U.PORTO

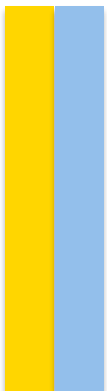
MESTRADO INTEGRADO EM MEDICINA

TROP2 and Nectin-4 expression in renal cell carcinoma and association with clinicopathological features

Ana Luísa Silva Mariz

M

2026



TROP2 and Nectin-4 expression in renal cell carcinoma and association with clinicopathological features

Dissertação no âmbito do Mestrado Integrado em Medicina

Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto

Junho, 2026

Estudante:
Ana Luísa Silva Mariz
Estudante do Mestrado Integrado em Medicina do Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto
Endereço de correio eletrónico institucional: up202004434@up.pt

Orientador:
Doutor João Pedro da Silva Machado Lobo
Professor Auxiliar Convidado, Departamento de Patologia e Imunologia Molecular,
Instituto de Ciências Biomédicas Abel Salazar- Universidade do Porto
Assistente Hospitalar, Anatomia Patológica, **Instituto Português de Oncologia do Porto Francisco Gentil, E.P.E.**

TROP2 and Nectin-4 expression in renal cell carcinoma and association with clinicopathological features

Dissertação no âmbito do Mestrado Integrado em Medicina do Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto

A Autora:

Assinado por: **Ana Luísa Silva Mariz**
Num. de Identificação: 14905955
Data: 2026.06.03 10:51:23+01'00'

Sob orientação de:

Assinado por: **João Pedro da Silva Machado Lobo**
Num. de Identificação: 13540895
Data: 2026.06.03 13:09:13 +0200

Declaração de Integridade Científica

Eu, Ana Luísa Silva Mariz, com o número de estudante 202004434, estudante do 6º ano do Mestrado Integrado em Medicina no Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, declaro que esta dissertação é da minha autoria e que não foi utilizada previamente em qualquer outro curso ou unidade curricular, desta ou outra instituição. Todas as referências a outros autores estão devidamente identificadas, de acordo com as normas de referenciação. Tenho conhecimento de que a prática de plágio ou autoplágio constitui uma infração académica.

Assinado por: **Ana Luísa Silva Mariz**
Num. de Identificação: 14905955
Data: 2026.06.03 10:52:19+01'00'

Financial support

This study was funded by a grant from the Research Centre of Portuguese Oncology Institute of Porto

RESUMO

Introdução: O carcinoma de células renais (RCC) representa cerca de 2-3% de todas as neoplasias, sendo os seus subtipos mais comuns o carcinoma de células claras (ccRCC), carcinoma papilar (pRCC) e carcinoma cromófobo (chRCC). Embora o RCC localizado tenha, em geral, um bom prognóstico, a doença metastática apresenta um prognóstico menos favorável, mesmo no paradigma atual de novas terapêuticas dirigidas. Neste contexto, os "Antibody–Drug Conjugates" (ADCs) representam uma oportunidade de terapêutica personalizada, nomeadamente os dirigidos à Nectina-4 e TROP-2, que têm sido estudados como potenciais alvos terapêuticos nas neoplasias uroteliais.

Objetivos: O objetivo deste estudo é avaliar a imunoexpressão de Nectina-4 e TROP-2 num grupo de doentes com ccRCC primário e metastático, assim como em tumores primários de pRCC e chRCC, com vista ao estabelecimento de um racional biológico para o estudo das potenciais aplicações de ADCs e relacionar a expressão destes marcadores com características clinicopatológicas.

Materiais e Métodos: Imunohistoquímica para TROP-2 e Nectina-4 foi realizada numa coorte de cinquenta e sete casos de carcinoma de células renais, e avaliada de acordo com a intensidade e percentagem de expressão.

Resultados: A expressão de TROP-2 foi mais elevada nos grupos de pRCC e chRCC, enquanto uma maior expressão de Nectina-4 foi observada no grupo de ccRCC. Comparando a expressão destes marcadores em lesões primárias e metastáticas, verificou-se uma perda de expressão de TROP-2 e Nectina-4 no grupo de lesões metastáticas. Adicionalmente, a baixa expressão de TROP-2 associou-se a um estadio patológico mais elevado e a uma maior taxa de progressão de doença, enquanto que a expressão elevada de Nectina-4 se associou a estadios patológicos mais baixos e menor grau ISUP. O nível de expressão de TROP-2 e de Nectina-4 não demonstrou um impacto estatisticamente significativo na sobrevivência global e livre de doença.

Discussão e conclusão: Este estudo demonstrou um padrão de expressão de Nectina-4 e TROP-2 distinto entre os diferentes subtipos estudados de RCC, com uma maior expressão de TROP-2 nos subtipos pRCC e chRCC. Embora nenhum destes biomarcadores tenha demonstrado um impacto significativo na sobrevivência global ou livre de doença, uma maior sobrevivência foi observada em tumores com elevada expressão, para ambos os marcadores. Estes achados podem ser úteis para o estabelecimento de racionalidade biológica pré-clínica na investigação de terapias baseadas em ADCs dirigidos a TROP-2 e Nectina-4 em grupos selecionados.

ABSTRACT

Introduction: Renal cell carcinoma (RCC) represents about 2-3% of all malignancies, with the most common subtypes being clear cell renal cell carcinoma (ccRCC), papillary renal cell carcinoma (pRCC) and chromophobe renal cell carcinoma (chRCC). Although localized RCC has an overall good prognosis, metastatic RCC has a poorer prognosis, even in the advent of new targeted treatment options. In this setting, Antibody–Drug Conjugates (ADCs) represent a unique personalized therapeutic opportunity, namely, those that target Nectin-4 and TROP-2, which have been recently studied, mainly as new treatment oportunities in urothelial cancer.

Aims: The aim of this study was to assess the immunoexpression of Nectin-4 and TROP-2 in a cohort of patients with both primary and metastatic ccRCC, as well as primary pRCC and chRCC, in order to possibly establish a biological rationale for further studying the applicability of available ADCs, as well as to study these markers' expression in relation to clinicopathological features.

Material and Methods: Immunohistochemistry for TROP-2 and Nectin-4 was carried out in a cohort of fifty-seven RCC cases, and evaluated for intensity and percentage of positive staining.

Results: TROP-2 expression was higher in pRCC and chRCC, whereas high Nectin-4 expression was more common in ccRCC. When comparing expression of these markers in primary versus metastatic lesions, an overall loss of expression was found in the metastasis subgroup. Furthermore, low TROP-2 was associated with higher pathological stage and increased disease progression rate, while high Nectin-4 expression was associated with lower pathological stage and lower ISUP grade. Neither TROP-2 nor Nectin-4 expression showed a statistically significant impact on overall or disease-free survival.

Discussion and conclusion: Our study demonstrated distinct expression patterns across the studied subtypes of RCC, with a higher TROP2 expression in pRCC and chRCC. Although neither biomarker demonstrated a statistically significant impact on overall or disease-free survival, consistently longer survival was observed in tumors with high expression of both markers. These findings may aid in providing a preclinical biological rationale for further investigation of ADC-based therapies targeting TROP-2 and Nectin-4 in selected RCC subgroups.

LIST OF ABBREVIATIONS

ADCs- Antibody–Drug Conjugates
ccRCC- clear cell renal cell carcinoma
chRCC- chromophobe renal cell carcinoma
DFS- Disease-free survival
FDA- Food and Drug Administration
IPO_Porto- Instituto Português de Oncologia- Porto
ISUP- International Society of Urological Pathology
OS- Overall survival
pRCC- papillary renal cell carcinoma
RCC- Renal Cell Carcinoma
TROP-2 - Trophoblast Cell Surface Antigen 2
WHO- World Health Organization

TABLE OF CONTENTS

TABLES INDEX

FIGURES INDEX

INTRODUCTION

Renal cell carcinoma (RCC) represents about 2-3% of all malignancies[1-3]. It represents the 14th most incident malignancy and 16th most deadly cancer, with a higher prevalence in males and in countries with a higher developmental index[3,4].

