Somatic Mutations and Deletions of the E-Cadherin Gene Predict Poor Survival of Patients With Gastric Cancer

Giovanni Corso, Joana Carvalho, Daniele Marrelli, Carla Vindigni, Beatriz Carvalho, Raquel Seruca, Franco Roviello, and Carla Oliveira

See accompanying editorial on page 838; listen to the podcast by Dr Ford at www.jco.org/podcasts

ABSTRACT

Purpose
The prognosis of gastric cancer (GC) is poor, and the molecular pathogenesis players are vastly unknown. Surgery remains the primary option in GC treatment. The aim of this study was to investigate the impact of somatic CDH1 alterations in prognosis and survival of patients with GC.

Patients and Methods
A series of patients with sporadic and familial GC (diffuse and intestinal; n = 246) were analyzed for somatic CDH1 mutations, promoter hypermethylation, and loss of heterozygosity (LOH) by polymerase chain reaction sequencing. E-cadherin protein expression was determined by immunohistochemistry. Associations between molecular, clinicopathologic, and survival data were analyzed.

Results
CDH1 somatic alterations were found in approximately 30% of all patients with GC. Both histologic types of sporadic GC displayed LOH in 7.5%, mutations in 1.7%, and hypermethylation in 18.4% of patients. Primary tumors from hereditary diffuse GC, lacking germline CDH1 alterations, showed exclusively CDH1 promoter hypermethylation in 50% of patients. Familial intestinal GC (FIGC) tumors showed LOH in 9.4% and hypermethylation in 17.0%. CDH1 alterations did not associate with a particular pattern of E-cadherin expression. Importantly, the worst patient survival rate among all GCs analyzed was seen in patients with tumors carrying CDH1 structural alterations, preferentially those belonging to FIGC families.

Conclusion
CDH1 somatic alterations exist in all clinical settings and histotypes of GC and associate with different survival rates. Their screening at GC diagnosis may predict patient prognosis and is likely to improve management of patients with this disease.

J Clin Oncol 31:868-875. © 2013 by American Society of Clinical Oncology

INTRODUCTION

Gastric cancer (GC) is the second leading cause of cancer-related deaths worldwide, affecting close to one million people per year.1 The majority of patients with GC present with advanced disease at diagnosis (stages III and IV), rendering the prognosis less than 25%.2-4 Most patients with GC are asymptomatic during the early stages of disease, thus delaying the initial diagnosis. Although, some studies have pinpointed improvements on patient survival with perioperative and adjuvant treatment modalities, surgical resection is still the primary curative treatment for localized GC; however, less than 50% of patients are eligible for resection.5,6 During the last decade, several genetic and epigenetic changes underlying gastric carcinogenesis have been elucidated.2,4 Nonetheless, their clinical usefulness is limited because of the lack of systematic genetic-clinical correlations.

GC is histologically classified into two major types, intestinal and diffuse.7 The intestinal type is characterized by well-differentiated glandular structures, whereas the diffuse type consists of individually infiltrating neoplastic cells that may have signet ring morphology, does not form glandular structures, and has a poorer patient prognosis.7,11 The majority of GCs (90%) appear in a sporadic setting. The remaining 10% of GCs show familial clustering, and of these, only 1% to 3% constitute hereditary syndromes.12 Among GCs with familial aggregation and with histology information, the following specific syndromes can be identified: familial intestinal GC...
(FIGC) and hereditary diffuse GC (HDGC; Online Mendelian Inheritance in Man No. 137215).3,13 So far, no germline defects have been associated with FIGC, whereas germline mutations and deletions of E-cadherin (CDH1) are the underlying genetic defect in 45% of families with clinical diagnosis of HDGC.14-16

Furthermore, 70% of CDH1 mutation–negative HDGC probands display germline monoallelic CDH1 RNA downregulation (allelic imbalance) reinforcing the role of CDH1 locus in this disease.17 HDGC tumors appear when complete somatic CDH1 inactivation is acquired, leading to reduced or absent E-cadherin expression.18,19 This occurs through second hit mechanisms, pursuing the Knudson’s model of tumor suppressor gene inactivation.20,21 CDH1 promoter hypermethylation is the most frequent second hit inactivation mechanism in HDGC primary tumors (50% to 70% of tumors), whereas a second mutation or deletion (loss of heterozygosity [LOH]/intrinsic deletions) was less frequently identified.22-24

