Tumor marker kinetics predict outcome in patients with relapsed disseminated non-seminomatous germ-cell tumors

C. Massard1*, A. Kramar2, J. Beyer3, J. T. Hartmann4, A. Lorch5, J. L. Pico1, G. Rosti6, J. P. Droz7 & K. Fizazi1

1Department of Medical Oncology, Institut Gustave Roussy, University of Paris Sud, Villejuif; 2Unit of Biostatistics, Centre Oscar Lambret, Lille, France; 3Department of Cancer Medicine, Vivantes Klinikum am Urban, Berlin; 4Cancer Center North, Christian-Albrechts-Universität zu Kiel, Kiel; 5Genitourinary Medical Oncology, Department of Urology, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany; 6Department of Cancer Medicine, Ospedale Ca’ Foncello, Treviso, Italy; 7Department of Cancer Medicine, Centre Léon-Bérard, Lyon, France

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Background: An early serum tumor marker (TM) decline during chemotherapy was shown to independently predict survival in patients with poor-prognosis disseminated non-seminomatous germ-cell tumors (NSGCTs). The aim of this study was to assess whether a TM decline (TMD) also correlates with the outcome in the salvage setting.

Patients and methods: Data regarding 400 patients with progressive or relapsed disseminated NSGCTs after first-line chemotherapy prospectively accrued onto two phase III clinical trials were obtained. Serum alpha-fetoprotein (AFP) and/or human chorionic gonadotropin (hCG) were assessed at baseline and after 6 weeks of chemotherapy. A total of 297 patients, 185 and 112 in the training and validation sets, with initially abnormal TMs for whom a change from baseline could be established were used for this analysis.

Results: An unfavorable decline in either AFP or hCG was predictive of progression-free survival (PFS) [hazard ratio, HR = 2.15, (95% CI 1.48–3.11); P < 0.001; 2-year PFS rate: 50% versus 26%] as was the Lorch prognostic score (LPS). In the multivariate analysis, an unfavorable TMD, stratified based on the LPS, was an independent adverse prognostic factor for PFS and OS.

Conclusion: An unfavorable TMD during the first 6 weeks after chemotherapy is associated with a poorer outcome in patients with relapsed disseminated NSGCTs.

Key words: germ-cell tumor, high-dose chemotherapy, prognosis, relapse, tumor marker

introduction

Since the late 1970s, disseminated germ-cell tumors (GCTs) have been a paradigm for curable metastatic cancer, but ~20% of patients still do not achieve cure after first-line cisplatin-based chemotherapy [1, 2]. Salvage chemotherapy, combined with surgical resection of residual metastatic disease, results in a long-term cure rate of ~25% in this setting. A regimen combining cisplatin and ifosfamide plus a third drug [vinblastine, etoposide, or paclitaxel (Taxol)] is the recommended standard [1]. Randomized trials failed to establish the superiority of a single cycle of high-dose (HD) chemotherapy plus stem-cell rescue over the conventional-dose chemotherapy or that of multiple cycles versus a single cycle of HD chemotherapy [3, 4]. HD chemotherapy is, therefore, currently regarded as an option in this setting, with favorable results only being reported in non-randomized experiences [5, 6]. In contrast with the first-line chemotherapy setting [International Germ Cell Cancer Collaborative Group (IGCCCG) 1997], validated and universally accepted prognostic factors have only recently become available for relapsed disseminated GCT [7].

Either serum alpha-fetoprotein (AFP) or human chorionic gonadotropin (hCG), or both the markers are elevated in the majority of patients with relapsed disseminated non-seminomatous germ-cell tumors (NSGCTs). An early decline in serum tumor markers (TMs) was previously shown to predict survival independently of the validated prognostic factors in patients with poor-prognosis disseminated NSGCTs according to the IGCCCG [8–10]. To our knowledge, these factors have not been studied in a large cohort of patients with relapsed NSGCTs. The primary objective of this study was to assess and subsequently validate, in an external independent dataset, whether an early decline in serum TMs is associated with the outcome in the salvage setting.
patients and methods

patients

Data regarding patients with relapsed or progressive disseminated NSGCTs after first-line chemotherapy for metastatic disease included in two phase III trials were collected. The eligibility criteria for this study were evidence of a disseminated NSGCT, only one previous line of conventional-dose platin-based chemotherapy, and available serum AFP and/or hCG values at baseline and after two cycles of chemotherapy (6 weeks).