RCC is a very heterogeneous disease, with several distinct histologic subtypes acknowledged in the World Health Organization (WHO) Classification. Of these subtypes, the most common are clear cell renal cell carcinoma (ccRCC), papillary renal cell carcinoma (pRCC) and chromophobe renal cell carcinoma (chRCC), making up most cases of RCC[5]. Some of the risk factors for RCC, that are common across all subtypes, include smoking, obesity and arterial hypertension[6].

The histological subtype is a key determinant of prognosis, with ccRCC having a more aggressive course, increased mortality, and more advanced staging, while pRCC and chRCC generally have a more indolent course. Additionally, other histological prognosis factors for renal cell carcinoma include tumour grade, lymphovascular invasion, tumour necrosis, invasion of the collecting system, and presence of rhabdoid and/or sarcomatoid changes[7-13].

Treatment of localized RCC is surgical (based on partial or radical nephrectomy). However, the treatment of metastatic RCC is much more challenging in daily practice. RCC is overall remarkably chemo- and radioresistant, being non-responsive to conventional chemo/radiotherapy. Neoadjuvant and adjuvant therapy consists mainly of antiangiogenic targeted agents, mTOR inhibitors and immunotherapies (including PD-1 and PD-L1 inhibitors), which are particularly relevant in clear cell renal cell carcinoma, as it is most often identified in more advanced stages, not being eligible for complete tumoral and metastasis surgical resection[14-21]. Moreover, for pRCC and chRCC histologies, metastatic disease is often hard to treat, as there is a lack of approved treatments for non-clear cell RCC histologies, which is a gap in the field.

Despite new targeted treatment options, patients develop drug resistance and eventually succumb to the disease, highlighting the necessity for novel treatment targets. In this setting, Antibody–Drug Conjugates (ADCs) represent a unique personalized therapeutic opportunity, for their ability to target specific tumor antigens, selectively delivering a cytotoxic agent specifically to the cells that express them, leading to a lower systemic toxicity and fewer adverse effects from systemic treatment[22].

ADCs are composed of an antibody, a linker, and a cytotoxic payload. Desired features of ADCs include high antibody specificity, a stable linker, and a potent cytotoxic payload. Their use has been increasing in recent years, with applications in different malignancies, namely breast and urothelial cancer[23-25]. Some examples of ADCs that showed positive results in clinical trials for the treatment of urothelial cancers include those that target Nectin-4, as well as Trophoblast Cell

Surface Antigen 2 (TROP-2), leading to FDA (United States' Food and Drug Administration) approval of enfortumab-vedotin and sacituzumab-govitecan, respectively, for the treatment of this disease; although FDA approval of sacituzumab-govitecan in this setting has since been withdrawn[26-30]. Enfortumab-vedotin is, in fact, in combination with pembrolizumab, a first-line treatment option for metastatic urothelial cancer[25-28].

Nectin-4 is a type I transmembrane polypeptide that shows little expression in healthy adult tissues, but it is highly enriched in embryonic and placental tissues, as well as in various solid tumors. Its role in malignant tissues is unknown, but it may be involved in DNA repair and in the migration, adhesion, and proliferation of tumor cells[32]. TROP-2 is a transmembrane glycoprotein with high expression in many malignancies and not in non-malignant tissues. Its role includes regulation of cell growth, regeneration, and proliferation[33].

To date, Nectin-4 and TROP-2 expression in RCC has only been evaluated in a small number of studies (which will be discussed below), highlighting the need for further knowledge on the potential applicability of TROP-2 and Nectin-4 targeted therapies in RCC.

The aim of this study was to assess the immunoexpression of Nectin-4 and TROP-2 in a cohort of patients with both primary and metastatic ccRCC, as well as primary pRCC and chRCC, in order to possibly establish a biological rationale for further studying the applicability of available ADCs. Furthermore, we intended to study the expression of these markers in relation to clinicopathological features, namely overall and disease-free survival.

MATERIALS AND METHODS

Patient series

Fifty-seven RCC cases were obtained retrospectively from the Department of Pathology of a single institution (IPO_Porto). We retrieved 28 metastatic ccRCC cases, with available primary specimens and respective metastasis specimens, and 29 non-ccRCC (pRCC and chRCC), regardless of metastisation, as long as primary specimens (and respective metastasis specimen if applicable) were available. Clinicopathological data were collected from the available reports.

Formalin-fixed and paraffin-embedded tissue samples from primary surgical specimens (partial or radical nephrectomies) and metastatic material (either surgical specimens from metastasectomies or percutaneous biopsies) were retrieved from the archive of the Department of Pathology of IPO_Porto. All available histologic slides were reviewed by a Uropathologist with expertise in renal neoplasms. Cases with a lack of clinical information or insufficient representative histologic material were excluded.

Tumor morphology was evaluated according to the 5th Edition of the WHO Classification of Urinary and Male Genital Tumors[5]. Furthermore, the following specific morphological features were assessed: a) tumor grading, which applies to ccRCC and pRCC, according to the International Society of Urological Pathology (ISUP)[10], and b) presence of sarcomatoid and/or rhabdoid areas.

Ethical approval was obtained from the Ethics Committee of the institution (IPO_Porto CES.019_26).

Immunohistochemistry for Nectin-4 and TROP-2

Four-micrometer sections were ordered from the selected paraffin blocks for immunohistochemistry. Slides were stained with anti-Nectin-4 (clone EPR15613-68 Abcam) and anti-TROP-2 (clone SP295 Abcam) antibodies, in automated stainers used in diagnostic routine. Positive controls included skin and placental tissues, respectively, embedded on each slide as external controls.

TROP-2 and Nectin-4 immunoexpression (as illustrated in **Figure 1**) was evaluated by an experienced Uropathologist, and quantified as 0-3 for intensity (0=null, 1=low, 2=intermediate, 3=high), and percentage of positive staining (as reported in *Alves et al*[38]).

For categorization of cases, Nectin-4-high and TROP-2-high cases required that at least 5% of tumor cells expressed the markers, with an intensity of 2 or 3. Other cases were classified as Nectin-4-low and TROP-2-low.

Statistical analysis

Statistical analysis was carried out using SPSS software (version 30.0, IBM, New York, NY, USA). Categorical variables were compared using the chi-squared test or Fisher's exact test, as appropriate. Mann-Whitney non-parametric test was used to compare quantitative independent variables within Nectin-4-high and Nectin-4-low RCC cases, TROP-2-high and TROP-2-low RCC cases. Overall survival (OS) was censored in the absence of death at the last date of follow-up. Duration of follow-up was calculated from the date of surgery to either the date of death or the last known follow-up. Disease-free survival (DFS) was censored in the absence of recurrence at the last date of follow-up. Survival analysis, OS and DFS, was carried out using Kaplan-Meier curves, with differences between groups analyzed via a Log-Rank test. Differences were considered statistically significant if $p<0.05$.

RESULTS

The study included a total of 57 renal cell carcinoma (RCC) cases, encompassing 28 ccRCC (49.1%), 18 pRCC (31.5%), and 11 chRCC (19.4%) cases (**Table I**). The median age at diagnosis for the overall cohort was 62.6 years (range: 30–89 years). Patients harboring chRCC presented the highest median age at diagnosis (70.0 years), whereas pRCC patients were younger (56.9 years). Overall, there was a predominance of male patients (68.4%), particularly within ccRCC (72.4%) and pRCC (77.7%), whereas chRCC showed a predominance of female patients (63.6%). Regarding pathological stage, most tumors were classified as pT3 (50.9%), especially within the ccRCC subgroup, in which 64.3% of tumors were classified as pT3 (**Table I**). In contrast, pRCC and chRCC more frequently presented with pT1 tumors, accounting for 50.0% and 45.4% of cases, respectively. No lymph node metastases were identified at diagnosis in any patient. Distant metastatic disease at diagnosis was observed in 7.0% of the cohort, including 7.2% of ccRCC cases and 11.1% of pRCC cases, whereas no metastatic disease was observed in chRCC. Disease progression occurred in 54.4% of patients and was predominantly characterized by distant progression (49.1%). ccRCC showed the highest rate of disease progression (78.6%), whereas most chRCC cases remained progression-free (90.9%) (**Table I**). Regarding ISUP grade, grade 3 tumors represented the most frequent category (36.8%), particularly in ccRCC, in which 50.0% of tumors were classified as grade 3. No patients received neoadjuvant therapy, while adjuvant therapy was administered exclusively in ccRCC cases, accounting for 50.0% of this subgroup.

