Preclinical and clinical activity of sunitinib in patients with cisplatin-refractory or multiply relapsed germ cell tumors: a Canadian Urologic Oncology Group/German Testicular Cancer Study Group cooperative study

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Background: The objective of the study was to investigate the activity of sunitinib in a cell line model and subsequently in patients with cisplatin-refractory or multiply relapsed germ cell tumors (GCT).

Methods: The effect of sunitinib on cell proliferation in cisplatin-sensitive and cisplatin-refractory GCT cell lines was evaluated after 48-h sunitinib exposure by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, and IC50 (concentration that causes 50% inhibition of growth) doses were determined. Sunitinib was subsequently administered at a dose of 50 mg/day for 4 weeks followed by a 2-week break to 33 patients using a Simon two-stage design.

Results: Sunitinib demonstrated comparable dose-dependent growth inhibition in cisplatin-sensitive and cisplatin-resistant cell lines, with IC50 between 3.0 and 3.8 μM. Patient characteristics were as follows: median of 2 (1–6) cisplatin-containing regimens; high-dose chemotherapy 67%; late relapse 33%; and cisplatin refractory or absolute cisplatin refractory 54%. Toxic effects included fatigue (39%), anorexia (21%), diarrhea (27%), mucositis (45%), nausea (33%), hand–foot syndrome (12%), dyspepsia (27%), and skin rash (18%). No unexpected side-effects were observed.

Thirty-two of 33 patients were assessable for response. Three confirmed partial responses (PRs) and one unconfirmed PR were seen for a total response rate of 13%. Median progression-free survival (PFS) was 2 months, with a 6-month PFS rate of 11%.

Conclusions: Sunitinib shows in vitro activity in cisplatin-resistant GCT cell lines. Modest clinical activity in heavily pretreated GCT patients was observed.

Key words: angiogenesis, cisplatin resistance, germ cell tumor, relapse, sunitinib

Introduction

Although the vast majority of patients with metastatic germ cell tumors (GCT) are cured, those with cisplatin-refractory relapse or relapse after high-dose chemotherapy (HD-CT) still exhibit a very poor prognosis, and <5% of these patients will achieve long-term survival [1]. For these patients, the evaluation of new active drugs and treatment combinations remains a priority.

Various agents have been evaluated in intensively pretreated or cisplatin-refractory patients. As single agents, only paclitaxel, gemcitabine, oxaliplatin, and orally administered etoposide have been shown to be active, with selected patients achieving long-term survival [2–6]. Different combinations of these agents demonstrated response rates up to 51%, including 10%-15% long-term survivors, although duration of remission is usually moderate [7–10].

The molecular mechanisms involved in cisplatin resistance are still incompletely understood. Mismatch repair deficiency and microsatellite instability are thought to be associated with cisplatin resistance in human GCT [11]. Preliminary studies suggest that vascular endothelial growth factor (VEGF) may play an important role in development and metastasizing of...
GCT [12–14]. A substantially higher VEGF and platelet-derived growth factor (PDGF) expression has been found in patients with GCT compared with normal testis tissue in several studies, indicating that VEGF and PDGF expression could play an important role in tumor angiogenesis, tumor progression, and metastases [12–15]. A significantly higher progression-free survival (PFS) rate was seen in a murine testicular cancer xenograft model when an angiostatic agent was combined with carboplatin or cisplatin as compared with either carboplatin–cisplatin or the angiostatic agent alone [16, 17].

Sunitinib is an orally administered small molecule and a potent multityrosine kinase inhibitor for VEGF receptor, PDGF receptor, as well as the stem cell factor receptor c-KIT. Sunitinib has demonstrated clinical activity in several tumors including thymic carcinoma and pancreatic neuroendocrine tumors, and is currently approved for the treatment of gastrointestinal stromal tumors and renal cell carcinoma.

Based on this rationale, we used a cell line model to investigate the preclinical activity of sunitinib in different GCT cell lines with defined levels of cisplatin resistance by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Having observed in vitro activity of sunitinib in cisplatin-resistant GCT cell lines at the same level as in their cisplatin-sensitive counterparts, we carried out a phase II study in patients with cisplatin-refractory or multiply relapsed GCT.

**patients and methods**

**preclinical study**

Sunitinib was kindly provided by Pfizer Inc., Berlin, Germany, and was dissolved in dimethyl sulfoxide (DMSO). Cisplatin was obtained from Teva, Radebeul, Germany, and was dissolved in 0.9% saline.