The IT94 randomized trial was a European trial initiated by the Institut Gustave Roussy which accrued 280 patients between February 1994 and September 2001. It compared four 3-weekly cycles of cisplatin, ifosfamide, and etoposide or vinblastine (VIP or VEP) with three cycles of the same regimen followed by a single-HD chemotherapy cycle consisting of carboplatin, etoposide, and cyclophosphamide (CarboPEC) with hematopoietic support. The details of the trial design, chemotherapy doses, and results have previously been reported [3].

The German Testicular Cancer Study Group (GTCSG) conducted a randomized phase III trial between September 1999 and October 2004 in 216 patients of whom 181 patients were included for first-salvage treatment and included in the current analysis [4]. Treatment in arm A consisted of one cycle of conventional-dose cisplatin 20 mg/m², etoposide 75 mg/m², and ifosfamide 1.2 g/m² for 5 days (VIP) plus three additional cycles of HD carboplatin 1500 mg/m² and etoposide 1500 mg/m² (CE) administered in three divided doses over 3 days followed by reinfusion of autologous peripheral-blood progenitor cells, 2 days later. Cycles were to be repeated at 21-day intervals. Treatment in arm B was identical to that in the experimental arm of the IT94 trial.

Baseline TMs were typically obtained just before the initiation of chemotherapy. The total number of eligible patients in this study was 400: 250 and 150 in the training and validation sets, respectively.

For the purposes of this study, 297 eligible patients with initial abnormal AFP and/or hCG TM and for whom a change from baseline at cycle 2 could be established were used for this analysis. Thus, data on 185 (74%) patients from the IT94 trial were available for the TMD analysis (either AFP or hCG elevated at baseline and available at cycle 2) (88 in the conventional chemotherapy arm and 97 in the HD chemotherapy arm), and those patients represent the training dataset. External validation was carried out on the 112 (75%) patients included in the GTCSG trial with an initially elevated marker (55 patients in the multiple HD chemotherapy arm and 57 patients in the single HD chemotherapy arm).

tumor marker decline

The TM response to treatment can fall into 6 possible categories: patients with initially normal values can either stay normal (N – N) or increase (N – AN); patients with initially abnormal values can normalize (AN – N), decrease rapidly (short half-life), decrease slowly (long half-life) or increase (AN – AN).

The TM half-life (HL) for AFP and hCG was estimated for patients with initially abnormal marker values: 106 (43%) patients for AFP and 123 (54%) patients for hCG in the training set. Patients with both normal AFP and hCG levels at baseline were not included in the analysis as detailed below.

HL mantle for marker M was calculated as the time necessary for AFP and hCG to achieve one-half of their initial value. It was calculated in two steps: (i) the rate of change in marker values, ∆M = (M0 – M1)/(t1 – t0), between cycle 2 (M1 at t1) and baseline (M0 at baseline t0); and (ii) HL mantle = −0.5 × M0/∆M.

For each marker, patients were classified as achieving a favorable marker decline if HL mantle was ≤6 and 3 weeks for AFP and hCG, respectively. These cut-off points were chosen to correspond to cycle evaluations as well as to median values observed in the dataset. This classification has the advantage of being practical since it only requires a comparison of the AFP value at 6 weeks (after two cycles of treatment) with the initial value. In order to qualify for a favorable decline, the AFP value should be less than half the initial value. Similar reasoning can be used for hCG at 3 weeks. The ‘favorable tumor marker decline group’ included patients with normal TM values at cycle 2 or a favorable decline. The ‘unfavorable tumor marker decline group’ included patients whose marker values either increased or who exhibited an insufficient marker decline (>6 and >3 weeks, respectively). Each marker was evaluated separately and in combination.

Patients were classified unfavorably if either AFP or hCG classified patients in the unfavorable category. Patients with only one favorable marker and missing information for the other marker were considered favorable.

statistical analysis

Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan–Meier method. PFS was calculated from the date of enrollment to the first date of progression, or relapse. Patients who never experienced progression or a relapse were censored at the date of the last follow-up or the date of death. OS was calculated from the date of enrollment to the date of death. The prognostic value of the following variables was evaluated in the training set: TM at baseline and at cycle 2, a TMD, the primary tumor site, previous response to first-line treatment, time since relapse, and the International Prognostic Factor Study Group (IPFSG) score [7]. The log-rank test was used for univariate comparisons. A P value of ≤0.05 was considered statistically significant. The relative influence of a TMD was estimated with Cox’s proportional hazards model adjusted for the Lorch prognostic score via the likelihood ratio statistic (LR).