The evaluation of TROP-2 and Nectin-4 expression revealed significant differences among RCC histological subtypes (**Table II**). Overall, 40.9% of tumors were classified as TROP-2-high and 59.1% as TROP-2-low. TROP-2 expression varied significantly according to histological subtype ($p<0.001$). Most ccRCC cases demonstrated low TROP-2 expression (89.3%), whereas high TROP-2 expression predominated in pRCC (72.2%) and chRCC (63.6%). Statistical analysis demonstrated that ccRCC contributed significantly to the excess of TROP-2-low tumors, whereas pRCC contributed significantly to the excess of TROP-2-high tumors. Regarding Nectin-4, 31.6% of tumors were classified as Nectin-4-high and 68.4% as Nectin-4-low (**Table II**). High Nectin-4 expression was more frequently observed in ccRCC (42.9%), followed by pRCC (33.3%), whereas all chRCC cases (100%) demonstrated low Nectin-4 expression ($p=0.034$).

To further investigate the behavior of these biomarkers during metastatic progression, we compared TROP-2 and Nectin-4 expression between primary tumors and matched metastatic lesions in ccRCC (**Tables III and IV**). Among the 28 ccRCC cases, matched metastatic material was available for 22 patients. Regarding TROP-2 expression, 18 primary tumors (90.9%) were classified as TROP-2-low and four (9.1%) as TROP-2-high (**Table III**). However, all metastatic lesions (100%)

demonstrated low TROP-2 expression. Therefore, none of the metastatic lesions retained high TROP-2 expression, despite four primary tumors initially showing high expression. Similarly, regarding Nectin-4 expression, 15 primary tumors (68.2%) were classified as Nectin-4-high and seven (31.8%) as Nectin-4-low (**Table IV**). Nevertheless, all metastatic lesions (100%) showed low Nectin-4 expression. These findings suggest that both TROP-2 and Nectin-4 expression may be lost during metastatic progression in ccRCC.

We next searched for clinicopathological features associated with TROP-2 expression in RCC (**Table V**). TROP-2-high tumors were significantly associated with lower pathological stage (p=0.021). Among TROP-2-high tumors, 52.2% were classified as pT1 and only 26.1% as pT3, whereas among TROP-2-low tumors, 67.6% were classified as pT3. Moreover, low TROP-2 expression was significantly associated with disease progression (p=0.001). Disease progression occurred in 73.5% of TROP-2-low tumors, compared to only 26.1% of TROP-2-high tumors. Conversely, the absence of progression was more frequent among TROP-2-high tumors (73.9%) compared to TROP-2-low tumors (26.5%). No significant associations were observed between TROP-2 status and age (p=0.371), sex (p=1.000), metastatic disease at diagnosis (p=1.000), ISUP grade (p=0.377), or sarcomatoid/rhabdoid differentiation (p=0.352).

Similarly, the analysis of Nectin-4 expression revealed significant associations with pathological stage and ISUP grade (**Table VI**). Tumors with high Nectin-4 expression were more frequently classified as pT1 (55.6%) and less frequently as pT3 (33.3%), whereas Nectin-4-low tumors more frequently presented as pT3 disease (59.0%) (p=0.036). Regarding ISUP grade, no Nectin-4-high tumors were classified as grade 4, whereas 28.5% of Nectin-4-low tumors were classified as grade 4 (p=0.011). Grade 1 tumors were more frequent among Nectin-4-high tumors (11.1%) compared to Nectin-4-low tumors (3.6%). No significant associations were observed between Nectin-4 status and age (p=0.514), sex (p=0.542), metastatic disease at diagnosis (p=0.584), disease progression (p=0.574), or sarcomatoid/rhabdoid differentiation (p=1.000).

Survival analyses were subsequently performed to evaluate the prognostic impact of TROP-2 and Nectin-4 expression on OS and DFS (**Figures 2–5**). A total of 21 deaths were recorded in the cohort, with a median overall survival of 9.5 years (95% CI: 7.3–11.7 years) (**Figures 2 and 3**). Among TROP-2-high tumors, seven deaths were documented, and median overall survival was 11.0 years (95% CI: 4.8–17.1 years), whereas TROP-2-low tumors showed 14 deaths and a median overall survival of 8.8 years (95% CI: 4.8–12.8 years) (**Figure 2**). However, no statistically significant differences were identified between groups (p=0.554). Similarly, Nectin-4-high tumors showed seven deaths and a median overall survival of 11.0 years (95% CI: 7.9–14.1 years), whereas Nectin-4-low tumors demonstrated 14 deaths and a median overall survival of 8.8 years (95% CI: 5.6–11.9 years) (**Figure 3**). Nevertheless, no statistically significant differences were observed (p=0.714).

Regarding DFS, 31 recurrence events were documented, with a median disease-free survival of 2.4 years (95% CI: 1.6–3.2 years) for the overall cohort (**Figures 4 and 5**). Among TROP-2-high tumors, six recurrences occurred, and median disease-free survival was 2.6 years (95% CI: 0.0–4.9 years), whereas TROP-2-low tumors demonstrated 25 recurrences and a median disease-free survival of 2.4 years (95% CI: 1.9–2.9 years) (**Figure 4**). No statistically significant differences were identified between groups (p=0.918). Regarding Nectin-4 expression, 11 recurrence events occurred among Nectin-4-high tumors, which demonstrated a median disease-free survival of 4.6 years (95% CI: 2.3–6.9 years), whereas Nectin-4-low tumors showed 20 recurrence events and a median disease-free survival of 2.1 years (95% CI: 1.2–3.0 years) (**Figure 5**). Despite this, statistical significance was not achieved (p=0.611).

DISCUSSION

The therapeutic landscape of RCC has undergone substantial transformation over recent decades, driven largely by the advent of targeted therapies and immune checkpoint inhibitors. Despite these significant advances, treatment resistance in the metastatic setting remains a major clinical challenge, particularly in advanced or recurrent disease, underscoring an urgent need for novel therapeutic strategies. In this context, ADCs represent a unique and increasingly relevant therapeutic opportunity, combining the antigen specificity of monoclonal antibodies with the cytotoxic potency of chemotherapy payloads to achieve selective tumor cell killing while minimizing systemic toxicity[22]. Although ADCs have dramatically reshaped the therapeutic landscape of urothelial carcinoma – most notably through the introduction of enfortumab-vedotin, targeting Nectin-4 – and of breast cancer – through the introduction of sacituzumab-govitecan, targeting TROP-2, data regarding the expression of these biomarkers across histological subtypes of RCC remain remarkably limited[26-30]. As such, this study sought to address this gap by characterizing the immunoexpression of TROP-2 and Nectin-4 in the three most common RCC subtypes, namely ccRCC, pRCC, and chRCC, as well as in matched metastatic lesions of ccRCC, thereby trying to provide a biological rationale for the potential application of ADC-based therapies in selected RCC subgroups.

