HLA-DRB4*01:01	21	35	ICIKVIRFDDRDEYL	Consensus	0.78	0.01	0.01	147	0.78	83	6.47
HLA-DRB4*01:01	196	210	KNGLMNHQIRYLSKF	Consensus	3.10	1879.06	48.25	288	1.99	43.20	3.10
HLA-DRB4*01:01	14	28	ISLILPIICIKVIRF	Consensus	4.42	1.93	12.17	115	0.55	58.10	4.42
HLA-DRB4*01:01	13	27	LISLILPIICIKVIR	Consensus	5	0.10	5.13	175	1.01	65	5
HLA-DRB4*01:01	199	213	LMNHQIRYLSKFDKY	Consensus	5.01	3.30	13.97	522	4.24	65.10	5.01
HLA-DRB4*01:01	7	21	STCCLVLISLILPII	Consensus	5.13	0.10	5.13	58	0.22	304.40	19.67
HLA-DRB4*01:01	8	22	TCCLVLISLILPIIC	Consensus	5.13	0.10	5.13	62	0.23	257.70	17.41
HLA-DRB4*01:01	9	23	CCLVLISLILPIICI	Consensus	5.13	0.10	5.13	61	0.23	219.20	15.38
HLA-DRB4*01:01	10	24	CLVLISLILPIICIK	Consensus	5.13	0.10	5.13	70	0.28	411.90	24.20
HLA-DRB4*01:01	11	25	LVLISLILPIICIKV	Consensus	5.13	0.10	5.13	112	0.53	428.40	24.82
HLA-DRB4*01:01	12	26	VLISLILPIICIKVI	Consensus	5.13	0.10	5.13	116	0.56	110.70	8.58
HLA-DRB4*01:01	195	209	RKNGLMNHQIRYLSK	Consensus	5.31	1879.06	48.25	275	1.88	68.70	5.31
HLA-DRB4*01:01	197	211	NGLMNHQIRYLSKFD	Consensus	5.31	2713.61	50.69	292	2.03	68.70	5.31
HLA-DRB4*01:01	22	36	CIKVIRFDDRDEYLL	Consensus	5.34	0.11	5.34	451	3.55	122.20	9.39
HLA-DRB4*01:01	198	212	GLMNHQIRYLSKFDK	Consensus	6.40	3.30	13.97	510	4.13	82.10	6.40

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB5*01:01	201	215	NHQIRYLSKFDKYTV	Consensus	0.52	82	0.52	8.40	1.90	5.30	0.11
HLA-DRB5*01:01	202	216	HQIRYLSKFDKYTVT	Consensus	0.53	84	0.53	10.70	2.57	5.30	0.11
HLA-DRB5*01:01	200	214	MNHQIRYLSKFDKYT	Consensus	0.55	86	0.55	10	2.37	5.30	0.11
HLA-DRB5*01:01	199	213	LMNHQIRYLSKFDKY	Consensus	0.59	90	0.59	12.10	2.96	5.30	0.11
HLA-DRB5*01:01	198	212	GLMNHQIRYLSKFDK	Consensus	0.60	92	0.60	14.80	3.66	5.30	0.11
HLA-DRB5*01:01	49	63	STFDFIKNTCANPKM	Consensus	0.97	131	0.97	81.50	13.59	4.10	0.55
HLA-DRB5*01:01	50	64	TFDFIKNTCANPKMK	Consensus	0.99	134	0.99	26.30	6.19	4.10	0.55
HLA-DRB5*01:01	48	62	YSTFDFIKNTCANPK	Consensus	1	135	1	76.60	13.10	4.10	0.55
HLA-DRB5*01:01	51	65	FDFIKNTCANPKMKC	Consensus	1.02	137	1.02	24.30	5.80	4.10	0.55
HLA-DRB5*01:01	116	130	GGIGQSYCLLVFKKK	Consensus	1.05	112	0.81	15.40	3.81	3.50	1.05
HLA-DRB5*01:01	117	131	GIGQSYCLLVFKKKE	Consensus	1.05	112	0.81	13.30	3.27	3.50	1.05
HLA-DRB5*01:01	118	132	IGQSYCLLVFKKKER	Consensus	1.05	74	0.46	9.60	2.25	3.50	1.05
HLA-DRB5*01:01	119	133	GQSYCLLVFKKKERR	Consensus	1.05	38	0.18	8.50	1.93	3.50	1.05
HLA-DRB5*01:01	120	134	QSYCLLVFKKKERRE	Consensus	1.05	38	0.18	10.80	2.60	3.50	1.05
HLA-DRB5*01:01	121	135	SYCLLVFKKKERRED	Consensus	1.05	47	0.25	14.20	3.51	3.50	1.05
HLA-DRB5*01:01	122	136	YCLLVFKKKERREDA	Consensus	1.05	47	0.25	32	7.22	3.50	1.05
HLA-DRB5*01:01	52	66	DFIKNTCANPKMKCT	Consensus	1.07	143	1.07	29.30	6.74	4.10	0.55
HLA-DRB5*01:01	158	172	KKTDAYGMAYKFDKN	Consensus	1.15	153	1.15	16.70	4.12	4.60	0.30
HLA-DRB5*01:01	157	171	PKKTDAYGMAYKFDK	Consensus	1.16	154	1.16	19.10	4.67	4.60	0.30
HLA-DRB5*01:01	159	173	KTDAYGMAYKFDKND	Consensus	1.18	156	1.18	18.20	4.47	4.60	0.30
HLA-DRB5*01:01	160	174	TDAYGMAYKFDKNDN	Consensus	1.19	158	1.19	17.40	4.28	4.60	0.30
HLA-DRB5*01:01	161	175	DAYGMAYKFDKNDNW	Consensus	1.22	161	1.22	26.80	6.29	4.60	0.30
HLA-DRB5*01:01	203	217	QIRYLSKFDKYTVTR	Consensus	1.64	206	1.64	13.20	3.24	5.30	0.11
HLA-DRB5*01:01	204	218	IRYLSKFDKYTVTRE	Consensus	1.66	208	1.66	22.10	5.34	5.30	0.11
HLA-DRB5*01:01	69	83	ATHFVLDFSDPKKRC	Consensus	2.22	31	0.13	9.50	2.22	1.90	4.34

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB5*01:01	45	59	ELLYSTFDFIKNTCA	Consensus	2.47	300	2.47	32.90	7.38	2.60	2.44
HLA-DRB5*01:01	68	82	NATHFVLDFSDPKKR	Consensus	2.52	30	0.13	10.50	2.52	1.90	4.34
HLA-DRB5*01:01	53	67	FIKNTCANPKMKCTN	Consensus	2.77	333	2.77	29.80	6.83	4.10	0.55
HLA-DRB5*01:01	54	68	IKNTCANPKMKCTNN	Consensus	2.84	341	2.84	55.40	10.67	4.10	0.55
HLA-DRB5*01:01	70	84	THFVLDFSDPKKRCI	Consensus	2.85	31	0.13	11.70	2.85	1.90	4.34
HLA-DRB5*01:01	46	60	LLYSTFDFIKNTCAN	Consensus	3.23	384	3.23	38.90	8.38	2.60	2.44
HLA-DRB5*01:01	14	28	ISLILPIICIKVIRF	Consensus	3.26	388	3.26	861.10	40.93	2.60	2.68
HLA-DRB5*01:01	47	61	LYSTFDFIKNTCANP	Consensus	3.36	401	3.36	52.10	10.27	2.60	2.44
HLA-DRB5*01:01	163	177	YGMAYKFDKNDNWSI	Consensus	3.41	406	3.41	48	9.72	4.60	0.30
HLA-DRB5*01:01	17	31	ILPIICIKVIRFDDR	Consensus	3.63	429	3.63	891.30	41.45	2.60	2.68
HLA-DRB5*01:01	15	29	SLILPIICIKVIRFD	Consensus	3.65	431	3.65	882	41.28	2.60	2.68
HLA-DRB5*01:01	162	176	AYGMAYKFDKNDNWS	Consensus	3.72	440	3.72	35.50	7.83	4.60	0.30
HLA-DRB5*01:01	280	294	ADEPITYAFVKVFSN	Consensus	3.73	385	3.24	197.60	21.85	2.10	3.73
HLA-DRB5*01:01	281	295	DEPITYAFVKVFSNP	Consensus	3.73	415	3.49	231.80	23.62	2.10	3.73
HLA-DRB5*01:01	282	296	EPITYAFVKVFSNPD	Consensus	3.73	82	0.52	47.40	9.64	2.10	3.73
HLA-DRB5*01:01	283	297	PITYAFVKVFSNPDH	Consensus	3.73	83	0.53	29.40	6.76	2.10	3.73
HLA-DRB5*01:01	284	298	ITYAFVKVFSNPDHN	Consensus	3.73	82	0.52	21.20	5.14	2.10	3.73
HLA-DRB5*01:01	285	299	TYAFVKVFSNPDHNE	Consensus	3.73	94	0.62	17.10	4.21	2.10	3.73
HLA-DRB5*01:01	286	300	YAFVKVFSNPDHNEP	Consensus	3.73	96	0.64	24.30	5.80	2.10	3.73
HLA-DRB5*01:01	71	85	HFVLDFSDPKKRCIS	Consensus	3.74	55	0.29	15.10	3.74	1.90	4.34
HLA-DRB5*01:01	18	32	LPIICIKVIRFDDRD	Consensus	3.85	456	3.85	1080.80	44.46	2.50	2.68
HLA-DRB5*01:01	16	30	LILPIICIKVIRFDD	Consensus	3.93	465	3.93	921.60	41.97	2.60	2.68
HLA-DRB5*01:01	241	255	SFLDVYWFQESVKHK	Consensus	4.08	208	1.66	16.50	4.08	1.80	4.69
HLA-DRB5*01:01	216	230	TREMVVKHRAKKFTM	Consensus	4.14	109	0.77	16.80	4.14	1.80	4.69
HLA-DRB5*01:01	66	80	TNNATHFVLDFSDPK	Consensus	4.34	44	0.22	42.30	8.91	1.90	4.34