In the sporadic context, the frequency of CDH1 somatic mutations is controversial, and percentages between 3% and greater than 50% have been reported for GCs with a diffuse component.25-28 LOH at the CDH1 locus was noted in diffuse and intestinal histotypes, with rates ranging from 11% to 39% and 36% to 46%, respectively.29,31 Epigenetic silencing through CDH1 promoter hypermethylation has been reported in frequencies that vary from 50% to 83% in diffuse tumors and from 6.25% to 50% in intestinal tumors.28,32 Importantly, the presence of concomitant CDH1 inactivation mechanisms was rarely described in this setting.28,29,33 Nevertheless, most of these studies have been limited by the series size, the type of molecular mechanisms studied, different methodologic approaches, and the lack of extensive correlations with clinical parameters, thus hampering potential translation of genetic data into medical practice.34

The goal of this study was to analyze the impact of somatic CDH1 alterations in prognosis and survival of patients with GC. In this study, we performed a comprehensive analysis of somatic CDH1 mutations, LOH, and promoter hypermethylation in 246 patients with sporadic and familial GC. Additionally, we analyzed the relationship between somatic alterations underlying CDH1 inactivation and survival rates, clinicopathologic characteristics of patients with GC, and E-cadherin expression in tumors.

**Patients and Methods**

**Patients**

All 246 patients with GC enrolled onto this study were admitted at the Division of General Surgery and Surgical Oncology, University of Siena (Siena, Italy) and were classified as having sporadic (n = 174) or familial (n = 72) GC. Patients with familial GC were classified as having HDGC (n = 19) or FIGC (n = 53) using clinical criteria defined by the International Gastric Cancer Linkage Consortium17 (Appendix Table A1, online only). The presence of CDH1 germline mutations was discarded in all patients with HDGC in this cohort. Patients with GC classification more than pT1 were excluded from the study (Appendix, online only).

**DNA Extraction**

Tissue areas for DNA extraction were histologically verified to contain a minimum of 70% to 80% of neoplastic cells. DNA was isolated from frozen material using the Puregene DNA Purification Kit (Genta Systems, Minneapolis, MN).

**CDH1 Promoter Hypermethylation Analysis**

CDH1 promoter methylation analysis was carried out in 160 base pairs upstream of the translation start site, encompassing 17 CpG sites, as previously described17 (Appendix, online only). Primer sequences are listed in Appendix Table A2 (online only).

**CDH1 Somatic Mutation Screening**

Fifty nanograms of tumor DNA from 86 diffuse GCs were subjected to mutation screening at the CDH1 hotspot region (exons 7 to 10), as previously described.35 Each mutation was confirmed by an independent polymerase chain reaction amplification followed by sequencing analysis. Primer sequences are listed in Appendix Table A2 (online only).

**LOH Analysis**

LOH analysis was performed using three intragenic CDH1 markers (the promoter −161/C/A [rs16260], the exon 13 2076T/C [rs1801552], and the 3′-untranslated region single-nucleotide polymorphisms [rs1801026]) and three proximal and distal microsatellite markers (D16S3025, D16S496, and D16S3067) flanking the CDH1 locus, as described.17 Only informative markers were considered for LOH analysis, and positive samples were repeated twice (Appendix). Primer sequences are listed in Appendix Table A2 (online only).

**E-Cadherin Immunohistochemistry**

Formalin-fixed, paraffin-embedded tissues comprising 207 GCs were stained with mouse E-cadherin monoclonal antibody HECD-1 (dilution 1:200; Zymed, San Francisco, CA) followed by incubation with avidin-biotin complex and diaminobenzidine. E-cadherin immunoreactivity was evaluated on tumor and normal tissues considering the predominant expression pattern—normal (complete membrane staining), aberrant (cytoplasmic and heterogeneous staining), or absent (no staining).