Our analysis demonstrated significant differences in TROP-2 and Nectin-4 expression profiles across RCC histological subtypes, consistent with the well-established molecular and biological heterogeneity of these tumors. TROP-2-high expression was predominantly observed in pRCC (72.2%) and chRCC (63.6%), whereas the vast majority of ccRCC cases (89.3%) showed low TROP-2 expression. These findings are in line with those reported by *Kessler et al*., who demonstrated significantly elevated TROP-2 mRNA and protein expression in pRCC compared to ccRCC and chRCC in a cohort of 88 RCC cases, with 90.9% of pRCC specimens exhibiting moderate-to-strong membranous staining by immunohistochemistry[36]. Similarly, in the large-scale tissue microarray study by *Dum et al*. encompassing more than 18,000 tumor samples across 150 tumor types, pRCC demonstrated positivity for TROP-2 in approximately 75% of cases – a substantially higher proportion than clear cell RCC – and low TROP-2 expression was significantly associated with higher Fuhrmann grade and higher pT stage in pRCC[39]. *Mikuteit et al*. have also reported TROP-2 expression in a subset of chRCC cases, further corroborating the differential expression across RCC subtypes[37]. The distinct expression profiles identified in our cohort reinforce that RCC subtypes harbor distinct molecular profiles, with potential implications for biomarker-guided therapeutic selection. This could be particularly relevant since targeted therapies for non-clear cell RCC histological types are scarce and, despite being rarer, metastatic disease from these forms of RCC

is difficult to manage and behaviour is challenging to predict. Our results support the putative use of ADCs targeting TROP-2 in non-clear cell RCC histologies, namely pRCC, as discussed below.

Beyond subtype-specific expression patterns, our analysis revealed significant associations between TROP-2 status and certain clinicopathological features. Low TROP-2 expression was significantly associated with higher pathological stage (p=0.021) and increased disease progression rate (p=0.001). Among TROP-2-low tumors, 67.6% were classified as pT3, whereas more than half of TROP-2-high tumors presented as pT1 disease. Furthermore, disease progression occurred in 73.5% of TROP-2-low cases, compared to only 26.1% of TROP-2-high cases. These findings parallel those reported by *Dum et al.*, who observed that low TROP-2 expression in pRCC was significantly associated with higher pT stage and higher tumor grade, suggesting that TROP-2 loss may accompany tumor progression[39]. Interestingly, *Kessler et al.* also reported a significant association between TROP-2 expression and local tumor burden within the pRCC subgroup, albeit without a statistically significant association with metastatic status[36]. These data suggest that TROP-2 expression may serve not only as a potential therapeutic target but also as a marker of less aggressive tumor biology in RCC, at least in certain histological subtypes, pending validation in larger studies, as the acquired data is still scarce.

From a therapeutic perspective, the existence of a high proportion of TROP-2-high cases in pRCC is particularly relevant, given that this subtype remains an area of significant unmet clinical need. Unlike ccRCC, for which multiple approved targeted agents and immune checkpoint inhibitor combinations exist, effective systemic therapy options for metastatic pRCC remain limited[39]. The functional relevance of TROP-2 expression in RCC was demonstrated by *Kessler et al.*, who showed that sacituzumab-govitecan – a TROP-2-directed ADC approved for urothelial cancer and triple-negative breast cancer — induced dose-dependent growth inhibition in TROP-2-positive RCC cell lines (Caki-1), whereas TROP-2-negative cell lines (769-P) showed resistance, confirming the on-target mechanism of action[36]. These preclinical observations, combined with the high prevalence of TROP-2 expression in pRCC documented both in our cohort and in the existing literature, provide a compelling rationale for the prospective evaluation of TROP-2-directed ADC therapies in patients with metastatic pRCC. In the future, studies should aim to test metastatic tumor samples from these histologies to further validate this hypothesis.

Regarding Nectin-4, our results demonstrated that high expression was more frequently observed in ccRCC (42.9%), while all chRCC cases (100%) showed low Nectin-4 expression, and approximately one-third of pRCC cases (33.3%) demonstrated high expression. Previous studies evaluating Nectin-4 in RCC subtypes have reported heterogeneous findings. *Zschäbitz et al.* evaluated Nectin-4 expression in a large multicentre cohort of pRCC (n=297) and demonstrated that 44.1% of pRCC specimens exhibited moderate-to-strong positivity[34]. In that study, Nectin-4

expression was not confirmed as a prognostic marker in pRCC overall, although a statistically significant survival advantage was observed in Nectin-4-positive type 1 pRCC, a designation no longer at use in the current WHO Classification[34]. *Mikuteit et al.* reported Nectin-4 positivity in 18.5% of chRCC specimens, also without significant associations with clinicopathological aggressiveness or survival[35]. In contrast, all chRCC cases in our cohort showed low Nectin-4 expression. These discrepancies may reflect differences in cohort characteristics, antibody clones, scoring systems, or positivity thresholds applied – limitations inherent to the comparison of immunohistochemical studies across institutions. Our results are nonetheless concordant with the broader observation that Nectin-4 is expressed in a non-negligible proportion of non-urothelial tumors, supporting its investigation as a potential therapeutic target beyond urothelial carcinoma.

In our cohort, high Nectin-4 expression was associated with lower pathological stage and lower ISUP grade. More than half of Nectin-4-high tumors were classified as pT1 (55.6%), whereas 59.0% of Nectin-4-low tumors presented as pT3 disease (p=0.036). Furthermore, no Nectin-4-high tumors were classified as ISUP grade 4, whereas 28.5% of Nectin-4-low tumors belonged to this category (p=0.011). Although neither TROP-2 nor Nectin-4 expression showed a statistically significant impact on overall or disease-free survival in our cohort, likely reflecting the limited sample size and number of events, both Nectin-4-high and TROP-2-high tumors consistently demonstrated longer survival outcomes, with a particularly pronounced difference in disease-free survival for Nectin-4-high tumors (median 4.6 versus 2.1 years). These trends are consistent with the hypothesis that preserved expression of these ADC targets may reflect less dedifferentiated tumor biology in RCC. Importantly, however, the absence of a clear-cut prognostic impact does not diminish the potential therapeutic relevance of these biomarkers, as has been well established in urothelial cancer, where Nectin-4 and TROP-2 expression levels have not been shown to predict benefit from enfortumab-vedotin and sacituzumab-govitecan, respectively, across all expression levels[29,40]. The results may also mean, however, that treatments targeting metastatic disease (which is the setting of disease in need of novel targeted therapies), may be less effective, given the downregulation of expression of the tested markers.

Regarding metastatic specimens, the present study shows a complete loss of both TROP-2 and Nectin-4 expression in metastatic lesions from ccRCC, even in cases in which the matched primary tumor exhibited high expression. Among the 22 matched primary-metastatic pairs analysed, all metastatic lesions demonstrated low Nectin-4 expression, despite 68.2% of primary tumors being classified as Nectin-4-high. Similarly, all metastatic lesions showed low TROP-2 expression, despite four primary tumors initially demonstrating high expression. These findings suggest that metastatic progression in ccRCC may be accompanied by significant downregulation – or complete loss – of ADC-relevant surface antigens. This phenomenon is not without precedent in

the urothelial cancer literature. *Klümper et al*. demonstrated a significant decrease in membranous Nectin-4 expression during metastatic spread in urothelial carcinoma, with nearly 40% of metastatic samples lacking protein expression, and showed that weak or absent Nectin-4 expression in metastatic lesions was associated with significantly shorter progression-free survival on enfortumab-vedotin[41]. These observations have important translational implications: biomarker assessment based exclusively on primary tumor tissue may not accurately reflect the antigen profile of metastatic disease – the actual therapeutic target in the advanced setting. Our data therefore may support the need for biomarker re-evaluation in metastatic samples prior to ADC-based treatment, whenever clinically feasible, even though, as previously mentioned, in urothelial cancer, Nectin-4 and TROP-2 expression levels have not been shown to predict benefit from enfortumab-vedotin and sacituzumab-govitecan, respectively, across all expression levels[29,40].

Our study has some limitations. Firstly, this was a retrospective, single-centre study conducted in a relatively limited cohort, particularly regarding subgroup analyses stratified by histological subtype. The small number of events in survival analyses may have precluded the detection of statistically significant prognostic associations despite clear numerical trends. Secondly, even though immunohistochemistry is a practical and widely available method for biomarker assessment, differences in antibody clones, antigen retrieval protocols, scoring systems, and positivity thresholds across studies may contribute to inter-study variability, limiting direct comparisons. Thirdly, the matched primary-metastatic analysis was restricted to ccRCC. Finally, functional in vitro or in vivo validation of the therapeutic potential of ADC-based therapies in RCC cell lines was beyond the scope of the present study.