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB5*01:01	67	81	NNATHFVLDFSDPKK	Consensus	4.34	42	0.20	24.60	5.86	1.90	4.34
HLA-DRB5*01:01	72	86	FVLDFSDPKKRCISS	Consensus	4.34	55	0.29	24.50	5.84	1.90	4.34
HLA-DRB5*01:01	214	228	TVTREMVVKHRAKKF	Consensus	4.42	110	0.78	18	4.42	1.80	4.69
HLA-DRB5*01:01	215	229	VTREMVVKHRAKKFT	Consensus	4.42	113	0.82	18	4.42	1.80	4.69
HLA-DRB5*01:01	243	257	LDVYWFQESVKHKPK	Consensus	4.54	141	1.05	18.50	4.54	1.80	4.69
HLA-DRB5*01:01	217	231	REMVVKHRAKKFTMD	Consensus	4.69	116	0.85	32.70	7.35	1.80	4.69
HLA-DRB5*01:01	218	232	EMVVKHRAKKFTMDF	Consensus	4.69	189	1.49	62.80	11.59	1.80	4.69
HLA-DRB5*01:01	219	233	MVVKHRAKKFTMDFS	Consensus	4.69	337	2.80	94.10	14.78	1.80	4.69
HLA-DRB5*01:01	242	256	FLDVYWFQESVKHKP	Consensus	4.69	150	1.12	20.20	4.93	1.80	4.69
HLA-DRB5*01:01	244	258	DVYWFQESVKHKPKL	Consensus	4.69	140	1.04	20.90	5.08	1.80	4.69
HLA-DRB5*01:01	245	259	VYWFQESVKHKPKLP	Consensus	4.69	224	1.81	28.10	6.52	1.80	4.69
HLA-DRB5*01:01	246	260	YWFQESVKHKPKLPY	Consensus	4.69	249	2.01	32.40	7.29	1.80	4.69
HLA-DRB5*01:01	247	261	WFQESVKHKPKLPYI	Consensus	4.69	451	3.81	64.50	11.79	1.80	4.69
HLA-DRB5*01:01	11	25	LVLISLILPIICIKV	Consensus	4.93	584	4.93	2377.30	57.99	2.80	2.05
HLA-DRB5*01:01	135	149	DALVDIRGLKTCSLK	Consensus	5.04	598	5.04	406.10	30.31	2.50	2.68
HLA-DRB5*01:01	123	137	CLLVFKKKERREDAL	Consensus	5.05	64	0.38	128.50	17.54	1.80	5.05
HLA-DRB5*01:01	124	138	LLVFKKKERREDALV	Consensus	5.05	134	0.99	208.80	22.45	1.80	5.05
HLA-DRB5*01:01	287	301	AFVKVFSNPDHNEPR	Consensus	5.05	294	2.42	37.30	8.13	1.70	5.05
HLA-DRB5*01:01	288	302	FVKVFSNPDHNEPRL	Consensus	5.05	307	2.53	58.40	11.06	1.70	5.05
HLA-DRB5*01:01	10	24	CLVLISLILPIICIK	Consensus	5.21	616	5.21	3348.80	64.29	2.80	2.05
HLA-DRB5*01:01	213	227	YTVTREMVVKHRAKK	Consensus	5.28	146	1.10	21.80	5.28	1.30	6.60
HLA-DRB5*01:01	134	148	EDALVDIRGLKTCSL	Consensus	5.32	630	5.32	397.30	30.02	2.50	2.68
HLA-DRB5*01:01	73	87	VLDFSDPKKRCISSI	Consensus	5.42	225	1.82	244.90	24.26	1.60	5.42
HLA-DRB5*01:01	74	88	LDFSDPKKRCISSIH	Consensus	5.42	234	1.88	475.50	32.38	1.60	5.42
HLA-DRB5*01:01	12	26	VLISLILPIICIKVI	Consensus	5.46	647	5.46	1802.70	53.06	2.60	2.68

Supplementary data 12 (cont.).

Allele	Start	End	Peptide	Method used	Percentile rank	SMM align IC50 (nM)	SMM align rank	NN align IC50 (nM)	NN align rank	Sturniolo score	Sturniolo rank
HLA-DRB5*01:01	220	234	VVKHRAKKFTMDFSN	Consensus	5.48	650	5.48	338.10	28.02	1.80	4.69
HLA-DRB5*01:01	8	22	TCCLVLISLILPIIC	Consensus	5.49	651	5.49	8344.60	81.40	2.80	2.05
HLA-DRB5*01:01	9	23	CCLVLISLILPIICI	Consensus	5.84	695	5.84	7651.20	79.82	2.80	2.05
HLA-DRB5*01:01	240	254	ISFLDVYWFQESVKH	Consensus	6.12	260	2.10	25.90	6.12	1.20	7.46
HLA-DRB5*01:01	254	268	HKPKLPYIYYNGECL	Consensus	6.18	659	5.55	151.10	19.11	1.40	6.18
HLA-DRB5*01:01	255	269	KPKLPYIYYNGECLP	Consensus	6.18	712	6	155.20	19.37	1.40	6.18
HLA-DRB5*01:01	256	270	PKLPYIYYNGECLPS	Consensus	6.18	707	5.96	114.50	16.48	1.40	6.18
HLA-DRB5*01:01	257	271	KLPYIYYNGECLPSN	Consensus	6.18	694	5.83	90.90	14.50	1.40	6.18
HLA-DRB5*01:01	258	272	LPYIYYNGECLPSNK	Consensus	6.18	706	5.95	157.60	19.53	1.40	6.18
HLA-DRB5*01:01	212	226	KYTVTREMVVKHRAK	Consensus	6.54	413	3.47	28.20	6.54	-1	19.74
HLA-DRB5*01:01	137	151	LVDIRGLKTCSLKER	Consensus	6.79	803	6.79	429.90	31.04	2.50	2.68
HLA-DRB5*01:01	131	145	ERREDALVDIRGLKT	Consensus	7.03	786	6.65	742.30	38.68	1.20	7.03
HLA-DRB5*01:01	132	146	RREDALVDIRGLKTC	Consensus	7.03	806	6.82	614.70	35.91	1.20	7.03
HLA-DRB5*01:01	133	147	REDALVDIRGLKTCS	Consensus	7.03	803	6.79	500.90	33.08	1.20	7.03

Notes: The MHCII binding predictions were made on 07/16/2017 using the IEDB analysis resource Consensus tool. In terms of population coverage, the reference sets for class II should provide >99%. Results are presented by allele, and from lowest to highest percentile rank. Peptides predicted as good binders are all of those with a percentile ranking lower or equal to 10.