results

patient characteristics

A total of 297 eligible patients with at least evaluable TM at cycle 2 were included in this analysis, 185 and 112 in the training set and the external validation set, respectively. Patient characteristics were different between the two groups: age (median 29 versus 35 years, P < 0.001), mediastinal primary site (11% versus 3%, P = 0.008) and complete response to first-line treatment (32% versus 45%, P = 0.03). No patients had liver, bone nor brain metastases in the validation set as compared with 29% of patients in the training set. The IPFSG score identified better risk patients in the validation set (Table 1). Also, the median interval between cycles was shorter in the training set (42 versus 50 days, P < 0.001). This one week median difference was mainly due to the group of patients in the validation set who received HD chemotherapy during the second cycle. All patients had at least one elevated TM at baseline. At the end of cycle 2, 72% of patients had at least one elevated marker and there was no statistically significant difference between the two groups.

tumor marker decline

Changes in TMs during the first two cycles are displayed in Table 2. In the training set, 70 patients (pts) had initial normal AFP, in 3 of whom it increased to above normal and was considered unfavorable; 89 patients had decreased AFP (50 became normal, in 24 patients the AFP decreased by more than half the initial value at 6 weeks (short half-life); in 15 patients the AFP decreased by less than half the initial value at 6 weeks (long half-life); 14 patients had increased AFP from initially
above normal. Four other patients who had initially normal AFP were included for hCG evaluation. Similar details for hCG and for patients in the validation set are provided in Table 2.

In the training set, 82% and 59% of the patients achieved a favorable AFP and hCG decline, respectively, and 52% of these patients obtained a favorable decline for both the TMs. In the validation set, 75% and 56% of the patients achieved a favorable AFP and hCG decline, respectively, and 38% of these patients obtained a favorable decline for both the TMs.

prognostic factors (univariate analysis)
The median follow-up was 44.6 months: 46.0 and 40.3 months for eligible patients in the two datasets, respectively. Overall, there were 174 PFS events and 138 patients died. In the univariate analysis in the training set, the factors associated with a poorer PFS were an unfavorable change in AFP [hazard ratio, HR = 2.57 (95% CI 1.65 – 4.02); P < 0.001; 2-year PFS rate: 45% versus 17%] (Figure 1A), an unfavorable change in hCG [HR = 1.72 (95% CI 1.20 – 2.43); P = 0.004; 2-year PFS rate: 46% versus 29%] (Figure 1C), and an unfavorable change in either AFP or hCG [HR = 2.15 (95% CI 1.48 – 3.11); P < 0.0001; 2-year PFS rate: 50% versus 26%] as well as the IPFSG score (P = 0.045), a primary mediastinal tumor site (P = 0.015), and a previous incomplete response to first-line therapy (P = 0.028) (Table 3). However, an elevated baseline AFP and an elevated baseline hCG were not significantly associated with PFS.

Regarding OS, an unfavorable change in AFP [HR = 2.47 (95% CI 1.65 – 4.02); P < 0.001; 2-year OS rate: 65% versus 39%] (Figure 1D), an unfavorable change in hCG [HR = 2.42; P = 0.005; 2-year OS rate: 65% versus 39%] (Figure 1D), and an unfavorable change in either AFP or hCG [HR = 2.15 (95% CI 1.48 – 3.11); P < 0.0001; 2-year OS rate: 50% versus 26%] as well as the IPFSG score (P = 0.045), a primary mediastinal tumor site (P = 0.015), and a previous incomplete response to first-line therapy (P = 0.028) (Table 3). However, an elevated baseline AFP and an elevated baseline hCG were not significantly associated with OS.

did not exert an impact on OS (Table 3).

multivariate analysis

In the training set, the multivariate analysis for PFS identified an unfavorable change in AFP or hCG levels as an adverse
The IPFSG score was not significant when adjusted for TMD (LR = 2.20, \( P = 0.33 \)). A mediastinal primary, however, was statistically significant when adjusted for a TMD (LR = 4.67, \( P = 0.031 \)), but lost statistical significance after stratification based on the IPFSG score (LR = 2.86, \( P = 0.09 \)). A poor-risk group was thus defined with an unfavorable TM decline (TMD) and had a 2.03 (95% CI 1.37–3.02) relative risk of progression compared with patients with a favorable TMD (2-year PFS rate: 50% versus 26%) (Table 3). Patients with an unfavorable TMD had an increased risk of progression [HR = 2.60 (95% CI 1.36–4.94) and HR = 2.03 (95% CI 1.21–3.39)] in the intermediate- and high- risk IPFSG groups, respectively (Figure 2C and E). No significant difference was observed in the low- risk IPFSG group (Figure 2A). These results were similar for OS and were confirmed in an external validation set (Figure 2B, D, and F).