NTERA2 cells, first described by Andrews [18], were obtained from the ‘Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH’, Braunschweig, Germany. NCCIT cells, first described by Teshima et al. [19], were obtained from American Type Culture Collection, Manassas, VA. 2102Ep cells were kindly provided by L. Looijenga, Rotterdam, The Netherlands.

NCCIT and 2102Ep cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) and F12 medium (1 : 1) (Gibco-BRL; Invitrogen, Paisley, UK) containing 10% fetal calf serum (FCS) (Gibco-BRL; Invitrogen), and NTERA2 cells with DMEM supplemented with Glutamax-I (Gibco-BRL; Invitrogen) containing 10% FCS (Gibco-BRL; Invitrogen). Cells were incubated at 37°C in a humidified atmosphere with 5% CO₂.

From these well-established GCT cell lines, all derived from human embryonal carcinomas, cisplatin-resistant sublines exhibiting 5.2–12.2-fold resistance to cisplatin were generated in our laboratory over a period of 18 months by intermittent exposure to increasing doses of cisplatin. Moreover, a substantial increase in cisplatin resistance were generated in our laboratory over a period of 18 months by intermittent exposure to increasing doses of cisplatin. The cells were exposed to the drug for 48 h. Thereafter, the drug-containing medium was removed and 0.2 ml MTT solution (final concentration: 0.5 mg/ml MTT; Sigma, Hamburg, Germany) was added. The plates were incubated for 2 h before the medium was removed and 0.1 ml DMSO was added. The plates were agitated for 15 min before reading absorbance using a photometer (Victor2 Wallac 1420; Perkin Elmer Life Sciences, Wellesley, MA) at 570 nm. All experiments were carried out in triplicates and were repeated at least twice. The results are expressed as IC₅₀ (inhibitory concentration).

**clinical study**

Eligibility criteria for this open-label, multicenter phase II trial included histologically confirmed seminomatous or nonseminomatous GCT, and relapse within 2 months after cisplatin-based chemotherapy, tumor progression during or relapse after salvage HD-CT, tumor progression during salvage cisplatin-based chemotherapy, or ineligibility for cisplatin-based chemotherapy or HD-CT due to severe comorbidities. Patients had to have measurable disease and documented tumor progression on imaging. A sole elevation of alpha fetoprotein (AFP) or beta-human chorionic gonadotropin (ß-HCG) without disease on imaging was accepted as a measurable disease if an increase of at least 25% within 4 weeks before study inclusion had been observed. Further inclusion criteria consisted of a Karnofsky performance status ≥80% and a life expectancy ≥2 months. Adequate bone marrow function (neutrophils >1500/µl, platelet count >100 000/µl), liver function (bilirubin <1.5-fold upper normal limit), and renal function (serum creatinine ≤1.5-fold upper normal limit) were mandatory. All patients gave written informed consent. The study (clinical trial identifier: NCT00371553) was approved by the ethics committees of the British Columbia Cancer Agency and the University of Hamburg-Eppendorf, Germany, as well as by local ethics committees of all participating centers.

Sunitinib was given at a dose of 50 mg daily for 4 weeks followed by a 2-week break to form 6-week cycles. Dose reductions to 37.5 or 25 mg daily for 4 weeks on/2 weeks off were recommended for significant toxicity. No more than two dose reductions were permitted in any patient. If clinically deemed appropriate or necessary by the investigator, a continuous administration schedule utilizing 37.5 or 25 mg daily without breaks was allowed. Intrapatient reescalation back to the previous dose level was permitted in the absence of grade ≥3 hematologic or grade ≥2 non-hematologic toxicity in the previous cycle. Treatment was continued until disease progression, occurrence of unacceptable toxicity, intercurrent illness that prevented further administration of therapy, or patient’s decision to withdraw from the study.

Pretreatment evaluation consisted of medical history, assessment of the Karnofsky performance status, physical examination, electrocardiogram, Multi Gated Acquisition Scan or echocardiogram, routine laboratory, thyroid function tests, urinanalysis, tumor markers (AFP and ß-HCG), as well as radiological tumor assessments.