Supplementary data 13. LJL143 in silico analysis: MHC-I restricted-epitopes

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*01:01	93	101	9	NSDLTKNLY	Consensus	0.15	5.07	0.1	24.58	0.2
HLA-A*01:01	150	158	9	HVDSYGLPY	Consensus	0.15	3.42	0.1	68.01	0.2
HLA-A*01:01	270	278	9	DTDELMTYA	Consensus	0.3	33.74	0.2	326.25	0.4
HLA-A*01:01	219	228	10	FSIILPNEEY	Consensus	0.35	467.62	0.4	391.56	0.3
HLA-A*01:01	79	87	9	ISAIKLTSY	Consensus	0.45	911.48	0.3	578.83	0.6
HLA-A*01:01	108	116	9	GTDNCKLVF	Consensus	0.5	1546.69	0.5	531.56	0.5
HLA-A*01:01	268	277	10	VFDTDELMTY	Consensus	0.6	2874.54	0.9	376.53	0.3
HLA-A*01:01	176	185	10	DTRFSTEVFY	Consensus	0.65	1308.31	0.6	730.80	0.7
HLA-A*01:01	60	68	9	CSNNQTHFV	Consensus	0.75	1477.77	0.5	958.41	1
HLA-A*01:01	92	101	10	QNSDLTKNLY	Consensus	0.8	53.74	0.1	1523.35	1.5
HLA-A*01:01	78	87	10	CISAIKLTSY	Consensus	0.9	1185.91	0.6	1229.70	1.2
HLA-A*01:01	145	154	10	GADPLHVDSY	Consensus	0.9	1069.21	0.6	1221.24	1.2
HLA-A*01:01	149	158	10	LHVDSYGLPY	Consensus	0.95	7.18	0.1	1939.99	1.8

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-A*02:01	189	198	10	GLFNTQITYL	Consensus	0.25	26.87	0.2	21.50	0.3	-	-
HLA-A*02:01	11	19	9	GLISFGFIV	Consensus	0.4	14.44	0.3	20.28	0.4	1.32e-05	0.9
HLA-A*02:01	273	282	10	ELMTYALVKV	Consensus	0.75	135.80	0.7	50.52	0.8	-	-
HLA-A*02:01	39	47	9	TLFFASYGL	Consensus	0.8	13.22	0.3	44.58	0.8	7.18e-05	4.1

Supplementary data 13 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*02:03	189	198	10	GLFNTQITYL	Consensus	0.5	16.64	0.6	14.90	0.4
HLA-A*02:03	99	107	9	NLYCQTGGI	Consensus	0.65	7.84	0.4	23.55	0.9
HLA-A*02:03	274	282	9	LMTYALVKV	Consensus	0.65	12.07	0.5	19.36	0.8
HLA-A*02:03	11	19	9	GLISFGFIV	Consensus	0.75	17.78	0.6	23.22	0.9

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*02:06	121	129	9	RQIAANIEI	Consensus	0.5	12.58	0.3	18.47	0.7
HLA-A*02:06	12	21	10	LISFGFIVAV	Consensus	0.75	61.26	0.2	71.93	1.3

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*23:01	196	204	9	TYLAEEDSF	Consensus	0.25	45.31	0.2	51.81	0.3
HLA-A*23:01	183	191	9	VFYHKNGLF	Consensus	0.4	73.56	0.2	156.11	0.6
HLA-A*23:01	170	179	10	RYNIFKDTRF	Consensus	0.45	36.49	0.4	67.25	0.5
HLA-A*23:01	32	41	10	KYKEKDETLF	Consensus	0.55	211.52	0.6	63.05	0.5
HLA-A*23:01	142	151	10	RYIGADPLHV	Consensus	0.75	182.34	0.6	153.01	0.9
HLA-A*23:01	182	191	10	EVFYHKNGLF	Consensus	1.0	339.49	0.6	259.24	1.4

Supplementary data 13 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*03:01	39	48	10	TLFFASYGLK	Consensus	0.25	11.31	0.3	10.40	0.2
HLA-A*03:01	68	76	9	VLNFKTNKK	Consensus	0.3	35.71	0.2	73.25	0.4
HLA-A*03:01	273	281	9	ELMTYALVK	Consensus	0.4	116.66	0.3	122.97	0.5
HLA-A*03:01	128	136	9	EIYGIPAKK	Consensus	0.5	218.26	0.4	151.98	0.6
HLA-A*03:01	108	117	10	GTDNCKLVFK	Consensus	0.5	113.06	0.6	41.80	0.4
HLA-A*03:01	131	140	10	GIPAKKCSFK	Consensus	0.5	63.12	0.5	45.84	0.5
HLA-A*03:01	40	48	9	LFFASYGLK	Consensus	0.55	213.74	0.4	170.14	0.7
HLA-A*03:01	67	75	9	FVLNFKTNK	Consensus	0.55	126.43	0.3	191.34	0.8
HLA-A*03:01	13	22	10	ISFGFIVAVK	Consensus	0.55	37.14	0.5	60.28	0.6
HLA-A*03:01	64	72	9	QTHFVLNFK	Consensus	0.6	113.70	0.3	223.25	0.9
HLA-A*03:01	189	197	9	GLFNTQITY	Consensus	0.7	81.24	0.2	306.06	1.2
HLA-A*03:01	67	76	10	FVLNFKTNKK	Consensus	0.9	156.93	0.9	88.96	0.9
HLA-A*03:01	89	98	10	KINQNSDLTK	Consensus	0.9	131.57	0.7	108.94	1.1

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*11:01	80	89	10	SAIKLTSYPK	Consensus	0.15	12.30	0.1	9.86	0.2
HLA-A*11:01	108	117	10	GTDNCKLVFK	Consensus	0.25	21.10	0.3	14.82	0.2
HLA-A*11:01	220	229	10	SIILPNEEYK	Consensus	0.25	24.94	0.3	17.37	0.2
HLA-A*11:01	64	72	9	QTHFVLNFK	Consensus	0.35	11.50	0.3	40.02	0.4
HLA-A*11:01	39	48	10	TLFFASYGLK	Consensus	0.35	15.27	0.3	25.99	0.4
HLA-A*11:01	81	89	9	AIKLTSYPK	Consensus	0.5	35.15	0.4	54.99	0.6
HLA-A*11:01	89	98	10	KINQNSDLTK	Consensus	0.55	120.62	0.6	35.31	0.5
HLA-A*11:01	67	75	9	FVLNFKTNK	Consensus	0.65	39.11	0.4	96.01	0.9

Supplementary data 13 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*11:01	13	22	10	ISFGFIVAVK	Consensus	0.65	20.21	0.3	93.72	1
HLA-A*11:01	67	76	10	FVLNFKTNKK	Consensus	0.7	79.01	0.3	106.62	1.1
HLA-A*11:01	178	187	10	RFSTEVFYHK	Consensus	0.7	60.34	0.3	99.51	1.1
HLA-A*11:01	273	281	9	ELMTYALVK	Consensus	0.75	127.12	0.4	120.31	1.1
HLA-A*11:01	40	48	9	LFFASYGLK	Consensus	0.8	300.25	0.4	127.15	1.2
HLA-A*11:01	179	187	9	FSTEVFYHK	Consensus	0.8	45.45	0.4	132.53	1.2
HLA-A*11:01	68	76	9	VLNFKTNKK	Consensus	0.85	96.97	0.4	152.51	1.3
HLA-A*11:01	167	175	9	NVERYNIFK	Consensus	0.85	76.30	0.4	153.21	1.3
HLA-A*11:01	221	229	9	IILPNEEYK	Consensus	0.85	54.95	0.4	149.73	1.3

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*24:02	170	179	10	RYNIFKDTRF	Consensus	0.15	88.46	0.1	29.97	0.2
HLA-A*24:02	32	41	10	KYKEKDETLF	Consensus	0.5	382.06	0.8	41.18	0.2
HLA-A*24:02	196	204	9	TYLAEEDSF	Consensus	0.7	222.44	0.9	277.10	0.5
HLA-A*24:02	165	174	10	GWNVERYNIF	Consensus	0.75	426.47	0.8	295.61	0.7
HLA-A*24:02	142	151	10	RYIGADPLHV	Consensus	0.85	339.51	0.8	387.01	0.9
HLA-A*24:02	183	191	9	VFYHKNGLF	Consensus	0.95	307.54	0.9	584.29	1
HLA-A*24:02	9	17	9	IVGLISFGF	Consensus	1.0	1166.91	1	569.68	1
HLA-A*24:02	248	256	9	KYPYIHYNG	Consensus	1.0	339.37	0.9	613.24	1.1

Supplementary data 13 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*26:01	241	249	9	ETMRKKPKY	Consensus	0.2	93.23	0.1	202.12	0.3
HLA-A*26:01	182	191	10	EVFYHKNGLF	Consensus	0.2	14	0.1	446.36	0.3
HLA-A*26:01	78	87	10	CISAIKLTSY	Consensus	0.45	2481.02	0.3	833.09	0.6
HLA-A*26:01	176	185	10	DTRFSTEVFY	Consensus	0.45	503.67	0.1	1006.21	0.8
HLA-A*26:01	122	130	9	QIAANIEIY	Consensus	0.5	364.14	0.3	541.51	0.7
HLA-A*26:01	8	17	10	SIVGLISFGF	Consensus	0.55	208.59	0.1	1223.74	1
HLA-A*26:01	182	190	9	EVFYHKNGL	Consensus	0.6	732.30	0.4	636.22	0.8
HLA-A*26:01	176	184	9	DTRFSTEVF	Consensus	0.65	1117.51	0.5	651.04	0.8
HLA-A*26:01	275	283	9	MTYALVKVF	Consensus	0.7	669.09	0.4	773.77	1
HLA-A*26:01	235	243	9	DVYWFQETM	Consensus	0.9	1207.83	0.5	1017.68	1.3