**Statistical Analysis**

Analyses were performed using commercially available statistical software (SPSS, version 14.0; SPSS, Chicago, IL). Statistical associations between clinicopathologic characteristics and CDH1 somatic alterations were assessed using the χ² test for categorical variables and the t test or analysis of variance for continuous variables. Survival curves were estimated using the Kaplan-Meier method and were compared using the log-rank test. Multivariate analysis was performed using a Cox proportional hazards regression model by considering the following risk factors: sex, age (older v the median age or younger), tumor location (other v antrum), Lauren histotype (nonintestinal v intestinal), depth of tumor invasion (pT2-4 v pT1), lymph node involvement (pN1-3 v pN0), presence of systemic metastasis (M1 v M0), and R category (R1 or R2 v R0). Postoperative mortality was assessed, with deaths unrelated to tumor recurrence considered censored observations at the time of death. P < .05 was considered statistically significant.

**Results**

After screening of somatic CDH1 promoter hypermethylation, LOH, and mutations in exons 7 to 10, GCs were clustered in the following groups regarding CDH1 alterations: patients with methylation only (named epigenetic); patients with LOH or mutation, with/without methylation (named structural); and patients without CDH1 alterations (named negative).

**CDH1 Structural and Epigenetic Alterations in Overall GC**

Overall, 77 (31.3%) of 246 GCs carried somatic CDH1 alterations. Epigenetic alterations were found in 51 (20.7%) of 246 GCs, and structural alterations were detected in 26 (10.6%) of 246 GCs. Specifically within structural alterations, LOH alone was detected in 18
(7.3%) of 246 GCs, mutation alone was found in three (1.2%) of 246 GCs (c.1109A>G [p.Asp370Gly], c.IVS9+5G>A, and c.1105_1106 insACCAAC), and five (2%) of 246 GCs presented LOH concomitantly with CDH1 promoter hypermethylation (Table 1, Appendix Figs A1 and A2, online only).

Patients with tumors with CDH1 structural alterations displayed poorer overall survival (P = .017; Fig 1; all cases) than patients with negative tumors or tumors carrying CDH1 epigenetic alterations.

Table 1. Epigenetic and Structural CDH1 Alterations in Patients With Sporadic and Familial GCs

<table>
<thead>
<tr>
<th>Setting and Histologic Type</th>
<th>No. of Patients</th>
<th>Epigenetic Methylation Only</th>
<th>Structural</th>
<th>Overall Frequency of CDH1 Alterations*</th>
<th>Negative Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporadic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal</td>
<td>107</td>
<td>15</td>
<td>10</td>
<td>9.3</td>
<td>0†</td>
</tr>
<tr>
<td>Diffuse/mixed</td>
<td>67</td>
<td>17</td>
<td>3</td>
<td>4.5</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>174</td>
<td>32</td>
<td>13</td>
<td>7.5</td>
<td>3</td>
</tr>
<tr>
<td>Familial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIGCs§</td>
<td>53</td>
<td>9</td>
<td>5</td>
<td>9.4</td>
<td>0†</td>
</tr>
<tr>
<td>HDGC§</td>
<td>19</td>
<td>10</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>19</td>
<td>5</td>
<td>6.9</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>246</td>
<td>51</td>
<td>18</td>
<td>20.7</td>
<td>7</td>
</tr>
</tbody>
</table>

Abbreviations: FIGC, familial intestinal gastric cancer; GC, gastric cancer; HDGC, hereditary diffuse gastric cancer; LOH, loss of heterozygosity.

*This frequency was calculated assuming that intestinal GCs were negative for somatic CDH1 mutations (three of 174 GCs; 1.7%).

†Intestinal GCs were assumed to lack CDH1 somatic mutations.

‡Methylation and LOH occurred concomitantly.

§A statistically significant association was found when comparing FIGCs or HDGCs for three classes of alterations—patients with epigenetic alterations only, patients with structural alterations, and negative patients (P = .005, Fisher’s exact test; Appendix Table A3, online only)—because more than 50% of HDGC tumors carry CDH1 promoter methylation. LOH was defined when the LOH index was greater than or less than 1.04 (proportion) for D16S3025, 1.0 (proportion) for D16S496, and 1.06 (proportion) for D16S3067 as reference.