CONCLUSION

In conclusion, our study demonstrates that TROP-2 and Nectin-4 show distinct and subtype-specific expression patterns across the three main histological subtypes of RCC. TROP-2-high expression was predominantly observed in pRCC and chRCC, whereas ccRCC cases were largely TROP-2-low. In contrast, Nectin-4-high expression was more frequently identified in ccRCC, while all chRCC cases demonstrated low expression. Low TROP-2 expression was significantly associated with higher pathological stage and increased disease progression, while low Nectin-4 expression was associated with higher pathological stage and ISUP grade. However, both biomarkers were uniformly lost in metastatic lesions of ccRCC, irrespective of their expression in matched primary tumors, highlighting the dynamic nature of ADC target expression during metastatic progression. Although neither biomarker demonstrated a statistically significant impact on overall or disease-free survival – likely reflecting the limited size of the cohort – longer survival trends were observed in tumors with high expression of both markers. Collectively, these findings provide a preclinical biological rationale for further investigation of ADC-based therapies targeting TROP-2 and Nectin-4 in selected RCC subgroups, particularly pRCC, and support the need for prospective studies evaluating the clinical applicability of these therapeutic strategies in renal neoplasms.

TABLES

Table I. Clinicopathological features of histologic renal cell carcinoma subtypes

Clinicopathological feature	**Categories**	**RCC** n= 57 (100)	**ccRCC** n=28 (49.1)	**pRCC** n=18 (31.5)	**chRCC** n= 11 (19.4)
Age at diagnosis (years) Median (range)	–	62.6 (30-89)	63.9 (50-89)	56.9 (30-83)	70.0 (30-83)
Sex n (%)	Male	39 (68.4)	21 (72.4)	14 (77.7)	4 (36.4)
	Female	18 (31.6)	7 (27.6)	4 (22.3)	7 (63.6)
pT n (%)	pT1	20 (35.1)	6 (21.4)	9 (50.0)	5 (45.4)
	pT2	8 (14.0)	4 (14.3)	1 (5.5)	3 (27.3)
	pT3	29 (50.9)	18 (64.3)	8 (44.5)	3 (27.3)
	pT4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
N at diagnosis n (%)	N0	57 (100)	28 (100)	18 (100)	11 (100)
	N1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
M at diagnosis n (%)	M0	53 (93.0)	26 (92.8)	17 (88.9)	11 (100)
	M1	4 (7.0)	2 (7.2)	1 (11.1)	0 (0.0)
Disease progression n (%)	No progression	26 (45.6)	3 (10.7)	13 (72.2)	10 (90.9)
	Locoregional	3 (5.3)	3 (10.7)	0 (0.0)	0 (0.0)
	Distant	28 (49.1)	22 (78.6)	5 (27.8)	1 (9.1)
Distant metastasis location[a] n (%)	Lung	13 (22.8)	9 (32.1)	4 (22.2)	0 (0.0)
	Adrenal gland	5 (8.8)	5 (17.9)	0 (0.0)	0 (0.0)
	Liver	4 (7.0)	3 (10.7)	0 (0.0)	1 (9.1)
	Thyroid	2 (3.5)	1 (3.6)	1 (5.6)	0 (0.0)
	Bone	6 (10.5)	6 (21.4)	0 (0.0)	0 (0.0)
	Peritoneum	1 (1.8)	1 (3.6)	0 (0.0)	0 (0.0)
	Breast	1 (1.8)	1 (3.6)	0 (0.0)	0 (0.0)
	Larynx	1 (1.8)	0 (0.0)	1 (5.6)	0 (0.0)
	Non-regional lymph node	6 (10.5)	4 (14.3)	2 (11.1)	0 (0.0)
ISUP grading n (%)	G1	3 (5.2)	1 (3.6)	2 (11.1)	-
	G2	14 (24.6)	9 (32.1)	5 (27.8)	-
	G3	21 (36.8)	14 (50.0)	7 (38.9)	-
	G4	8 (33.4)	4 (14.3)	4 (22.2)	-
Neoadjuvant therapy n (%)	Yes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	No	57 (100)	28 (100)	18 (100)	11 (100)
Adjuvant therapy n (%)	Yes	14 (24.6)	14 (50.0)	0 (0.0)	0 (0.0)
	No	43 (75.4)	14 (50.0)	18 (100)	11 (100)

Table II. Expression of TROP-2 and Nectin-4 in RCC and respective histologic subtypes.

	ccRCC n=28 (49.1)	**pRCC** n=18 (31.5)	**chRCC** n=11 (19.4)	**Total** n=57	**p-value***
TROP-2-low	25 (89.3)	5 (27.8)	4 (36.4)	34 (59.1)	**<0.001**
TROP-2-high	3 (10.7)	13 (72.2)	7 (63.6)	23 (40.9)	
Nectin-4-low	16 (57.1)	12 (66.7)	11 (100)	39 (68.4)	**0.034**
Nectin-4-high	12 (42.9)	6 (33.3)	0 (0.0)	18 (31.6)	

*Chi-squared or Fisher's exact tests with adjusted standardized residuals were used to assess associations and identify contributing cells.

Table III. TROP-2 expression in primary surgical specimen as compared to metastasis of ccRCC.

		Primary		
		TROP-2-low n=18 (90.9)	TROP-2-high n=4 (9.1)	Total n=22
Metastasis	TROP-2-low	18 (100)	4 (100)	22 (100)
	TROP-2-high	0 (0.0)	0 (0.0)	0 (0.0)

Highlighted cell (light grey) represents discordant cases.

Table IV. Nectin-4 expression in primary surgical specimen as compared to metastasis of ccRCC.

		Primary		
		Nectin-4-low n=7 (31.8)	Nectin-4-high n=15 (68.2)	Total n=22
Metastasis	Nectin-4-low	7 (100)	15 (100)	22 (100)
	Nectin-4-high	0 (0.0)	0 (0.0)	0 (0.0)

Highlighted cell (light grey) represents discordant cases.

Table V. Relationship between TROP-2 status in primary tumor specimens and specific clinicopathologic features in renal cell carcinoma

Clinicopathologic feature	Categories	TROP-2-low n=34	TROP-2-high n=23	Total n=57	p-value*
Age at diagnosis (years) Median (range)	–	63.9 (30-89)	59.7 (42-86)	62.6 (30-89)	0.371
Sex n (%)	Male	23 (67.6)	16 (69.6)	39 (68.4)	1.000
	Female	11 (32.4)	7 (30.4)	18 (31.6)	
pT n (%)	pT1	8 (23.5)	12 (52.2)	20 (35.1)	**0.021**
	pT2	3 (8.8)	5 (21.7)	8 (14.0)	
	pT3	23 (67.6)	6 (26.1)	29 (50.9)	
	pT4	0 (0.0)	0 (0.0)	0 (0.0)	
N at diagnosis n (%)	N0	34 (100)	23 (100)	57 (100)	NA
	N1	0 (0.0)	0 (0.0)	0 (0.0)	
M at diagnosis n (%)	M0	32 (94.1)	21 (91.3)	53 (93.0)	1.000
	M1	2 (5.9)	2 (8.7)	4 (7.0)	
Disease progression[a] n (%)	No	9 (26.5)	17 (73.9)	26 (45.6)	**0.001**
	Yes	25 (73.5)	6 (26.1)	31 (54.4)	
ISUP grading[b] n (%)	G1	1 (3.3)	2 (12.5)	3 (6.5)	0.377
	G2	8 (26.7)	6 (37.5)	14 (30.4)	
	G3	16 (53.3)	5 (31.3)	21 (45.7)	
	G4	5 (16.7)	3 (18.7)	8 (17.4)	
Sarcomatoid and/or rhabdoid areas n (%)	No	32 (94.1)	23 (100)	55 (96.5)	0.352
	Yes	2 (5.9)	0 (0.0)	2 (3.5)	

*Calculated using Chi-squared test or Fisher's exact test when indicated for categorical variables, and Mann–Whitney U test for continuous variables.