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-A*30:01	72	81	10	KTNKKSCISA	Consensus	0.15	58.12	0.2	92.46	0.1	-	-
HLA-A*30:01	81	89	9	AIKLTSYPK	Consensus	0.3	8.53	0.2	13.83	0.3	0.0002	3.7
HLA-A*30:01	244	252	9	RKKPKYPYI	Consensus	0.4	31.79	0.4	24.43	0.4	5.06e-05	1.1
HLA-A*30:01	64	72	9	QTHFVLNFK	Consensus	0.5	9.08	0.2	29.24	0.5	3.87e-05	0.9
HLA-A*30:01	117	125	9	KKRKRQIAA	Consensus	0.5	70.36	0.5	10.89	0.2	7.48e-05	1.4
HLA-A*30:01	13	22	10	ISFGFIVAVK	Consensus	0.75	1121.86	0.8	302.65	0.7	-	-
HLA-A*30:01	213	222	10	KDIKKKFSII	Consensus	0.8	1166.42	1	274.75	0.6	-	-
HLA-A*30:01	138	146	9	SFKDRYIGA	Consensus	0.8	77.10	0.5	47.86	0.8	0.00105	18

Supplementary data 13 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*30:02	79	87	9	ISAIKLTSY	Consensus	0.35	71.98	0.2	113.62	0.5
HLA-A*30:02	121	130	10	RQIAANIEIY	Consensus	0.35	94.98	0.2	103.13	0.5
HLA-A*30:02	189	197	9	GLFNTQITY	Consensus	0.8	97.59	0.3	320.23	1.3
HLA-A*30:02	242	251	10	TMRKKPKYPY	Consensus	0.8	963.26	0.7	182.97	0.9
HLA-A*30:02	229	237	9	KRISFLDVY	Consensus	0.85	212.41	0.8	178.84	0.9
HLA-A*30:02	219	228	10	FSIILPNEEY	Consensus	0.95	938.54	0.7	233.55	1.2

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*31:01	170	178	9	RYNIFKDTR	Consensus	0.15	6.03	0.1	8.21	0.2
HLA-A*31:01	113	121	9	KLVFKKRKR	Consensus	0.65	24.20	0.4	47.37	0.9
HLA-A*31:01	40	49	10	LFFASYGLKR	Consensus	0.8	82.27	0.5	97.59	1.1
HLA-A*31:01	236	244	9	VYWFQETMR	Consensus	0.85	30.15	0.4	76.64	1.3

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-A*32:01	275	283	9	MTYALVKVF	Consensus	0.3	19.65	0.3	33.18	0.3	4.37e-07	0.2
HLA-A*32:01	13	21	9	ISFGFIVAV	Consensus	0.4	120.44	0.4	651.54	3.4	1.37e-06	0.2
HLA-A*32:01	175	184	10	KDTRFSTEVF	Consensus	0.5	4003.65	0.7	2123.34	0.3	-	-
HLA-A*32:01	217	225	9	KKFSIILPN	Consensus	0.5	1076.70	0.5	244.87	1.7	3.88e-06	0.5
HLA-A*32:01	230	238	9	RISFLDVYW	Consensus	0.5	90.23	0.3	55.97	0.5	1.62e-05	2.1
HLA-A*32:01	230	239	10	RISFLDVYWF	Consensus	0.75	774.76	0.4	3837.25	1.1	-	-

Supplementary data 13 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*33:01	235	244	10	DVYWFQETMR	Consensus	0.3	22.23	0.2	96.94	0.4
HLA-A*33:01	40	49	10	LFFASYGLKR	Consensus	0.4	184.73	0.3	116.82	0.5
HLA-A*33:01	110	119	10	DNCKLVFKKR	Consensus	0.4	238.47	0.4	114.16	0.4
HLA-A*33:01	41	49	9	FFASYGLKR	Consensus	0.7	121.48	0.3	198.63	1.1
HLA-A*33:01	236	244	9	VYWFQETMR	Consensus	0.7	29.44	0.3	189.26	1.1

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-A*68:01	128	136	9	EIYGIPAKK	Consensus	0.2	8.61	0.1	15.90	0.3
HLA-A*68:01	235	244	10	DVYWFQETMR	Consensus	0.2	7.87	0.1	15.42	0.3
HLA-A*68:01	64	72	9	QTHFVLNFK	Consensus	0.25	8.07	0.1	25.85	0.4
HLA-A*68:01	13	22	10	ISFGFIVAVK	Consensus	0.5	26.22	0.6	28.91	0.4
HLA-A*68:01	220	229	10	SIILPNEEYK	Consensus	0.5	51.77	0.6	25.35	0.4
HLA-A*68:01	179	187	9	FSTEVFYHK	Consensus	0.55	16.33	0.3	42.12	0.8
HLA-A*68:01	39	48	10	TLFFASYGLK	Consensus	0.65	14.18	0.4	51.76	0.9
HLA-A*68:01	167	175	9	NVERYNIFK	Consensus	0.8	21.86	0.5	65.09	1.1
HLA-A*68:01	80	89	10	SAIKLTSYPK	Consensus	0.9	23.88	0.6	67.76	1.2

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*07:02	285	293	9	NPESDGSRL	Consensus	0.8	373.33	0.5	215.22	1	2.34e-05	0.8
HLA-B*07:02	87	96	10	YPKINQNSDL	Consensus	0.9	214.71	0.5	286.30	1.3	-	-

Supplementary data 13 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-A*68:02	182	190	9	EVFYHKNGL	Consensus	0.4	4.42	0.2	27.96	0.4	8.91e-06	0.4
HLA-A*68:02	13	21	9	ISFGFIVAV	Consensus	0.5	15.58	0.5	34.24	0.5	3.34e-06	0.2
HLA-A*68:02	38	47	10	ETLFFASYGL	Consensus	0.65	2.88	0.1	52.64	1.2	-	-
HLA-A*68:02	275	284	10	MTYALVKVFT	Consensus	0.8	16.67	0.4	51.09	1.2	-	-
HLA-A*68:02	39	47	9	TLFFASYGL	Consensus	0.8	19.19	0.5	66.93	0.8	6.81e-05	3.7

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*08:01	214	222	9	DIKKKFSII	Consensus	0.2	23.81	0.2	44.31	0.2	3.31e-05	1.2
HLA-B*08:01	116	124	9	FKKRKRQIA	Consensus	0.3	28.64	0.2	70.23	0.3	2.53e-05	1
HLA-B*08:01	115	124	10	VFKKRKRQIA	Consensus	0.5	153.28	0.6	456.69	0.4	-	-
HLA-B*08:01	87	96	10	YPKINQNSDL	Consensus	0.7	177.25	0.6	661.64	0.8	-	-
HLA-B*08:01	1	9	9	MNSINFLSI	Consensus	0.7	353.95	0.5	184.74	0.7	3.6e-05	1.3
HLA-B*08:01	214	223	10	DIKKKFSIIL	Consensus	0.85	65.08	0.3	874.22	1.4	-	-
HLA-B*08:01	116	125	10	FKKRKRQIAA	Consensus	0.9	81.17	0.3	934.59	1.5	-	-
HLA-B*08:01	115	123	9	VFKKRKRQI	Consensus	0.9	253.31	0.5	256.77	0.9	0.000155	5.7

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*35:01	150	158	9	HVDSYGLPY	Consensus	0.5	13.45	0.2	21.56	0.5	0.000499	5.4
HLA-B*35:01	50	58	9	DPCQIVLGY	Consensus	0.8	43.43	0.3	45.98	0.8	0.00151	15
HLA-B*35:01	130	139	10	YGIPAKKCSF	Consensus	0.85	7540.54	1.5	126.15	0.2	-	-

Supplementary data 13 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*15:01	121	130	10	RQIAANIEIY	Consensus	0.1	6.93	0.1	0.50	0.1	-	-
HLA-B*15:01	7	15	9	LSIVGLISF	Consensus	0.3	17.33	0.1	46.03	0.3	4.47e-06	0.5
HLA-B*15:01	275	283	9	MTYALVKVF	Consensus	0.3	16.56	0.1	41.02	0.3	1.7e-05	2.1
HLA-B*15:01	6	15	10	FLSIVGLISF	Consensus	0.3	20.07	0.4	2.85	0.2	-	-
HLA-B*15:01	130	139	10	YGIPAKKCSF	Consensus	0.55	72.55	0.5	6	0.6	-	-
HLA-B*15:01	274	283	10	LMTYALVKVF	Consensus	0.65	6.11	0.1	14.40	1.2	-	-
HLA-B*15:01	78	87	10	CISAIKLTSY	Consensus	1.0	207.72	1.4	7.05	0.6	-	-
HLA-B*15:01	150	158	9	HVDSYGLPY	Consensus	1.0	550.94	1	751.62	4.6	7.78e-06	0.9
HLA-B*15:01	189	197	9	GLFNTQITY	Consensus	1.0	72.26	0.2	145.55	1	4.9e-05	7.3