The prognostic impact of a TMD was verified in an independent validation dataset and produced an estimated HR of 2.18 (95% CI 1.23–3.87, \( P = 0.005 \)). However, this estimated HR was less significant when adjusted based on the IPFSG score (HR = 1.72: 95% CI 0.89–3.30, \( P = 0.09 \), Harrell’s C index = 0.57) (data not shown). For patients with a favorable TMD, there was no statistically significant difference in PFS between the training and validation sets (\( P = 0.16 \)). However, patients with an unfavorable TMD had slightly better PFS rates in the validation set (2-year PFS: 26% versus 36%, \( P = 0.035 \)). With regard to OS, the results between the training and validation sets were not statistically significant: favorable TMD (70% versus 63%, \( P = 0.50 \)), and an unfavorable TMD (37% versus 51%, \( P = 0.12 \)). The clinical characteristics of patients in the validation set were different from those of patients in the training set. Patients in the training set were younger, more of

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**Figure 1.** Prognostic value of serum alpha-fetoprotein (AFP) and human chorionic gonadotropin (hCG) decline in terms of progression-free survival (PFS) and overall survival (OS) in the training set: (A) PFS, AFP decline; (B) OS, AFP decline; (C) PFS, hCG decline; and (D) OS, hCG decline.
them had a mediastinal primary tumor, an incomplete response to first-line chemotherapy, and a high-risk IPFSG score (Table 1), indicating an overall better prognosis of patients in the validation set. However, there was no difference in 2-year PFS (38% versus 44%, P = 0.10) nor 2-year OS (54% versus 55%, P = 0.83) between these two datasets. Also, baseline TMs were similar (Table 4).

discussion

This study, based on a large multi-institution collection of data regarding 400 patients with relapsed disseminated GCTs, showed that patients with an unfavorable AFP or HCG TMD during the first 6 weeks of salvage chemotherapy were independently associated with a worse PFS and OS. This result remained significant when adjusted for the IPFSG prognostic score, although the results were less pronounced in the validation set, probably due to the overall better prognosis and survival of these patients.

Various prognostic factors were previously proposed in relapsed GCTs. However, no prognostic classification including a multivariate analysis of a large series subsequently completed by an independent validation was available until recently. Only very recently, a large international retrospective study comprising 1594 patients from 38 countries established and validated prognostic factors in the salvage setting: non-pure seminoma histology, the primary tumor location, response to first-line treatment, a progression-free interval after first-line treatment, AFP and hCG levels as well as the presence of liver, bone, or brain metastases. These variables were combined to define prognostic subgroups with different outcomes [7].

In the present study, data on an early TMD were collected and tested in a Cox multivariate analysis together with pre-treatment prognostic factors in a large series of patients. Of critical importance, an unfavorable marker decline (AFP or HCG) was found to be independently associated with a poor prognosis. These results, albeit less pronounced, were later validated in an independent external validation set. That primary mediastinal NSGCT has a more aggressive behavior and a different biological background than other GCTs that have been consistently reported, and only exceptional cures have been described in patients failing first-line chemotherapy, most of them being obtained through salvage surgery rather than salvage chemotherapy [11–14]. It is, therefore, not surprising that this factor appeared as a strong predictor of a poor outcome in both this study and the recent international study [7]. This factor was not, however, found to be prognostically significant in the validation set due to the small number of patients with a mediastinal primary.