CDH1 Structural and Epigenetic Alterations in Different Clinical Settings and Histologic Types of GC

We evaluated the distribution of somatic CDH1 alterations among clinical settings (sporadic and familial) and histotypes (intestinal and diffuse) of GC and correlated these findings with overall patient survival. The overall frequency of CDH1 alterations was similar in sporadic (51 of 174 tumors; 29.3%) and familial (26 of 72 tumors; 36.1%) tumors (Table 1). Specifically within the sporadic...
setting, 32 (18.4%) of 174 GCs displayed epigenetic alterations and 19 (10.9%) of 174 GCs presented structural alterations. Within the familial setting, 19 (26.4%) of 72 GCs harbored epigenetic alterations, whereas seven (9.7%) of 72 GCs displayed structural alterations (Table 1; Appendix Table A3, online only). No significant correlation was observed between GC clinical settings and overall/specific CDH1 alterations (P > .05; Appendix Table A4, online only). However, patients with tumors with CDH1 structural alterations, from both sporadic and familial settings, had a poorer survival rate than patients with negative tumors or with CDH1 epigenetic alterations (P = .046 and P = .002, respectively; Fig 1).

Regarding GC histotypes independent of the clinical setting, the overall frequency of CDH1 alterations was 27.5% (44 of 160 GCs) in intestinal GCs and 38.4% (33 of 86 GCs) in diffuse GCs (Table 2). Whereas within intestinal tumors, 15% carried epigenetic alterations and 12.5% carried structural alterations, in diffuse tumors, 31.4% carried epigenetic alterations and 7% carried structural alterations. Although diffuse tumors carried epigenetic alterations more often than structural alterations (P = .007; Table 2), these molecular features did not confer different overall survival (P = .230, Fig 1). In contrast, and although intestinal tumors carried similar frequencies of epigenetic and structural alterations, Kaplan-Meier plots showed that patients with intestinal tumors carrying structural alterations had a lower probability of survival than patients with intestinal tumors negative for or carrying CDH1 epigenetic alterations (P = .041; Fig 1). In fact from the 26 GCs with structural alterations, 20 (76.9%) were of the intestinal type (P = .007; Table 3).

We observed that within sporadic and familial settings, patients with tumors carrying structural alterations had worse overall survival. Moreover, we also verified that patients with intestinal-type tumors carrying structural alterations, independent of the clinical setting, had worse overall survival. Therefore, we next assessed whether the worse overall survival of patients with intestinal tumors with structural alterations was a feature of familial and/or sporadic settings.

Intestinal tumors belonging to sporadic and familial (FIGC) settings did not differ in the frequency of epigenetic (14% sporadic, 17% FIGC) and structural alterations (12.1% sporadic, 13.2% FIGC; Table 1, Appendix Table A3, Fig 2). Nonetheless, the Kaplan-Meier plots showed statistical differences in terms of overall survival only for patients belonging to FIGC families. The patients with FIGC carrying tumors with structural alterations had the poorest survival rate (P < .001; Fig 2). Although it did not reach statistical significance (P = .148), patients with intestinal tumors belonging to the sporadic setting and carrying structural alterations also had a poorer survival rate compared with patients carrying negative/epigenetic alterations (Table 1, Appendix Table A3, Fig 2).

Within diffuse-type tumors, tumors belonging to the sporadic setting displayed epigenetic (25.4%) and structural (8.9%) alterations, whereas HDGC tumors (52.6%) harbored exclusively epigenetic alterations (Table 1, Appendix Table A3). Kaplan-Meier plots showed no association between the type of CDH1-specific alterations and overall survival of patients with sporadic diffuse or HDGC tumors (P = .259 and P = .631, respectively; Fig 2). However, despite the lack of statistical significance, the few patients with sporadic diffuse-type tumors carrying structural alterations had the lowest survival rates (P = .259; Fig 2).

**CDH1 Structural and Epigenetic Alterations and Clinicopathologic Features of GC**

We next tried to understand whether the presence of overall or specific CDH1 alterations correlated with clinicopathologic parameters of tumors and patients (Table 3). This analysis revealed that patients with tumors carrying overall CDH1 alterations displayed significantly more frequently lymph node metastases (P = .021) and more advanced tumors (P = .029), in particular invasive stage III. The other clinicopathologic parameters, including age, sex, liver or peritoneal metastases, radicality of resection, extent of gastrectomy, lymphadenectomy, death of invasion, familial aggregation, and even Lauren histotype, were not significantly associated with the presence of overall CDH1 alterations (Table 3).