**definitions**

Disease was considered cisplatin refractory when at least tumor stabilization or a remission had been achieved during cisplatin-based chemotherapy, but tumor progression occurred again within 4 weeks of the last cisplatin-based chemotherapy. Disease was considered absolutely cisplatin refractory when tumor progression had occurred while patients were receiving cisplatin-based therapy [21, 22].

**response/toxicity assessment**

Response assessments were carried out on day 28 of each 6-week cycle. Response was evaluated according to RECIST version 1.0 criteria [23]. In addition, patients with reduction of the size of a tumor lesion and normalization of previously elevated tumor markers were considered partial remission with tumor marker normalization (PR negative). Patients with...
a partial remission of tumor lesions without complete tumor marker
normalization were considered a marker-positive partial remission (PR
positive). If elevated markers were the only evidence of progressive disease,
a decrease of at least 90% was required for a PR. Levels of serum tumor
markers were measured every 3 weeks. All responses as well as the diagnosis
of a stable disease had to be confirmed after a 4-week interval.
Toxicity was assessed after each cycle and graded by the National Cancer
Institute—Common Terminology Criteria for Adverse Events version 3.0.

statistical considerations
The primary end point of this study was response rate. Tolerability, PFS,
and overall survival (OS) time represented secondary end points. An
optimal Simon two-stage design was used to determine the number of
patients required [24]. Assuming a response rate of clinical interest
of >20%, a minimal response rate of 5%, a probability of 5% for rejecting
an active drug combination (type II error), and a probability of 20% to
further evaluate an ineffective drug combination (type I error), 16 patients
had to be enrolled into the first cohort. If no response to study therapy was
observed among the first 16 patients, the study was to be terminated. If at
least one objective remission occurred, the study was to be continued with
a second cohort of 16 patients.

results
proliferation and cytotoxicity assays
MTT assay. After long-term exposure to cisplatin, resistance
levels substantially increased in all three cell lines: NTERA2,
2102Ep, and NCCIT (Figure 1). Whereas the IC50 values of the
sensitive parental cell lines were 0.45 µM [±0.1 standard
deviation (SD)], 0.75 µM (±0.1 SD), and 1.75 µM (±0.1 SD),
respectively, the three cisplatin-resistant sublines exhibited IC50
values of 5.1 µM (±0.1 SD) in NTERA-2R, 4.9 µM (±0.1 SD)
in 2102Ep-R, and 4.7 µM (±0.2 SD) in NCCIT-R (P < 0.001
for all). Sunitinib substantially decreased proliferation of all
GCT cell lines in comparison with control cells treated with the
solvent DMSO. By MTT assay, IC50 values for sunitinib were
within a narrow range in all three cell lines: NTERA2 3.8 (±0.1
SD), NTERA2-R 3.5 (±0.1 SD), 2102Ep 3.0 (±0.1 SD),
2102Ep-R 3.3 (±0.4 SD), NCCIT 3.7 (±0.2), and NCCIT-R 3.4
(±0.6 SD) (Figure 1). Apart from NTERA2-R, which were
slightly more sensitive to sunitinib than NTERA2 (P = 0.02),
there was no difference in sensitivity to sunitinib between
cisplatin-sensitive parental cell lines and their cisplatin-resistant
sublines, demonstrating that there is no cross-resistance of
cisplatin and sunitinib in GCT cell lines in vitro.

clinical study
Thirty-three patients with heavily pretreated or cisplatin-
refractory GCT were entered into the study between February
2007 and January 2010. Patient characteristics are listed in
Table 1.
Fifteen percent of patients had a primary mediastinal GCT. All
patients were heavily pretreated with a median number of 2
(range, 1–6) platinum-based regimens. Sixty-six percent of
patients had relapsed after HD-CT, and 33% were considered
late relapses (≥2 years after initial therapy). Fifty-four percent of
patients were considered cisplatin refractory or absolutely
refractory.
A total of 66 cycles of sunitinib were administered with
a median number of 1 cycle per patient (range, 1–9). No
complete remission was observed. Three patients achieved
a partial remission (9%) (Tables 2 and 3). Time to progression
for these three patients was 5.0, 6.4, and 12.2 months. One
additional seminoma patient achieved a response but
subsequently went off study before a confirmatory scan was
carried out (Table 3). Stable disease was recorded for 41% of
patients with a median duration of 2.3 months (range 1.6–6.5
months).
After a median follow-up of 14.4 months (range, 6–18
months), all patients are off study. In 30 patients, treatment
termination was due to progression; 2 patients refused further
treatment, and 1 patient was stopped due to
hyperbilirubinemia. Median PFS for all patients was 2.0 months
[95% confidence interval (CI) 1.4–2.60], with 11% and 3.7% of
patients being progression free at 6 and 12 months, respectively
(Figure 2). Median OS was 3.8 months (95% CI 3.0–6.6), with
36.4% and 9.9% of patients being alive at 6 and 12 months,
respectively.
Sunitinib treatment was feasible and toxicity in these heavily
pretreated patients was generally acceptable. However,
treatment duration was short in the majority of patients and
thus toxicity assessment, and in particular evaluation of long-
term toxicity, is limited (Table 4). The most common
sunitinib-related toxic effects included fatigue (39%), anorexia
(21%), diarrhea (27%), mucositis (45%), nausea/vomiting
(24%), and dyspepsia (27%). Grade 3/4 toxic effects were rare
and no unexpected side-effects were observed.