[a] Locoregional or Distant

[b] Used for ccRCC and pRCC

Table VI. Relationship between Nectin-4 status in primary tumor specimens and specific histopathologic features in renal cell carcinoma

Clinicopathologic feature	Categories	Nectin-2-low n=39	Nectin-2-high n=18	Total n=57	p-value*
Age at diagnosis (years) Median (range)	–	63.2 (42-89)	61.7 (30-79)	62.6 (30-89)	0.514
Sex n (%)	Male	28 (71.8)	11 (61.1)	39 (68.4)	0.542
	Female	11 (28.2)	7 (38.9)	18 (31.6)	
pT n (%)	pT1	10 (25.6)	10 (55.6)	20 (35.1)	**0.036**
	pT2	6 (15.4)	2 (11.1)	8 (14.0)	
	pT3	23 (59.0)	6 (33.3)	29 (50.9)	
	pT4	0 (0.0)	0 (0.0)	0 (0.0)	
N at diagnosis n (%)	N0	39 (100)	18 (100)	57 (100)	NA
	N1	0 (0.0)	0 (0.0)	0 (0.0)	
M at diagnosis n (%)	M0	37 (94.9)	16 (88.9)	53 (93.0)	0.584
	M1	2 (5.1)	2 (11.1)	4 (7.0)	
Disease progression n (%)	No	19 (48.7)	7 (38.9)	26 (45.6)	0.574
	Yes	20 (51.3)	11 (61.1)	31 (54.4)	
ISUP grading[a] n (%)	G1	1 (3.6)	2 (11.1)	3 (6.5)	**0.011**
	G2	7 (25.0)	7 (38.9)	14 (30.4)	
	G3	12 (42.9)	9 (50.0)	21 (45.7)	
	G4	8 (28.5)	0 (0.0)	8 (17.4)	
Sarcomatoid and/or rhabdoid areas n (%)	No	37 (94.9)	18 (100)	55 (96.5)	1.000
	Yes	2 (5.1)	0 (0.0)	2 (3.5)	

*Calculated using Chi-squared test or Fisher's exact test when indicated for categorical variables, and Mann–Whitney U test for continuous variables.

[a] Locoregional or Distant

[b] Used for ccRCC and pRCC

FIGURES

Fig 1. Representative histological features of clear cell renal cell carcinoma **(A)**, papillary renal cell carcinoma **(B)**, chromophobe renal cell carcinoma **(C)**, and liver metastasis of clear cell renal cell carcinoma **(D)**. Representative immunohistochemical staining demonstrating strong membranous TROP-2 expression in chromophobe renal cell carcinoma **(E-G)** and papillary renal cell carcinoma **(H)**. Representative immunohistochemical staining demonstrating strong membranous Nectin-4 expression in papillary renal cell carcinoma **(I-K)**, with absence of Nectin-4 expression in clear cell renal cell carcinoma **(L)**.

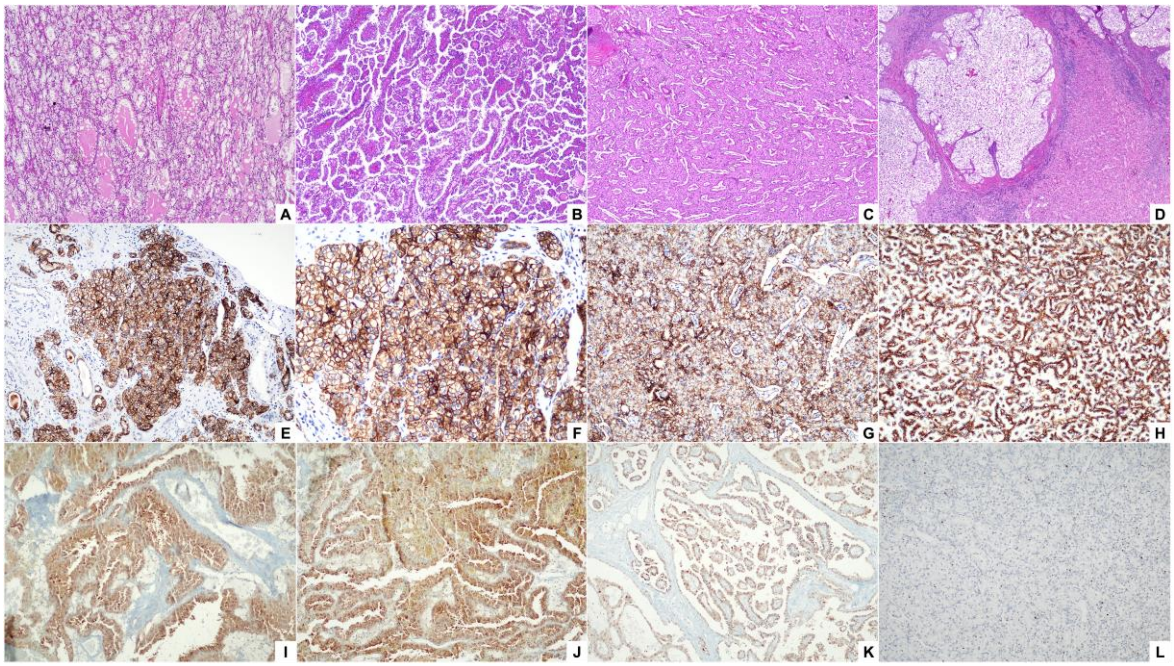


Figure 2. Kaplan-Meier curves regarding overall survival, stratified for TROP-2 status.

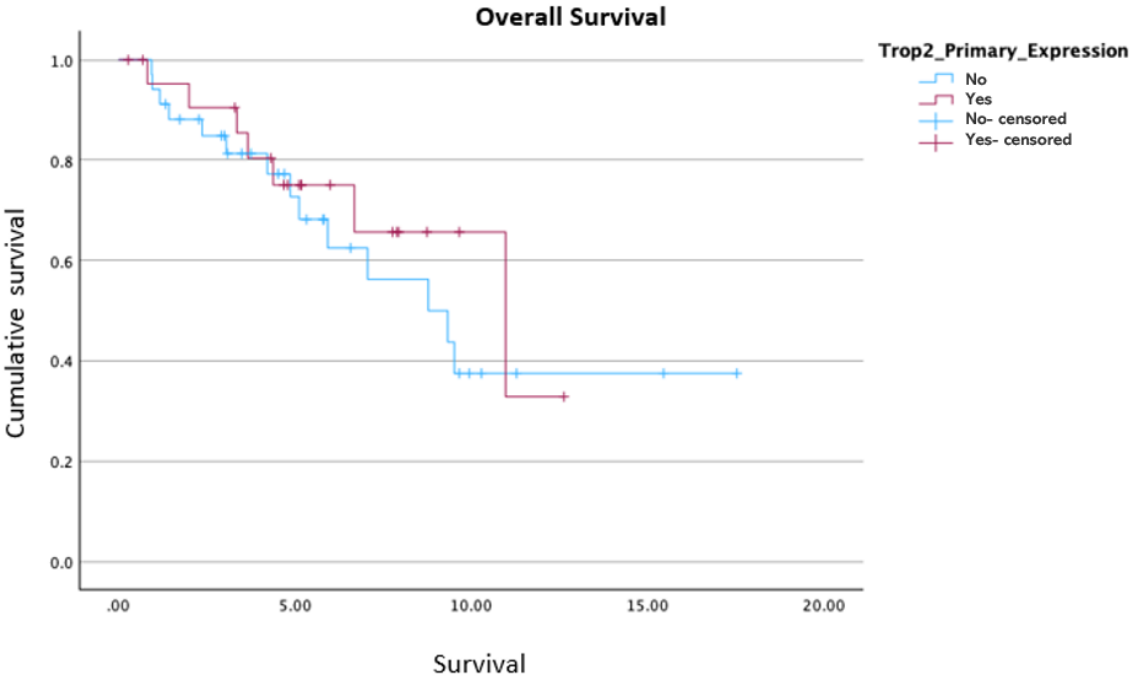


Figure 3. Kaplan-Meier curves regarding overall survival, stratified for Nectin-4 status.