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-B*44:02	37	45	9	DETLFFASY	Consensus	0.25	201.52	0.2	249.80	0.3
HLA-B*44:02	260	269	10	NENKTCELVF	Consensus	0.35	158.80	0.4	95.70	0.3
HLA-B*44:02	34	42	9	KEKDETLFF	Consensus	0.4	99.01	0.1	506.52	0.7
HLA-B*44:02	162	171	10	QEHGWNVERY	Consensus	0.6	695.98	0.5	261.15	0.7
HLA-B*44:02	240	249	10	QETMRKKPKY	Consensus	0.65	171.74	0.4	311.09	0.9
HLA-B*44:02	36	45	10	KDETLFFASY	Consensus	0.8	85.52	0.3	424.51	1.3
HLA-B*44:02	199	208	10	AEEDSFSEAR	Consensus	0.8	3873.31	1.3	77.96	0.3
HLA-B*44:02	260	268	9	NENKTCELV	Consensus	1.0	2075.58	0.8	850.35	1.2

Supplementary data 13 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-B*44:03	37	45	9	DETLFFASY	Consensus	0.15	53.71	0.1	88.82	0.2
HLA-B*44:03	162	171	10	QEHGWNVERY	Consensus	0.5	177.23	0.6	208.96	0.4
HLA-B*44:03	260	269	10	NENKTCELVF	Consensus	0.55	190.16	0.6	247.21	0.5
HLA-B*44:03	240	249	10	QETMRKKPKY	Consensus	0.7	502.70	1.2	123.05	0.2

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*51:01	223	231	9	LPNEEYKRI	Consensus	0.2	253.95	0.1	349.65	0.2	0.000121	8.7
HLA-B*51:01	1	9	9	MNSINFLSI	Consensus	0.6	7658.86	0.5	3094.78	1.8	1.02e-05	0.6
HLA-B*51:01	10	18	9	VGLISFGFI	Consensus	0.6	9399.63	0.6	2241.97	1.2	2.82e-06	0.2
HLA-B*51:01	156	165	10	LPYQFDQEHG	Consensus	0.8	11047.67	0.8	3451.83	0.8	-	-

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*53:01	249	258	10	YPYIHYNGEC	Consensus	0.75	3301.03	1.3	106.62	0.2	-	-
HLA-B*53:01	156	164	9	LPYQFDQEH	Consensus	1.0	1157.35	1	891	2.6	0.000183	0.9
HLA-B*53:01	158	166	9	YQFDQEHGW	Consensus	1.0	360.59	0.7	288.32	1	0.03	68

Supplementary data 13 (cont.).

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank
HLA-B*57:01	275	283	9	MTYALVKVF	Consensus	0.25	14.12	0.2	90.33	0.3
HLA-B*57:01	230	238	9	RISFLDVYW	Consensus	0.35	130.94	0.3	123.83	0.4
HLA-B*57:01	136	145	10	KCSFKDRYIG	Consensus	0.4	311.68	0.6	137.53	0.2
HLA-B*57:01	231	239	9	ISFLDVYWF	Consensus	0.4	17.39	0.3	184	0.5
HLA-B*57:01	230	239	10	RISFLDVYWF	Consensus	0.5	29.21	0.3	357.61	0.7
HLA-B*57:01	7	15	9	LSIVGLISF	Consensus	0.85	472.44	0.4	715.85	1.3
HLA-B*57:01	79	87	9	ISAIKLTSY	Consensus	0.9	542.55	0.4	758.26	1.4

Allele	Start	End	Length	Peptide	Method used	Percentile rank	ANN IC50 (nM)	ANN rank	SMM IC50 (nM)	SMM rank	Combib_Sid08 score	Combib_Sid08 rank
HLA-B*58:01	230	238	9	RISFLDVYW	Consensus	0.3	16.43	0.3	31.28	0.3	9.2e-05	6.1
HLA-B*58:01	231	239	9	ISFLDVYWF	Consensus	0.3	65.91	0.3	69.39	0.5	1.55e-06	0.2
HLA-B*58:01	7	15	9	LSIVGLISF	Consensus	0.4	93.05	0.3	128.91	0.9	4.27e-06	0.4
HLA-B*58:01	275	283	9	MTYALVKVF	Consensus	0.4	37.28	0.3	35.59	0.4	4.71e-05	3.2
HLA-B*58:01	79	87	9	ISAIKLTSY	Consensus	0.9	225.87	0.5	149.38	0.9	0.000468	23

Note: The MHCI binding predictions were made on 11/25/2016 using the IEDB analysis resource Consensus tool which combines predictions from ANN aka NetMHC (4.0), SMM and Comblib. In terms of population coverage, the reference sets for class II should provide >97%. Results are presented by allele, and from lowest to highest percentile rank. Peptides predicted as good binders are all of those with a percentile ranking lower or equal to 1.

III. DISCUSSION & PERSPECTIVES

The epidemiological complexity of human leishmaniasis makes disease control extremely dependent on an immune prophylactic approach (Kamhawi, 2017). Vaccination, and the consequent generation of long-lasting anti-*Leishmania* immunity, is the only viable way to decrease the population at risk, and consequently disease incidence and prevalence. Being most of the different leishmaniases zoonosis, and all of them vector-borne diseases, to expect the eradication of the pathogenic agents is unrealistic at the least. Parasite species are expected to circulate in the wild between their respective vectors and animal reservoirs (Shimozako *et al*, 2017). Consequently, the elimination of possible transmission events is also difficult to achieve, as is consequently to abolish human infection (generally). Vaccination will therefore mainly impact infection establishment, and consequently disease development: this is realistically the ultimate goal to consider when we think of anti-*Leishmania* vaccines. Furthermore, anti-*Leishmania* vaccines will probably work mostly individually, and not so much at the population level. While for other vaccine-preventable diseases, vaccine coverage is important both individually (protection of the immunized individual) and collectively (protection of susceptible individuals within a given population) (Giubilini, 2018), considering leishmaniasis the impact of the potential generation of "herd immunity" statuses will be negligible (perhaps only potentially relevant when we think of anthroponotic leishmaniasis).

But is the development of a vaccine for leishmaniasis something achievable? An easy task is not for sure. However, the scientific community agrees that is not an impossible one (Cecilio *et al*, 2018). Truth be told, after more than 50 years of anti-*Leishmania* vaccines' research, there is still no vaccine available to humans. Still, the protection results observed following leishmanization campaigns (in humans) (Marzinowsky and Schurenkova, 1924; Senekji and Beattie, 1941), together with the data gathered after the clinical use of veterinary vaccines for leishmaniasis (in dogs) (Moreno, 2019) point to the hypothesis that the most needed anti-*Leishmania* vaccine for humans will eventually be a reality.

1. One vaccine or several vaccines?

As we stand, in the anti-*Leishmania* vaccines field, there is no wrong or right answers to the question above. The fact that there is still no vaccine available for humans makes every vaccine candidate on the vaccine-development pipeline equally valid and essential. In line

with this notion, the economic viability (and the profitability potential) of the first anti-*Leishmania* vaccine for humans (Lee *et al*, 2012; Bacon *et al*, 2013), higher thinking of a pan-*Leishmania* vaccine in comparison with a vaccine against a specific parasite species (with the associated geographic restrictions), should not be a major factor to consider. Still, and because leishmaniasis is a NTD, vaccines should be as cheap as possible (Bethony *et al*, 2011).

This dissertation focuses on two quite distinct vaccine candidates. First, their final target populations are different. The first candidate was envisioned as a vaccine against visceral leishmaniasis, better applicable to South America, since the sand fly-derived antigen used was a protein from the saliva of *L. longipalpis* sand flies. The second one was thought of as a large-spectrum vaccine (a proof-of-principle). Furthermore, also their composition is distinct. While the first candidate was multivalent in nature (composed of three different recombinant proteins) and adjuvanted, the second one was monovalent and in theory self-adjuvanted (DNA-vaccine).