discussion
Despite the overall success of cisplatin-based chemotherapy in
patients with metastatic GCT, the prognosis for cisplatin-
refractory patients remains very poor. Gemcitabine–oxaliplatin and paclitaxel–gemcitabine or gemcitabine–oxaliplatin–paclitaxel are the most commonly used regimens for these patients resulting in response rates of 25%–40%, with selected patients achieving prolonged survival [7–9, 25]. Limited clinical data exist regarding targeted therapies in GCT [26, 27]. Based on previous reports on the potential role of VEGF and VEGFR and the very high expression of tyrosine kinases in GCT, we investigated the multi tyrosine kinase inhibitor sunitinib preclinically and subsequently clinically in cisplatin-resistant GCT [28]. Analysis of the efficacy of sunitinib in GCT cell lines in vitro demonstrated inhibition of proliferation (MTT assay) at an IC50 in the range of 3.0–3.8 μM after 48 h of drug exposure. There was no difference between cisplatin-sensitive and cisplatin-resistant cell lines, indicating that there is no cross-resistance between sunitinib and cisplatin. Furthermore, this dose range for in vitro activity is at the lower end of previously determined doses in different other tumor cell lines including renal cell carcinoma, nasopharyngeal carcinoma, or lung cancer, where IC50 doses of 3–10 μM have been reported [29–31]. An IC50 of ~6 μM has previously been reported for the human yolk sac tumor cell line 1411H after 24-h treatment [17]. As was the case in our three cell lines and their cisplatin-resistant sublines, no cross-resistance between cisplatin and sunitinib was observed by Castillo-Avila et al. [17] who, in addition to direct action on receptor tyrosine kinases, also did observe a strong antiangiogenic effect of sunitinib in GCT xenografts. This suggests that in GCT cells, resistance to cisplatin does not confer resistance to sunitinib.

Despite this promising preclinical activity of sunitinib in cisplatin-resistant GCT cells, shown both in vitro in our analysis and in vivo by Castillo-Avila et al. [17], sunitinib exhibited only modest activity with three confirmed temporary partial responses (PRs) in patients with cisplatin-refractory or multiply relapsed GCT and thus our study failed to meet its primary end point of a 20% response rate.

This study was conducted in a prognostically unfavorable group of patients, comparable with those treated within our previous trials evaluating the role of paclitaxel, gemcitabine, and oxaliplatin, or the combination of gemcitabine–paclitaxel in relapsed GCT [7–9]. Thirty-three percent of the patients presented with a late relapse, 15% initially had presented with a primary mediastinal GCT, 66% were pretreated with HD-CT, and 54% of our patients were classified as cisplatin refractory