A more original finding was that an unfavorable decline in serum AFP and HCG levels was also independently predictive of poorer PFS and OS in the salvage setting. At least three large retrospective studies of patients with NSGCTs receiving first-line therapy retrospectively identified an unfavorable TMD as an independent prognostic factor in patients with a poor prognosis according to the IGCCCG [8–10]. This concept is currently being used prospectively in a multinational phase III trial testing a dose-dense chemotherapy regimen compared with the standard four-cycle bleomycin, etoposide, and cisplatin (BEP) regimen in patients with poor-prognosis NSGCTs and an unfavorable TMD (GETUG 13 trial, NCT00104676). Interestingly, in the present study focused on the salvage setting, baseline AFP and HCG were not prognostic factors for PFS and OS, whereas the declining serum AFP and HCG levels were. This may be due to the fact that serum AFP and HCG levels at baseline are usually less elevated in patients with relapsed GCTs compared with those undergoing first-line therapy, although the latter share a better prognosis. What seems to be more important is a change in marker levels during the first one or two cycles of chemotherapy, rather than initial marker values.

During the last decade, it has been common practice in many countries to use triplet conventional-dose cisplatin–ifosfamide-based chemotherapy in patients with favorable features in the salvage setting, and to use HD chemotherapy plus a stem-cell transplant in patients with adverse prognostic factors. For example, investigators from the Memorial Sloan-Kettering hospital added paclitaxel to cisplatin and ifosfamide (TIP regimen) in good-risk patients and developed a high-dose sequential regimen (TICE) in poor-risk patients [15, 16] which yielded promising results in phase II trials. A similar strategy was developed by the French GETUG group that recently completed two parallel phase II trials of the gemicitabine, ifosfamide, and cisplatin (GIP regimen) combination in good-risk patients [17], while using a sequential high-dose Taxif-2 regimen in patients with adverse factors [18]. A large randomized, phase III trial is planned to compare standard-dose chemotherapy with HD chemotherapy in relapsed GCT.

### Table 3. Lorch prognostic score and tumor marker change in the training set and in the validation set

<table>
<thead>
<tr>
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<th>Training set</th>
<th>Validation set</th>
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<tbody>
<tr>
<td></td>
<td>E/N</td>
<td>2-year PFS (%)</td>
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<tr>
<td><strong>AFP decline</strong></td>
<td></td>
<td></td>
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<tr>
<td>Favorable</td>
<td>81/145</td>
<td>45</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>26/32</td>
<td>17</td>
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<tr>
<td><strong>hCG decline</strong></td>
<td></td>
<td></td>
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<tr>
<td>Favorable</td>
<td>54/96</td>
<td>46</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>47/67</td>
<td>29</td>
</tr>
<tr>
<td><strong>AFP/hCG decline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>50/97</td>
<td>50</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>64/88</td>
<td>26</td>
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<tr>
<td><strong>Mediastinal primary</strong></td>
<td></td>
<td></td>
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<tr>
<td>No</td>
<td>97/164</td>
<td>41</td>
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<tr>
<td>Yes</td>
<td>17/21</td>
<td>19</td>
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<tr>
<td><strong>Prior response</strong></td>
<td></td>
<td></td>
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<tr>
<td>Complete</td>
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<td>53</td>
</tr>
<tr>
<td>Incomplete</td>
<td>82/125</td>
<td>31</td>
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<tr>
<td><strong>Lorch score</strong></td>
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<td></td>
</tr>
<tr>
<td>Very low/low</td>
<td>9/17</td>
<td>50</td>
</tr>
<tr>
<td>Intermediate</td>
<td>38/67</td>
<td>44</td>
</tr>
<tr>
<td>High</td>
<td>36/61</td>
<td>40</td>
</tr>
<tr>
<td>Very high</td>
<td>27/34</td>
<td>18</td>
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E, number of events; AFP, alpha-fetoprotein; hCG, human chorionic gonadotropin; PFS, progression-free survival.
Figure 2. Progression-free survival (PFS) and overall survival (OS) according to the Lorch risk group and tumor decline in the training set: (A) PFS, low risk; (B) OS, low risk; (C) PFS, intermediate risk; (D) OS, intermediate risk; (E) PFS, high risk; (F) OS, high risk.
patients (TIGER project) [19]. In the TIGER trial, TMD may prospectively be assessed and validated as an additional prognostic refinement after initiation of treatment.

In conclusion, this study provides evidence that the early evaluation of declining serum AFP and hCG levels during salvage chemotherapy has an independent prognostic value for progression or relapse in patients with initially elevated AFP or hCG levels. Patients with an insufficient TMD have a two-fold risk of progression or relapse compared with those with a sufficient TMD. These patients may benefit from HD chemotherapy or new therapeutic strategies, although this will need to be confirmed.

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disclosure

The authors have declared no conflicts of interest.

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