Both have advantages and disadvantages, thinking particularly, in anti-*Leishmania* vaccines, but also generally, in vaccines *per se*. Again comparing polyvalent with monovalent vaccines, it is straightforward to conclude that the second ones are easier to produce and to control (Schlingmann *et al*, 2018). As a matter of fact, each element of a multivalent vaccine have to be considered individually in terms of production and the associated application of exhaustive quality control measures, required for any human pharmaceutical commercial product (Smith *et al*, 2011). Still, the multivalency is not something to completely disregard. Multivalency has the advantage of modularity (Schlingmann *et al*, 2018). Each component of the vaccine can, due to this, be included/excluded from the vaccine composition, meaning that the formula may be modified allowing to obtain tailored-based vaccines to use in particular contexts (for particular populations) - e.g. we cannot forget that endemic and non-endemic individuals basal immune responses may be different, meaning that a good vaccine for the first may not necessarily be the best for the second population. This discussion can also be applied to the vaccine spectrum. Still, although tailored-made vaccines may be essential for particular populations (e.g. not covered by the large-spectrum vaccines – it is probably utopic to consider that a single anti-*Leishmania* vaccine will be equally effective in the entirety of the human population), the ones with a broader spectrum are in theory a general better option. Although in the NTDs list leishmaniasis appears as a single item (Hotez *et al*, 2020),

leishmaniasis is a spectrum of diseases caused by as many as 20 etiologic agents, whose relevance is not equal, due to important epidemiologic factors, such as disease burden, but not only. This means that some leishmaniasis have the potential to be more neglected than others, paralleling the issue recognized for malaria, comparing the relevance of *P. falciparum*-caused disease, with the ones caused by *P. vivax, P. malariae* and *P. ovale* infections (Hotez *et al*, 2020). In this sense, a broad-spectrum vaccine (ideally a pan-*Leishmania* vaccine) will be potentially "less discriminatory", while in parallel more profitable. Additionally, even a broad-spectrum antigen can be combined with others (taking again advantage of modularity). One may argue that to expose individuals to (epidemiologically) irrelevant immunogens may not be a wiser option: e.g. in line with the second vaccine candidate, to expose individuals from South America to sand fly salivary epitopes which they would be potentially exposed only in the event of a visit to Africa. However, and as what happens with many broad-spectrum vaccines (e.g. HPV or pneumococcal vaccines), the risk of the potential "unnecessary immunogenicity" is outweighed by the benefit of protecting a higher frequency of the potentially affected populations (Schlingmann *et al*, 2018).

Still on the topic of advantages/disadvantages of the studied vaccine candidates in this dissertation, we need to consider the traditional ones associated with the second and third generations of anti-*Leishmania* vaccines (the multivalent and the engineered vector-based anti-*Leishmania* vaccines, respectively) (Cecilio *et al*, 2018). From the safety standpoint, and mostly due to the existence of precedents, recombinant proteins'-based vaccines will be accepted more easily by the regulatory entities than DNA vaccines (Myhr, 2017). However, this (theoretical) precautionary response, may change with time or on the other hand, show to have been a prudent and wise option. This will depend on the data gathered through the monitoring of the clinical use of DNA vaccines in the context of veterinary medicine (Ferraro *et al*, 2011) [and perhaps also human medicine, soon (Hobernik and Bros, 2018)]. Still, a DNA vaccine has the potential to give rise to a recombinant protein vaccine, using one of the many expression systems available, also rationally. Although the natural evolution of science (and the associated methodologies) makes us, newly formed scientists, think of protein production and purification as a potentially easy task, this is most certainly a misconception. The expression of chimeric proteins (such as the one we propose as a proof of principle of a future vector-based pan-*Leishmania* vaccine) may be quite a challenging task (Schmidt, 2017). Particularly if we want to reproduce the natural proteins' glycosylation patterns, known to be important factors for immune responses (Wolfert and Boons, 2013). However, we need to keep in mind that recombinant-protein based vaccines may not work

ideally without the use of adjuvants (Christensen, 2016), enhancers/modulators of immune responses, whose use will consequently imply a potential additional risk (Petrovsky, 2015). In fact, in the first manuscript reprinted in the objectives & results section of this dissertation we show precisely this: the inclusion of an adjuvant in the multivalent vaccine formulation was essential for the improvement of the vaccine immunogenic potential both considering humoral and cellular responses.

2. How should be an ideal anti-*Leishmania* vaccine composed?

Most of the anti-*Leishmania* vaccines, in line with the majority of research on *Leishmania* spp. and leishmaniasis, completely ignore the transmitting agent, the vector. As stressed in the introduction of this dissertation, for leishmaniasis (and every other vector-borne disease), the comprehension of the infectious process, the pathogenesis and the epidemiology all depend on the individual understanding of the etiologic agent, the host, and the vector, but also of their interactions with each other. In this sense, to disregard the vector in any *Leishmania*-related research, may potentially lead to conclusions that perhaps are irrelevant in the frame of the natural context, and therefore will not contribute to the advancement of the knowledge on leishmaniasis (they can potentially even harm such an advancement by acting as confounding factors).

Our focus was precisely related to this notion. Our goal was to design anti-*Leishmania* vaccines that included the vector. Sand fly saliva-based vaccines are not a new notion. Since Kamhawi *et al.* showed that the exposure to non-infected sand fly bites would protect from a posterior challenge *via* sand fly bites (Kamhawi *et al*, 2000), a few defined sand fly salivary proteins were explored in the context of anti-*Leishmania* vaccination (Cecilio *et al*, 2018). Here, we used one of those candidates (LJL-143), in combination with parasite-derived antigens as a proposal of a multivalent anti-*Leishmania* vaccine for VL. And trying to go one step further, considering that this antigen would mostly be relevant in South America, we decided to overcome the limitation of its potential narrow spectrum. We opted to fuse it (portions and not the full-length protein) to another sand fly-saliva studied vaccine, PdSP15,

generating a neo-antigen with a broader spectrum (at least geographically speaking) with the potential to be used not only in South America but also in Africa.

Overall we believe our data suggest that vector-based antigens should be part of the anti-*Leishmania* vaccines for humans, but not as a whole. Indeed, the anti-sand fly saliva immune response impacts *Leishmania* transmission via sand fly bites and consequently infection and disease, as we show in the second manuscript this dissertation focuses on, in the BALB/c mouse model [and as others have shown as well, with distinct sand fly salivary proteins (Kamhawi *et al*, 2014; Oliveira *et al*, 2015)]. However, time-wise this impact will be limited, considering the availability of the antigen. Sand fly saliva will be deposited on the skin only at the moment of transmission. Therefore, contrarily to parasite-derived antigens, expected to be found as long as parasites survive, sand fly-derived antigens presence will probably be restricted to the skin (and perhaps also draining lymph nodes) for a limited time, after which they will cease to exist, as will the associated vaccine-elicited secondary immune responses. The question that deserves to be asked is "are these anti-saliva responses enough to prevent disease development?". Usually, the favorable readouts of anti-*Leishmania* vaccine efficacy trials (with both vector and parasite-based antigens) are the decrease in parasite numbers together with milder pathological phenotypes, and not complete protection from disease (Teixeira and Gomes, 2013). Establishing a direct parallel to humans, in the form of a speculative exercise, having however in mind that to perform such a simple parallel is not realistic, we can ask another question. Will these vector-derived vaccines alone, and the early and potentially transient anti-sand fly saliva immunity effect, be sufficient to prevent human disease development (either cutaneous or visceral), or will they only contribute to the occurrence of milder clinical presentations? Both case scenarios are plausible. Assuming that the initial anti-saliva immune response will indirectly impact *Leishmania* parasites in the skin, in a greater enough extent, sufficient to reduce parasite numbers below the threshold required for pathogenicity, vector-based vaccines alone may be a good enough prophylactic agent. They may re-enforce a probable bottleneck of *Leishmania* infection establishment - known in other vector borne parasitic diseases (Sinnis and Zavala, 2012; Menard *et al*, 2013) - related with both transmitted parasite numbers and their early survival in the skin (Loeuillet *et al*, 2016)]. Perhaps, the deleterious and indirect effect of anti-saliva immunity on *Leishmania* parasites will delay parasite establishment long enough for the development of anti-*Leishmania* immunity (in the context of a primary immune response, expected to occur in naïve individuals), that will then by itself further control *Leishmania* parasites and prevent disease development. On the other way, on the

event of the indirect effect of anti-*Leishmania* won't impact parasite establishment sufficiently enough to prevent disease development, it still may impact disease severity, which by itself is not an unfavorable outcome. Although not ideal, this case scenario would still potentially contribute to the decrease in global disease burden, considering morbidity and the life-long consequences of non-fatal leishmaniases.

The same hypothetical exercise can be performed thinking on most of the anti-*Leishmania* vaccines proposed up to now, composed exclusively of parasite-derived antigens. Is the elicited anti-*Leishmania* immunity sufficient to prevent vector-transmitted parasites' establishment, in the context of infectious inoculums also composed of many individual immunomodulatory agents, not all parasite-derived (Rogers, 2012; Abdeladhim *et al*, 2014; Dey *et al*, 2018; Perez-Cabezas *et al*, 2019)? Or would most of the pre-clinically determined good vaccine candidates (so far), fail in the context of human clinical trials in the field [like the ALM vaccine (Cecilio *et al*, 2018)], or in a better case scenario contribute to "lower infections" and consequently to milder clinical pictures?