### Table 1. Patients’ characteristics (N = 33)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years (range)</td>
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<td>22–54</td>
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<td>Eastern Cooperative Oncology</td>
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<td>Group performance status</td>
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<tr>
<td>0</td>
<td>14</td>
<td>42</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>52</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Location of primary tumor</td>
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<td></td>
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<tr>
<td>Gonadal</td>
<td>26</td>
<td>79</td>
</tr>
<tr>
<td>Retroperitoneal</td>
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<td>0</td>
</tr>
<tr>
<td>Mediastinal</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Extragonadal not further specified</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Histology</td>
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<td></td>
</tr>
<tr>
<td>Pure seminoma</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Mixed nonseminomatous</td>
<td>29</td>
<td>88</td>
</tr>
<tr>
<td>Metastases at study entry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>20</td>
<td>61</td>
</tr>
<tr>
<td>Liver</td>
<td>11</td>
<td>33</td>
</tr>
<tr>
<td>Bones</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>Other</td>
<td>18</td>
<td>55</td>
</tr>
<tr>
<td>Visceral (liver, pancreas, colon)</td>
<td>25</td>
<td>76</td>
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<tr>
<td>Tumor markers at study entry</td>
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<tr>
<td>Median AFP value</td>
<td>9 kU/l (1–490 000)</td>
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<tr>
<td>Median beta-HCG value</td>
<td>111 U/l (0.3–39 000)</td>
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</tr>
<tr>
<td>Median LDH value</td>
<td>278 (110–2107)</td>
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<tr>
<td>Elevated TUM at baseline</td>
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<tr>
<td>AFP</td>
<td>15</td>
<td>48</td>
</tr>
<tr>
<td>HCG</td>
<td>18</td>
<td>62</td>
</tr>
<tr>
<td>LDH</td>
<td>18</td>
<td>60</td>
</tr>
<tr>
<td>Median no. of previous platin-based cycles</td>
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<tr>
<td>Previous high-dose chemotherapy</td>
<td>22</td>
<td>66</td>
</tr>
<tr>
<td>Refractoriness after previous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>platin-based chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute cisplatin refractory</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>Cisplatin refractory</td>
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<td>27</td>
</tr>
<tr>
<td>Not refractory</td>
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<td>45</td>
</tr>
<tr>
<td>Previous gemcitabine–oxaliplatin-based</td>
<td>21</td>
<td>64</td>
</tr>
<tr>
<td>regimen</td>
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<tr>
<td>Previous gemcitabine–paclitaxel-based</td>
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<td>15</td>
</tr>
<tr>
<td>regimen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late relapse (&gt;2 years after initial</td>
<td>11</td>
<td>33</td>
</tr>
<tr>
<td>chemotherapy)</td>
<td></td>
<td></td>
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### Table 2. Treatment efficacy (N = 33)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cycles</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>Median cycles per patient (range)</td>
<td>1</td>
<td>1–9</td>
</tr>
<tr>
<td>Response (n = 32), n (%)</td>
<td></td>
<td></td>
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<tr>
<td>Confirmed PR</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Unconfirmed PR</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Stable disease</td>
<td>13</td>
<td>41</td>
</tr>
<tr>
<td>Progression</td>
<td>15</td>
<td>47%</td>
</tr>
<tr>
<td>Time to progression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median, months (95% CI)</td>
<td>2</td>
<td>1.4–2.6</td>
</tr>
<tr>
<td>3 months PFS, % (95% CI)</td>
<td>26</td>
<td>13.8% to 47.5%</td>
</tr>
<tr>
<td>6 months PFS, % (95% CI)</td>
<td>11</td>
<td>5.8% to 31.6%</td>
</tr>
<tr>
<td>OS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median, months (95% CI)</td>
<td>3.8</td>
<td>3.0–6.6</td>
</tr>
<tr>
<td>3 months OS, % (95% CI)</td>
<td>66</td>
<td>52.4% to 84.9%</td>
</tr>
<tr>
<td>6 months OS, % (95% CI)</td>
<td>36</td>
<td>23.2% to 57.1%</td>
</tr>
<tr>
<td>9 months OS, % (95% CI)</td>
<td>17</td>
<td>6.4% to 35.9%</td>
</tr>
<tr>
<td>12 months OS, % (95% CI)</td>
<td>10</td>
<td>0.8% to 31.0%</td>
</tr>
</tbody>
</table>

PR, partial response; CI, confidence interval; PFS, progression-free survival; OS, overall survival.
In addition, the majority of patients had also been pretreated with gemcitabine, paclitaxel, and/or oxaliplatin or combinations of these drugs. The PR rate of 13% and disease stabilization rate of 41% with 11% of patients being progression free at 6 months suggest activity in this patient population but is lower than the response rates of 25%–45% recently reported for conventional combination chemotherapy such as oxaliplatin–gemcitabine (Table 2). Achieving a high response rate in refractory patients, as seen in studies with combination regimens, e.g. gemcitabine-oxaliplatin, is important because the induction of a response may subsequently allow the resection of residual masses and may thus be a chance to still achieve long-term survival. This is underlined by a proportion of ~10%–15% of long-term survivors in the trials with conventional chemotherapy [10]. No complete remission was observed in the current trial with sunitinib. Interestingly, however, three of the responding patients were classified as cisplatin refractory before sunitinib therapy. Two patients had refractory seminoma, which can harbor c-KIT mutations as a potential target for sunitinib [32, 33]. Further molecular analysis of these cases is warranted to potentially identify predictive factors for response to sunitinib.