When considering the specific type of CDH1 alterations in the different GC groups (epigenetic v structural v negative), we observed that patients carrying tumors displaying epigenetic CDH1 alterations more frequently had the diffuse histotype (P = .007) and 84.3% had lymph node metastases (P = .02). We also observed that patients carrying tumors with structural CDH1 alterations more frequently had intestinal histotype (20 of 26 patients, 76.9%; P = .007) and were mainly males (23 of 26 patients, 88.5%; P = .004), and 46.1% had been submitted to R1/R2 resections (compared with 21.6% with epigenetic alterations and 24.8% negative for alterations; P = .09; Table 3).

**CDH1 Structural and Epigenetic Alterations and E-Cadherin Immunoeexpression Pattern**

We also evaluated E-cadherin expression and correlated it with tumor histology and CDH1 alterations. Of 207 patients analyzed, 73.4% showed aberrant expression, 14.5% displayed complete loss of E-cadherin, and 12.1% retained the protein at the cell membrane (Table 4). In both sporadic and familial settings, there was a significant

---

**Table 2. Epigenetic and Structural CDH1 Alterations in Intestinal and Diffuse GCs**

<table>
<thead>
<tr>
<th>GC Histotype</th>
<th>No. of Patients</th>
<th>Epigenetic</th>
<th>Structural</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal</td>
<td>160</td>
<td>24 15.0</td>
<td>20 12.5</td>
<td>116 72.5</td>
</tr>
<tr>
<td>Diffuse</td>
<td>86</td>
<td>27 31.4</td>
<td>6 7.0</td>
<td>53 61.6</td>
</tr>
</tbody>
</table>

**Abbreviation:** GC, gastric cancer.
The association between complete loss of E-cadherin expression and diffuse histology (sporadic: \( P/H11021 .001 \); familial: \( P/H11005 .017 \); Table 4). In addition, there were no differences when comparing E-cadherin immunohistochemistry (IHC) pattern in GCs carrying overall or specific \( CDH1 \) alterations with the pattern in those without alterations (\( P/H11005 .479 \) and \( P/H11005 .275 \), respectively; Table 3). These results revealed that the \( CDH1 \) alterations analyzed do not generate a specific E-cadherin IHC pattern and, more importantly, that 60% of GCs without E-cadherin expression and approximately 70% of GCs with aberrant expression are negative for \( CDH1 \) alterations (Appendix Table A5, online only).

**DISCUSSION**

GC is a highly heterogeneous disease where even similar clinical and pathologic features lead to distinct outcomes.\(^7,9,11,36\) These observations indicated that staging systems, based on clinical and pathologic features, are limited in their capacity to stratify patients within the context of a highly heterogeneous disease like GC. This suggests that future efforts should focus on other potential targets and biomarkers for stratifying patients with GC and to identify therapies with greater clinical impact.
findings, may have reached their limit of usefulness and impelled the need for molecular biomarkers, as an added value, to predict patients’ outcome and treatment.

The present study encompasses a large series of sporadic and familial GCs (negative for CDH1 germline mutations) systematically analyzed for somatic CDH1 structural and epigenetic inactivating mechanisms. Furthermore, this study systematically correlates the molecular data with patient survival, clinicopathologic parameters, and E-cadherin IHC expression.

The main findings of the present study indicate the following: somatic CDH1 alterations occur in approximately 30% of all GCs analyzed (approximately 20% epigenetic, approximately 10% structural); CDH1 structural alterations underlie the worst survival rate of patients with GC overall; patients with FIGC with tumors with structural alterations had the worst overall survival rate; HDGC tumors harbored exclusively CDH1 promoter hypermethylation (approximately 50%); specific CDH1 alterations underlie distinct GC clinicopathologic features; and overall or specific CDH1 alterations are not associated with a distinct pattern of E-cadherin immunoeexpression. These and other findings will be discussed in more detail here.

The results herein reported may change the paradigm thus far described for CDH1 alterations in GC. In the sporadic context, E-cadherin abnormalities have been, to date, mainly associated with the diffuse type of GC, and CDH1 somatic mutations and promoter methylation have each been reported in more than 50% of these patients.26,27,29,33,34,37 In diffuse GCs, we report epigenetic alterations (methylation only) in 25.4%, somatic mutations in 4.5%, and LOH in 4.5% (structural alterations). These frequencies are lower than most earlier reported frequencies, but are similar to those recently reported in several independent series.26,30,31 Discrepancies are most likely a result of different methodologies used. Interestingly, we demonstrate that CDH1 somatic epigenetic and structural alterations are as frequent in intestinal as in diffuse GC, suggesting histotype independence.