The abovementioned questions and discussion justify our suggestion of the ideal anti-*Leishmania* vaccine for humans: a bivalent vaccine, composed of one vector-derived and one parasite-derived broad-spectrum antigens, adjuvanted and/or vehiculated if needed. The spectrum option needs no additional justification, considering the ones highlighted in the previous section. The combination of parasite- and vector-derived antigens gives a logical potential combination of two distinct immune mechanisms that impact parasites' establishment. The direct anti-*Leishmania* specific immune responses together with the indirect sand fly saliva-specific immune responses may act, either additively or better yet synergistically, and consequently theoretically better prevent disease development. The bivalency brings a modular potential that may allow either vaccine-formula alteration or the use of non-traditional immunization schemes. In the first reprinted manuscript, part of the objectives & results section of this dissertation, we show that more than an antigen, the sand fly salivary protein LJL143 demonstrates adjuvant-like properties. This protein potentiated the immunogenicity of the parasite-derived antigens included in the vaccine formulation, when a prime-boost immunization scheme was adopted (prime with the sand fly salivary protein, followed by two boosts with the whole vaccine formulation, including the sand fly-derived antigen). Thinking on the real application of these vaccines (and assuming the sand fly-derived vaccine component will also act as an adjuvant), while endemic individuals are expected to be "primed", since they have probably been sufficiently exposed to sand fly bites

and therefore sand fly salivary proteins (Oliveira *et al*, 2013), that is not the case considering non-endemic ones. Still, an anti-*Leishmania* vaccine can be as essential for this last population, in the situation of travel or relocation to endemic countries. In this context, the bivalency (modularity) may be extremely important, and the vaccine can potentially be administered in two different schemes: a traditional one for the endemic population, and a prime-boost one for the non-endemic population.

3. Thinking before testing

Most of the anti-*Leishmania* vaccines developed and explored over the years were tested empirically. While this option was the only one available in the "early ages" of vaccinology, in the present it should no longer be followed (Rueckert and Guzman, 2012), unless if justified by an extreme context (e.g. thinking of an emergent disease with pandemic potential such as the new COVID-19: the simplicity of the SARS-CoV-2 viral genome (Wu *et al*, 2020) and the consequently limited number of viral proteins - among them only 4 major structural proteins - together with the urge for a vaccine may justify an empirical approach). Therefore, today, (anti-*Leishmania*) vaccines should be designed and tested rationally, built on top of what we already know, while using the modern tools we have available (Rueckert and Guzman, 2012).

The works this dissertation focuses on are examples of rational vaccine-development. Although both studies focused on known antigens, most of them extensively studied, not only, but particularly in the context of anti-*Leishmania* vaccination, their rationales (and purposes) are quite different. The first study proposes a simple combination of different parasite- and vector-derived components (that would induce both anti-sand fly saliva and anti-*Leishmania* responses), while the second one proposes the generation of a neo-antigen with the objective of broadening the potentially limited spectrum of traditional sand fly salivary proteins (as the one used in the first study). Importantly, both vaccines were developed having in mind the final target population, humans.

As is widely accepted, although extremely important, pre-clinical investigation results' many times do not parallel to the clinical context. Human immune responses are different than murine immune responses [and even from the ones observed in non-human primates, whose genetic differences can be as low as 2% (Bjornson-Hooper *et al*, 2019)]. Therefore,

a molecule determined as a strong murine immunogen, may not be a (strong) human immunogen and *vice-versa*. This is particularly relevant thinking of cellular immunology, the one of higher interest for the fight against intracellular pathogens. T-cell epitopes are usually HLA-restricted, frequently in a species (and even strain) -specific fashion (Mestas and Hughes, 2004; Davis, 2008). And we show precisely this with both manuscripts that compose the objectives & results section of this dissertation. For instance, we show that while KMP-11 was the weakest immunogen in the context of the murine model we used, it was the one that induced a better response in the context of cell-proliferation experiments using PBMCs from cured VL human patients. Additionally, we also show that, for instance, *in silico* predictions for PdSp15 sand fly salivary protein suggest that it would be immunogenic in BALB/c but not in C57Bl/6 (or much more in the first mouse strain, considering only for that one relevant MHC-II restricted epitopes were predicted). The same applies for the human context: e.g. more than 20 MHC-II restricted epitopes were predicted for the allele HLA-DRB3*01:01, comparing with less than 5 predicted for the allele HLA-DRB1*12:01 - all irrelevant once they mapped to the protein signal peptide sequence. Although we tend to think that the simplest and perhaps most logical things are “common sense” they should still be the first notions passed to every new generation of future vaccinologists.

Considering all of the abovementioned, and in the form of a lesson learned from our studies, we propose the adoption of a defined logical strategy for a more rational development of anti-*Leishmania* vaccines’. Particularly considering new antigens, but not exclusively, an initial *in silico* characterization should be performed considering both the pre-clinical and clinical contexts idealized. This means that the potential for immunogenicity on both the target population and the pre-clinical model chosen to evaluate vaccine potential, should be evaluated *a priori* of the beginning of any *de facto in vivo* work. At least the most prevalent MHC-II alleles should be considered since we are developing T-cell vaccines. However, the analysis should be extended to MHC-I restricted epitopes if possible, and even B-cell epitopes can be considered, although the role of antibodies in anti-*Leishmania* immunity may be of limited relevance (Rodrigues *et al*, 2016). Even considering that *in silico* studies are predictions, and therefore potentially fallible (Schmidt *et al*, 2017; Ernst *et al*, 2018), this initial approach might be important for the adjustment of initial rationales deemed more likely to fail (sparing the time that would have been unnecessarily spent): e.g. i) if the chosen antigen is deemed weekly immunogenic in the context of the envisioned pre-clinical model, a different (better) model can be chosen; ii) if only a small portion of the chosen antigen is

determined to be relevant for its immunogenicity in the target population, different strategies can be thought of - e.g. a) the generation of a repetitive neo-antigen, contemplating just the immunogenic portion (multiple times), increasing the epitopes' availability (Zhang *et al*, 2002); b) the consideration of new antigens. This was basically the strategy used in the construction of the broad-spectrum vector-based neo-antigen (we propose as a proof of principle of a vector-based pan-*Leishmania* vaccine) that indeed retained the immunogenicity of the two "mother antigens" *in vivo*, results that validated the *in silico* predictions we performed. More and more, different studies use the available tools that allow *in silico* evaluation of the antigens immunogenic potential (Hegde *et al*, 2018; Kazi *et al*, 2018; Rana *et al*, 2018; Norman *et al*, 2019; Singh *et al*, 2019). Still, some use them from the perspective of a supplementary analysis (potentially incomplete when is focused on either pre-clinical or clinical contexts) (Bordbar *et al*, 2020) not taking advantage of the insight they can actually bring for rational vaccine design.

Validation of bioinformatics analysis should obviously be the next step to be undertaken, considering the "rational pipeline" we are proposing. Ideally, the first step of validation should be the assessment of the real immunogenic potential in humans. This does not mean that we propose the conduction of human clinical trials ahead of pre-clinical research (bypassing the essential pre-clinical demonstration of safety and potential of the vaccine candidate). This validation can be performed *ex vivo* using cells from individuals that were naturally exposed to parasites and mounted anti-*Leishmania* immune responses (resistant or cured individuals with a positive leishmanin skin test) (Sundar and Rai, 2002). In fact, we used this strategy to define (in part) our multivalent vaccine candidate antigen composition, as others have (Coler *et al*, 2015). In this context, an interesting result, that indirectly attests the potential human immunogenicity of the tested antigen, is the observation of specific T-cell proliferation events. These positive results not only indicate that the antigen is indeed immunogenic, but also, and more importantly, that even in the context of an infection will be "seen" by the host immune system, which may not always be the case (Finlay and McFadden, 2006).

The candidates that "pass" these two phases will then be ready to be tested pre-clinically (with potentially better chances of translation to the clinical context), while the ones that "fail" can be further modified (saving - thinking in the pre-clinical research context - time, money and most importantly unnecessary animal experimentation). The pre-clinical research phase, on one way will serve as an extra validation step (of the *in silico* predictions

performed having in mind the pre-clinical model), and on the other will allow the evaluation of the real vaccine potential (in terms of safety and efficacy), essential for the approval of any clinical evaluation protocol (WHO, 2005). Still, and once more it needs to be stressed that even the most methodologically and analytically correct pre-clinical evaluation performed in an inadequate animal model may potentially lead to erroneous conclusions. Again, and assuming the animal model was chosen based on the *in silico* research, every vaccine composed of vector-derived antigens or parasite-derived ones should be tested (at least once, before moving to clinical trials) in the context of natural transmission (infection *via* sand fly bites).