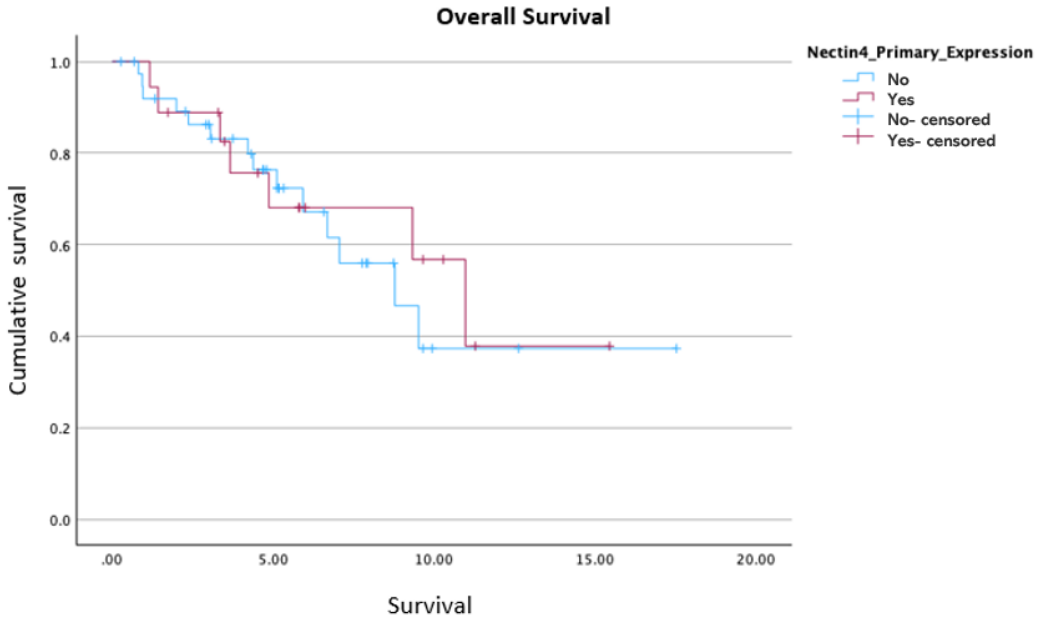


Figure 4. Kaplan-Meier curves regarding disease-free survival, stratified for TROP-2 status.

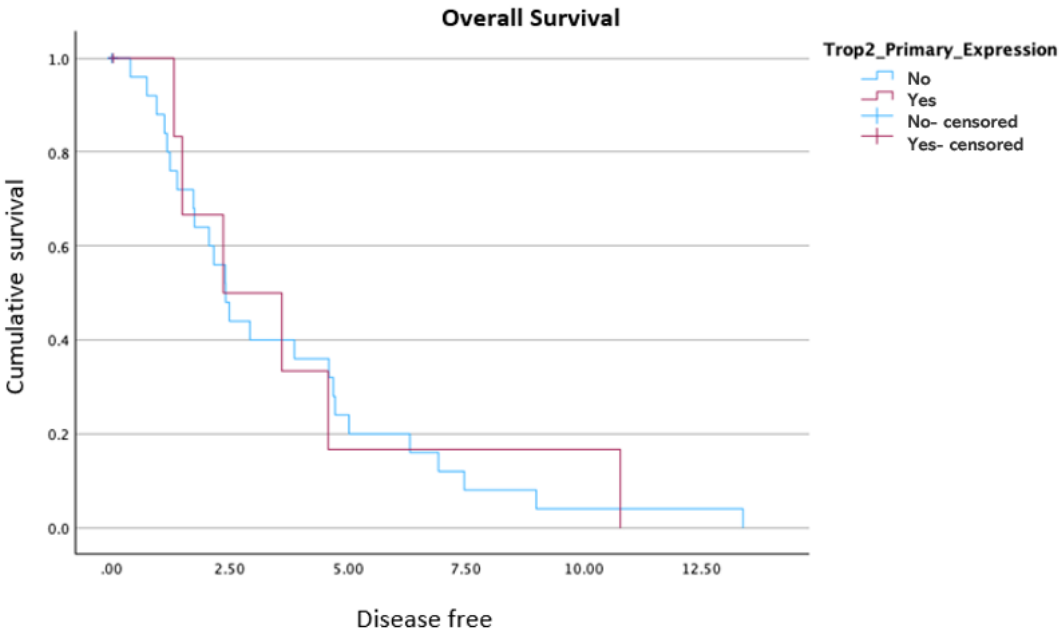
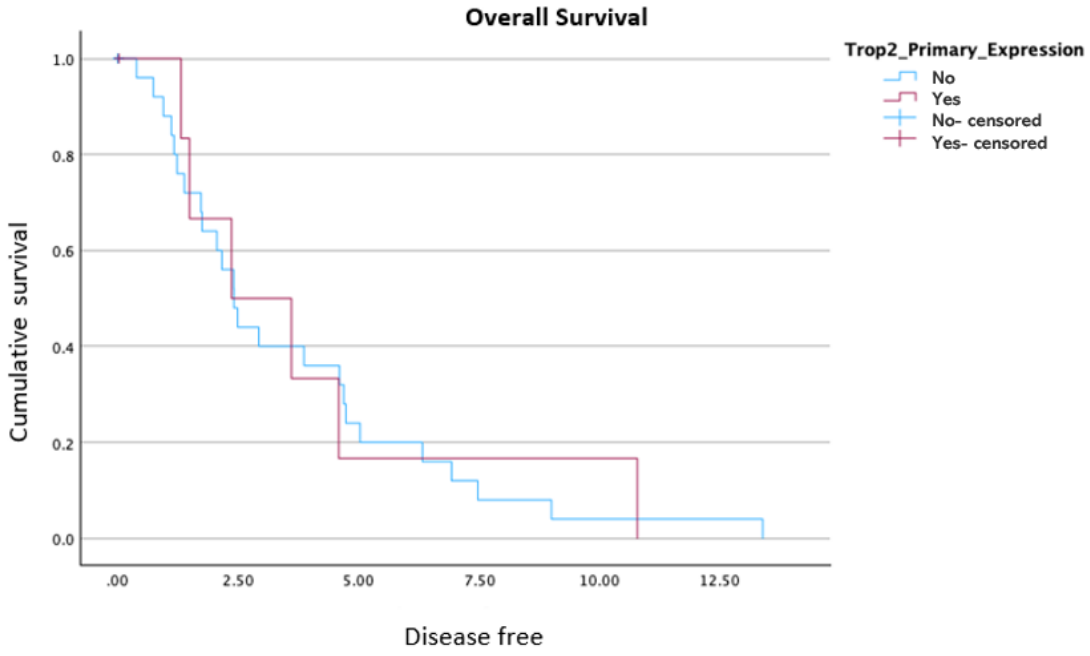


Figure 5. Kaplan-Meier curves regarding disease-free survival, stratified for Nectin-4 status.