Forty-one percent of patients in our study achieved disease stabilization, although the duration of stabilization was generally brief and nonresponding patients progressed rapidly, similar to the duration of stable disease seen in previous chemotherapy studies. It remains unclear whether off-target activity of sunitinib may have contributed to the activity seen in our study. However, any activity in refractory GCT patients is uncommon, and the overall control rate seen in this study is noteworthy and suggests that further investigation of antiangiogenic agents, in particular in combination with chemotherapy, is warranted.

Sunitinib was associated with acceptable toxicity in this heavily pretreated patient population. No unexpected toxic effects were observed, and myelotoxicity was not treatment limiting in these extensively pretreated patients. However, the majority of patients received sunitinib only for a short period of time, which limits our assessment of toxicity.

Our results are slightly better than results previously reported in another small exploratory phase II trial [34]. In this trial, some marker decline was seen; however, no objective response was observed, and the trial was stopped prematurely after enrollment of only 10 patients.

In summary, sunitinib appears to have good preclinical activity and antitumor responses were observed in refractory GCT suggesting a role of the VEGF pathway in GCT. Exploring the molecular characteristics of responding patients may allow the identification of a molecular profile suitable for sunitinib or treatment with other targeted therapies. However, taking into account the results by Feldman et al. [34] as well as our results, it is unlikely that sunitinib as a single agent will clinically play...

<p>| Table 3. Characteristics of patients achieving a confirmed or an unconfirmed PR to sunitinib |
|---------------------------------|---------------------------------|------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Primary histology</th>
<th>Prior therapy</th>
<th>Late relapse status</th>
<th>Site of metastases at study entry</th>
<th>Best response to sunitinib</th>
<th>Time to progression (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>007-007</td>
<td>Seminoma</td>
<td>BEP, TIP, HD-CT, Gem–Oxali</td>
<td>No</td>
<td>Refractory</td>
<td>PR</td>
<td>5.3</td>
</tr>
<tr>
<td>007-013</td>
<td>Nonseminoma</td>
<td>BEP, VIP, Gem–Oxali</td>
<td>No</td>
<td>Absolute refractory</td>
<td>PR-</td>
<td>6.4</td>
</tr>
<tr>
<td>007-014</td>
<td>Nonseminoma</td>
<td>PVB, VIP, actinomycin–cyclophosphamide</td>
<td>Yes</td>
<td>Sensitive</td>
<td>Marker relapse</td>
<td>12.2</td>
</tr>
<tr>
<td>04-001</td>
<td>Seminoma</td>
<td>BEP, VIP, HD-CT</td>
<td>No</td>
<td>Refractory</td>
<td>Lymph nodes</td>
<td>PR unconfirmed</td>
</tr>
</tbody>
</table>

BEP, Bleomycin, Etoposide, Cisplatin; HD-CT, high-dose chemotherapy; PR, partial response; PVB, Cisplatin, Vinblastine, Bleomycin; TIP, Paclitaxel, Etoposide, Cisplatin; VIP, Etoposide, Ifosfamide, Cisplatin.
a major role in cisplatin-refractory patients at standard doses and standard schedules unless the underlying mechanism of response to sunitinib is identified.

GCT as a highly chemotherapy-sensitive malignancy may provide an opportunity to combine sunitinib with classic cytotoxic cisplatin-based chemotherapy [17, 35]. Kramar et al. [36, 37] recently published preliminary data on the combination of oxaliplatin and bevacizumab. Five of eight patients with late relapse responded, suggesting some activity of a combination approach in this particular patient population. A major concern, however, with regard to combination treatment using a multitargeted kinase inhibitor like sunitinib and a cytotoxic drug is overlapping and often additive toxicity, e.g. myelosuppression [37, 38]. Further research to unravel the molecular biology of testicular GCT and in particular the mechanisms of chemotherapy resistance is urgently needed, but the identification of new targets will hopefully allow more tailored therapy approaches in GCT.

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References