4. Which readouts to look for?

The difficulty of ensuring (or predicting) translation from the pre-clinical to the clinical contexts, apart from reasons discussed throughout this dissertation, related to antigen selection/design and with the appropriateness of the animal models used is also a consequence of the recognized limited (or dubious) knowledge on the correlates of protection (that can be related, although not necessarily, to the determinants of resistance to *Leishmania*) (Gannavaram *et al*, 2014). This is first and foremost justified by the complexity of leishmaniasis. A spectrum of diseases implies a spectrum of pathogenic processes that will probably require distinct immune counteractive mechanisms to be resolved (Scott and Novais, 2016; Burza *et al*, 2018). In other words, the appropriately mounted immune response to eliminate parasites from a localized skin region is probably not equally appropriate to eliminate parasites dispersed through the reticuloendothelial system. Additionally, not only we need to understand the anti-parasitic responses in each of these different pathogenic processes, but also the regulatory responses (or lack of them) that will always balance the first ones for the good of re-reaching homeostasis (Marques *et al*, 2016; Cronkite and Strutt, 2018). The natural unsureness of the scientific community (and my own) in regard to this matter is exemplified by the notion that pro-inflammatory responses, that are deleterious for *Leishmania* parasites, are also the responsible for pathology in the context of MCL (Ronet *et al*, 2011).

This said, we can also consider that the immune responses required for protection in the context of vaccination, independently of parasite strain and disease manifestation, may be

similar. The transmission events (and processes) are expected to be similar for each of the as many as 20 leishmaniasis etiologic agents: all *Leishmania* species that cause disease to humans start the infectious process after being deposited on the host skin *via* a bite of a phlebotomine sand fly (Akhoundi *et al*, 2016). Therefore it is not absurd to consider that vaccine-mediated protection from disease may be dependent on early events after transmission, particularly on the mounting of effective immune responses at the site of the bite (Glennie *et al*, 2017). If this is the case, the correlates of protection should also be transversal to all disease manifestations. We cannot forget that the vaccines we are focusing on are supposed to be prophylactic, meaning they are required to work prior to infection establishment and disease development.

Maybe what needs to be done, is just to change the perspective. For instance, does it make sense to look for systemic responses later after transmission, if the ones expected to be relevant are the early (and perhaps local) responses? Also, and considering a vaccine composed of both vector- and parasite-derived antigens, should we restrict our focus on responses to parasites, or should we once more not disregard the vector from the equation when trying to establish good correlates of protection? While if we assume that the effect of the anti-saliva immune response in the context of anti-*Leishmania* vaccination is synergistic (or potentiator) of the effect of anti-parasitic responses, maybe to look only for the latter will be sufficient (once they will reflect the contribution of the two individual responses). On the other hand in the event of an additive effect of anti-saliva immunity for vaccine-mediated protection, probably these responses have to be accounted for in the investigation of proper correlates of protection.

Traditionally, in the anti-*Leishmania* vaccines field, the parasite-specific IFN-γ response is the main (and many times single) focus of the investigation on the correlates of protection (Okwor *et al*, 2012; Kima and Soong, 2013). This is a consequence of obvious reasons. As intracellular pathogens, *Leishmania* elimination will largely depend on pro-inflammatory cellular responses (Ismail *et al*, 2002; Tam and Jacques, 2014; Thakur *et al*, 2019). It is widely recognized that IFN-γ secretion by CD4+ T cells and the consequent (signaling-mediated) effect on the infected (or even bystander non-infected) APCs is essential to promote the generation of nitric (and oxygen) reactive species that are essential for parasite killing (Brandonisio *et al*, 2001; Resende *et al*, 2013; Olekhnovitch *et al*, 2014). Still, for every immune response, the environment is important (Trinchieri, 2015), meaning that looking for an individual parameter (even if extremely important) may not be sufficient. In our second

study, we show precisely this. Although *Leishmania*-specific IFN-γ secretion by CD4+ T cells determined *ex vivo* was consistently higher in vaccinated animals, the parasite numbers correlated, not with IFN-γ, but instead with IL-4 and IL-10 *Leishmania*-specific secretion (highlighting the importance of the need for a low regulatory context together with a strong IFN-γ response to achieve infection control – at least in this particular context). But can we extrapolate the environment from every experimental context? In the first work, reprinted in the objectives and results section of this dissertation, we assessed specific T cell responses trough cell proliferation experiments and looked at cytokine secretion to try to disclose the quality of immune responses. Importantly, in this context, mouse splenocytes were incubated with the vaccine antigens for 4 days. Does this experimental setting parallels the possible real context (thinking solely of the environment)? We believe that it may not. First, the immune responses' strength may depend on antigen dosages (which will be different, comparing in vitro settings with the natural ones) (George *et al*, 2003; Au-Yeung *et al*, 2014). Second, *in vitro* are closed systems (culture-plate wells) that do not allow cytokine diffusion, which may lead to consequent misleading readouts: e.g. the accumulation of high IFN-γ levels may result in a counteractive regulatory response not parasite-specific - something we possibly observed in the same study (Cope *et al*, 2011; Oyler-Yaniv *et al*, 2017). Third, these evaluations are usually static and therefore do not account for cytokine "consumption", meaning that the readout at a given time-point may be an underestimation of the response that happened (Han *et al*, 2012; Oyler-Yaniv *et al*, 2017).

The quest for the appropriate correlates of protection is not an exclusive challenge of anti-*Leishmania* vaccines (Plotkin, 2010). Still, and because the vaccines we need to prevent leishmaniasis are "not traditional" (most of the vaccines available for humans are antibody-based), most of the "regular correlates of protection" (e.g. antibody titers, antibody-mediated neutralization) (Plotkin, 2010) cannot be applied to the anti-*Leishmania* vaccines context. However, and although their relevance for *Leishmania* resistance is debatable at the least (Gardinassi and de Miranda Santos, 2016; Rodrigues *et al*, 2016), antibody responses should not be completely excluded. Antibodies may still be good surrogates of protection, or even if that does not turn to be true, they can be used to characterize populations (e.g. vaccine coverage).

All of the abovementioned, result in the last personal consideration written in this dissertation. Having in mind that there is still no vaccine available to use in the fight against human leishmaniasis, perhaps an extensive pre-clinical characterization from the

mechanistic standpoint is not extremely important. This does not mean that to understand the pre-clinical determinants of protection is not something important. But again, translation of pre-clinical data to the clinical context, may not be straightforward. In other words, maybe to define clear correlates of protection should be something to explore deeply in the clinical-trials context. Obviously this proposition would lose validity in the context of the existence of anti-*Leishmania* vaccines for humans. In this context, for each new vaccine candidate proposed, their potential advantages (comparing to the *de facto* vaccines) would need to be demonstrated pre-clinically (this requiring the clear understanding of the determinants of protection in the disease models used, which however might still not be similar - e.g. thinking of distinct protective mechanisms).

5. Future perspectives

In line with all the discussed above, the main future perspective is the generation of a large-spectrum vaccine candidate for human leishmaniasis: a bivalent formula, composed with one vector- and one parasite-derived large-spectrum antigens (adjuvanted if needed). These antigens can be designed based on the ones this dissertation focuses on.

Considering the parasite-derived antigens used, one is already a fusion protein, while the other is a single full-length protein. The same *in silico* approach we used to design the broad-spectrum vector-based antigen can be used to identify the relevant immunogenic portion(s) of KMP-11, the ones to fuse to the chimeric antigen LeishF3+. Obviously the approach proposed needs then to be validated, using for instance the same proliferation assays with human cells collected from former VL patients (cured), and even, thinking on the evaluation of a potential chimeric antigen broader spectrum, with human cells collected from former CL patients. These determinations should also be done using as controls the original full-length proteins, to understand if their immunogenicity was retained (or better yet improved) in the context of the fusion antigen. Importantly, these full-length proteins, the “mother antigens” of this proposed potential neo-antigen have a relatively high degree of conservation, considering all *Leishmania* species pathogenic to humans (Pascalis *et al*, 2003; Coler *et al*, 2015; de Mendonca *et al*, 2015), a determination that can be re-done considering the final protein sequences selected.

Considering the vector-derived antigen, many improvements should be done. First, we need to understand if the lack of a protective phenotype observed in the context of *L. major* transmission by *P. duboscqi* sand flies was indeed due to the choice of the mouse model of disease used (C57Bl/6 mice). If this is not the case, and the vaccine does not protect in the context of vector-transmission (*P. duboscqi*) to BALB/c mice, the choice of the PdSP15 portions to include in the chimeric antigen needs to be re-considered. Additionally, the same *in silico* analysis can be performed using different salivary proteins, from distinct relevant vectors, to increase even more the spectrum of the vector-derived antigen. Importantly, also considering efficacy determination, control groups immunized with the full length “mother antigens” should be considered, to understand the impact of our approach in the context of efficacy retention (do the relevant immunogenic portions chosen demonstrate a similar or better protective phenotype than the full-length salivary proteins?).

Finally, and in the event of the vector- and parasite-derived neo-antigens (hypothesized above) demonstrate good immunogenic and effective potentials in the pre-clinical context, they can be tested in combination, as a single vaccine, using both a traditional immunization scheme, but also a prime-boost one in order to understand if the reported potential adjuvant properties of sand fly salivary proteins are retained in the context of a chimeric antigen.

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