REFERENCES

1. Ferlay, J., et al., Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. Eur J Cancer, 2018. 103: p. 356-387.
2. Siegel, R.L., K.D. Miller, and A. Jemal, Cancer statistics, 2018. CA Cancer J Clin, 2018. 68(1): p. 7-30.
3. Bray, F., et al., Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2024. 74(3): p. 229-263.
4. Cirillo, L., S. Innocenti, and F. Becherucci, Global epidemiology of kidney cancer. Nephrology Dialysis Transplantation, 2024. 39(6): p. 920-928.
5. Moch, H., et al., The 2022 World Health Organization Classification of Tumours of the Urinary System and Male Genital Organs-Part A: Renal, Penile, and Testicular Tumours. Eur Urol, 2022. 82(5): p. 458-468.
6. Macleod, L.C., et al., Risk factors for renal cell carcinoma in the VITAL study. J Urol, 2013. 190(5): p. 1657-61.
7. Sun, M., et al., Prognostic factors and predictive models in renal cell carcinoma: a contemporary review. Eur Urol, 2011. 60(4): p. 644-61.
8. Zhang, L., et al., Tumor necrosis as a prognostic variable for the clinical outcome in patients with renal cell carcinoma: a systematic review and meta-analysis. BMC Cancer, 2018. 18(1): p. 870.
9. Fuhrman, S.A., L.C. Lasky, and C. Limas, Prognostic significance of morphologic parameters in renal cell carcinoma. Am J Surg Pathol, 1982. 6(7): p. 655-63.
10. Delahunt, B., et al., The International Society of Urological Pathology (ISUP) grading system for renal cell carcinoma and other prognostic parameters. Am J Surg Pathol, 2013. 37(10): p. 1490-504.
11. Paner, G.P., et al., Updates in the Eighth Edition of the Tumor-Node-Metastasis Staging Classification for Urologic Cancers. Eur Urol, 2018. 73(4): p. 560-569.
12. Dagher, J., et al., Clear cell renal cell carcinoma: validation of World Health Organization/International Society of Urological Pathology grading. Histopathology, 2017. 71(6): p. 918-925.
13. Leibovich, B.C., et al., Histological subtype is an independent predictor of outcome for patients with renal cell carcinoma. J Urol, 2010. 183(4): p. 1309-15.
14. Motzer, R.J., et al., Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. J Clin Oncol, 2009. 27(22): p. 3584-90.
15. Sternberg, C.N., et al., Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. J Clin Oncol, 2010. 28(6): p. 1061-8.
16. Escudier, B., et al., Phase III trial of bevacizumab plus interferon alfa-2a in patients with metastatic renal cell carcinoma (AVOREN): final analysis of overall survival. J Clin Oncol, 2010. 28(13): p. 2144-50.
17. Rini, B.I., et al., Phase III trial of bevacizumab plus interferon alfa versus interferon alfa monotherapy in patients with metastatic renal cell carcinoma: final results of CALGB 90206. J Clin Oncol, 2010. 28(13): p. 2137-43.
18. Motzer, R.J., et al., Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet, 2008. 372(9637): p. 449-56.
19. Motzer, R.J., et al., Nivolumab versus everolimus in patients with advanced renal cell carcinoma: Updated results with long-term follow-up of the randomized, open-label, phase 3 CheckMate 025 trial. Cancer, 2020. 126(18): p. 4156-4167.
20. Tannir, N.M., et al., Nivolumab plus ipilimumab versus sunitinib for first-line treatment of advanced renal cell carcinoma: extended 8-year follow-up results of efficacy and safety from the phase III CheckMate 214 trial. Ann Oncol, 2024. 35(11): p. 1026-1038.

21. Choueiri, T.K., et al., Cabozantinib plus Nivolumab and Ipilimumab in Renal-Cell Carcinoma. N Engl J Med, 2023. 388(19): p. 1767-1778.
22. Hafeez, U., et al., Antibody-Drug Conjugates for Cancer Therapy. Molecules, 2020. 25(20).
23. Krop, I.E., et al., Trastuzumab emtansine versus treatment of physician's choice in patients with previously treated HER2-positive metastatic breast cancer (TH3RESA): final overall survival results from a randomised open-label phase 3 trial. Lancet Oncol, 2017. 18(6): p. 743-754.
24. Modi, S., et al., Trastuzumab Deruxtecan in Previously Treated HER2-Positive Breast Cancer. N Engl J Med, 2020. 382(7): p. 610-621.
25. Rosenberg, J.E., et al., Pivotal Trial of Enfortumab Vedotin in Urothelial Carcinoma After Platinum and Anti-Programmed Death 1/Programmed Death Ligand 1 Therapy. J Clin Oncol, 2019. 37(29): p. 2592-2600.
26. Yu, E.Y., et al., Enfortumab vedotin after PD-1 or PD-L1 inhibitors in cisplatin-ineligible patients with advanced urothelial carcinoma (EV-201): a multicentre, single-arm, phase 2 trial. Lancet Oncol, 2021. 22(6): p. 872-882.
27. Powles, T., et al., Enfortumab Vedotin in Previously Treated Advanced Urothelial Carcinoma. N Engl J Med, 2021. 384(12): p. 1125-1135.
28. O'Donnell, P.H., et al., Enfortumab Vedotin With or Without Pembrolizumab in Cisplatin-Ineligible Patients With Previously Untreated Locally Advanced or Metastatic Urothelial Cancer. J Clin Oncol, 2023. 41(25): p. 4107-4117.
29. Powles, T., et al., Enfortumab Vedotin and Pembrolizumab in Untreated Advanced Urothelial Cancer. N Engl J Med, 2024. 390(10): p. 875-888.
30. Tagawa, S.T., et al., TROPHY-U-01: A Phase II Open-Label Study of Sacituzumab Govitecan in Patients With Metastatic Urothelial Carcinoma Progressing After Platinum-Based Chemotherapy and Checkpoint Inhibitors. J Clin Oncol, 2021. 39(22): p. 2474-2485.
31. A.G. van der Heijden (Chair), H.M.B., A. Carrion, R. Cathomas, E.M. Compérat, K. Dimitropoulos, J.A. Efstathiou, R. Fietkau, B. Kiss, A. Lorch, P. Mariappan (Vice-chair), R.P. Meijer, L.S. Mertens, M.I. Milowsky, Y. Neuzillet, V. Panebianco, M. Rink, G.N. Thalmann, EAU Guidelines on Muscle-invasive and Metastatic Bladder Cancer. EAU Guidelines Office, 2026.
32. Li, K., et al., Therapeutic prospects of nectin-4 in cancer: applications and value. Front Oncol, 2024. 14: p. 1354543.
33. Shvartsur, A. and B. Bonavida, Trop2 and its overexpression in cancers: regulation and clinical/therapeutic implications. Genes Cancer, 2015. 6(3-4): p. 84-105.
34. Zschäbitz, S., et al., Expression of nectin-4 in papillary renal cell carcinoma. Discov Oncol, 2022. 13(1): p. 90.
35. Mikuteit, M., et al., Expression of Nectin-4 in Chromophobe Renal Cell Carcinoma in a Multicenter Cohort: Early Prognostic and Therapeutic Considerations. Oncology, 2024. 102(6): p. 503-509.
36. Kessler, C., et al., TROP-2 overexpression in papillary renal cell carcinoma supports its potential as a therapeutic target for antibody-drug-conjugate therapy. World J Urol, 2025. 43(1): p. 522.
37. Mikuteit, M., et al., Trophoblast Cell Surface Antigen 2 (Trop2) in Chromophobe Renal Cell Carcinoma. Oncology, 2022. 100(6): p. 331-336.
38. Alves, M.B., et al., Expression of ENPP1 in Testicular Germ Cell Tumors: Exploring Its Role in the Pathobiology of Distinct Histotypes and in Prognosis. Pathobiology, 2026. 93(1): p. 12-22.
39. Dum, D., et al., Trophoblast Cell Surface Antigen 2 Expression in Human Tumors: A Tissue Microarray Study on 18,563 Tumors. Pathobiology, 2022. 89(4): p. 245-258.
40. Loriot, Y., et al., Sacituzumab Govitecan Demonstrates Efficacy across Tumor Trop-2 Expression Levels in Patients with Advanced Urothelial Cancer. Clin Cancer Res, 2024. 30(15): p. 3179-3188.
41. Klümper, N., et al., Membranous NECTIN-4 Expression Frequently Decreases during Metastatic Spread of Urothelial Carcinoma and Is Associated with Enfortumab Vedotin Resistance. Clin Cancer Res, 2023. 29(8): p. 1496-1505.

INSTITUTO DE **CIÊNCIAS BIOMÉDICAS ABEL SALAZAR**