In the familial setting, tumors from HDGC families lacking germline CDH1 mutations displayed exclusively CDH1 promoter methylation in frequencies comparable to those previously published for HDGC carriers of CDH1 germline mutations.23 We herein report for the first time, to the best of our knowledge, that tumors from FIGC families also present somatic CDH1 epigenetic and structural alterations in frequencies similar to those of patients with sporadic GC.

Importantly, GCs with epigenetic or structural CDH1 alterations or negative for CDH1 alterations revealed an unequal impact on patient survival. Patients with tumors with CDH1 structural alterations displayed a significantly poorer survival rate than patients with tumors negative for CDH1 alterations or patients with tumors with
epigenetic CDH1 alterations. In line with these findings, Gamboa-Domínguez et al. observed that patients with GC displaying CDH1 exon 8/9 deletions (structural) have a worse clinical evolution and a shorter overall survival. An adverse prognostic effect of CDH1 promoter hypermethylation in patients with diffuse GC has also been reported, but no structural alterations were reported in that series.\\n
When we explored in depth the survival rates of patients belonging to different clinical settings and histologic types, the most striking finding was that patients with FIGC with tumors with structural alterations had the worst overall survival. This observation indicates that CDH1 somatic alterations are a novel and unexpected feature of FIGCs and, more importantly, allows the stratification of patients with FIGC into subsets with completely different clinical outcomes. Available data on familial history associated with survival of patients with GC has remained largely conclusive.\n
However, patients with tumors with CDH1 structural alterations more often had the intestinal type (76.9%), and despite the generally accepted better prognosis, these patients had the worst survival, as previously discussed. Furthermore, these patients were mainly men and had been more frequently subjected to R1/2 radical resections, indicating the presence of a more aggressive disease. Therefore, screening of CDH1 alterations at the biopsy stage, concomitantly with histologic classification and both faster and more radical surgery, may bring benefit to the approximately 10% of patients with GC who belong to this specific group (Appendix Fig A3, online only).\n
In parallel, we also verified that approximately 68% of patients with altered E-cadherin expression were negative for CDH1 alterations, showing that IHC, per se, is not an efficient method to infer E-cadherin molecular alterations and indicating other transcriptional/post-transcriptional regulatory mechanisms. Following this idea, we have recently reported that miR-101 downregulation with consequent EZH2 upregulation constitutes an additional mechanism by which E-cadherin becomes dysfunctional, mainly in intestinal-type GC retaining allele(s) untargeted by classical CDH1 inactivating mechanisms.\n
In conclusion, our data provide, for the first time to our knowledge, a detailed analysis of somatic CDH1 alterations in different clinical settings and histologic types of GC, highlighting subsets of patients with distinct clinical outcomes. In particular, this work defined a group of patients (FIGC with CDH1 structural alterations) with the worst prognosis among all GCs analyzed. The presence of CDH1 epigenetic and structural alterations in a diagnostic/preoperative biopsy may provide clinical utility and improve patient management, particularly to infer the prognosis of GC and the pattern of tumor dissemination.

REFERENCES


AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Giovanni Corso, Joana Carvalho, Franco Roviello, Carla Oliveira
Financial support: Franco Roviello, Carla Oliveira
Provision of study materials or patients: Raquel Seruca, Franco Roviello, Carla Oliveira
Collection and assembly of data: Giovanni Corso, Joana Carvalho, Carla Vindigni, Raquel Seruca, Carla Oliveira
Data analysis and interpretation: Giovanni Corso, Joana Carvalho, Daniele Marrelli, Beatriz Carvalho, Carla Oliveira
Manuscript writing: All authors
Final approval of manuscript: All authors

© 2013 by American Society of Clinical Oncology. JOURNAL OF CLINICAL ONCOLOGY

Downloaded from ascopubs.org by 193.136.52.11 on January 19, 2018 from 193.136.052.011 Copyright © 2018 American Society of Clinical Oncology. All rights reserved.