Maintaining the dose intensity of ICE chemotherapy with a thrombopoietic agent, PEG-rHuMGDF, may confer a survival advantage in relapsed and refractory aggressive non-Hodgkin lymphoma

C. H. Moskowitz1,2,*, P. A. Hamlin2, J. Gabrilove1,3, J. R. Bertino2,4, C. S. Portlock2, D. J. Straus2, A. N. Gencarelli2, S. D. Nimer1 & A. D. Zelenetz2

1Hematology Service; 2Lymphoma Service, Division of Hematologic Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA; 3Current address: The James F. Holland Professor of Medicine, The Mount Sinai School of Medicine, The Department of Medicine, Division of Hematology/Oncology, New York, NY; 4Current address: Cancer Institute of New Jersey, Robert wood Johnson School of Medicine, New Brunswick, NJ, USA

Received 27 December 2006; revised 19 April 2007; accepted 31 May 2007

Introduction: HDT/ASCT is standard for relapsed and refractory DLCL patients responding to second-line chemotherapy. We incorporated a thrombopoietic agent into the ICE chemotherapy program to potentially: decrease platelet associated toxicities, augment stem cell collection and maintain dose intensity.

Methods: This randomized, double-blind, placebo-controlled phase I/II trial examines PEG-rHuMGDF versus placebo with ICE chemotherapy. Phase I compared three cohorts and defined a clinically effective dose (CED). Phase II evaluated the CED versus placebo. Outcome measures included safety, hematological end-points, stem cell collection and the impact of dose-intensity on outcome.

Results: Forty-one patients with primary refractory (16) or relapsed DLCL (25) were treated; Response rates for evaluable patients are: 75% (12/16) for placebo and 82% (18/22) for PEG-rHuMGDF. PEG-rHuMGDF treated patients had significantly less grade IV thrombocytopenia, higher median platelet nadirs, and less platelet transfusion per cycle. ICE dose intensity was improved with PEG-rHuMGDF versus placebo: 75 versus 42% ($P=0.008$). At 8.5 years median follow-up, overall and event-free survival are 47 and 31%, respectively. Patients treated on PEG-rHuMGDF versus placebo had improved survival (59 versus 31%, $P=0.06$).

Conclusion: PEG-rHuMGDF ameliorated thrombocytopenia, improved platelet recovery, and maintained ICE dose intensity. Potential survival advantages conferred by maintaining dose intensity require validation with newer thrombopoietic agents.

Key words: dose-intensity, ICE chemotherapy, non-Hodgkin’s lymphoma, refractory, relapsed, transplantation

Introduction

The initial treatment of diffuse large cell lymphoma (DLCL) with anthracycline-containing combination chemotherapy programs results in cure rates of 40–50% [1]. Effective second-line therapy is therefore paramount, as 50–60% of patients will be either refractory to initial therapy or will relapse after achieving a complete remission (CR). A prospective randomized study has determined that high dose chemoradiotherapy (HDT) and autologous stem cell transplantation (ASCT) is standard therapy for these patients [2]. Chemosensitivity to conventional dose second-line chemotherapy prior to HDT is recognized as a significant predictor of a favorable outcome following ASCT [2, 3]. Furthermore, the quality of that response, complete versus partial remission (PR), also is a factor in outcome [4]. An ideal second-line regimen should possess the following qualities: (i) induces a high response rate; (ii) have minimal non-hematological toxicity; and (iii) enable the adequate collection of autologous stem cells. The ICE (ifosfamide, carboplatin, etoposide) chemotherapy regimen was developed with these goals in mind [4].

The ICE chemotherapy regimen was designed as a dose-intensive and dose-dense cytoreductive and stem cell mobilization regimen for patients with non-Hodgkin’s lymphoma (NHL). We have reported our results in 163 patients treated with this regimen, demonstrating an overall response rate of 66% (24% complete and 42% partial response) with a median collection of $8.4 \times 10^6$ CD34-positive cells/kg following three cycles of chemotherapy [4]. These results are comparable with other pre-ASCT cytoreduction regimens such...
as DHAP, ESHAP or mini-TEAM [5–7]. Carboplatin, rather than cisplatin was incorporated to decrease the incidence of renal insufficiency and ifosfamide was administered as a 24-hour infusion to decrease the incidence of ifosfamide induced encephalopathy. Subsequent studies have demonstrated an improvement in response quality with the addition of rituximab to ICE chemotherapy [8], a strategy undergoing validation in a phase III setting [9].

Prior to rituximab, efforts to identify aspects of the ICE program amenable to improvement focused on potential supportive-care agents to ameliorate the toxicity profile. Hematopoietic growth factors have routinely been incorporated into the treatment program to maintain dose intensity and schedule. The dose limiting toxicity of ICE chemotherapy is principally thrombocytopenia; Grade III/IV thrombocytopenia (platelets < 50,000 /mm³) occurred in 29% of ICE cycles administered and required platelet transfusions in 30% of all patients [4]. This hematological toxicity caused 20% of cycles 2 and 3 of ICE to be delayed until platelet count recovered to greater than 50,000/mm³. The median time to deliver three cycles of ICE was 38 days instead of the planned 31 days. In the initial treatment of DLCL, maintenance of dose intensity and dose density has been demonstrated to correlate with improved survival in retrospective analyses and recently in prospective studies [10–15]. If the maintenance of dose intensity and schedule remain important during second-line therapy, treatment delays due to thrombocytopenia may have important consequences on the overall and complete response rates and ultimately upon survival measures.

Thrombopoietin (Mpl ligand) is a primary regulator of thrombopoiesis, with effects on stem cells, megakaryocyte progenitors, and thrombocytes. Pegylated-recombinant human megakaryocyte growth factor (PEG-rHuMGDF) has an amino acid sequence that is identical to the first 163 amino acids of native human thrombopoietin with a covalent bond to polyethylene glycol at the amino terminal. PEG-rHuMGDF has been shown to increase platelet counts both before and after chemotherapy administration [16, 17].

We designed this randomized, double-blind, placebo-controlled, dose-finding study of multi-cycle ICE chemotherapy and PEG-rHuMGDF support to ascertain a clinically effective dose (CED) of PEG-rHuMGDF in relapsed and refractory aggressive lymphoma patients. In addition, we sought to determine the safety of PEG-rHuMGDF in patients with aggressive NHL undergoing up to three cycles of ICE chemotherapy, the effect of PEG-rHuMGDF used in combination with filgrastim on platelet recovery, the number of platelet transfusions required, and on the yield of CD34+ cells obtained during leukapheresis. Importantly, the impact of PEG-rHuMGDF on the ability to deliver planned chemotherapy on schedule, with the goal of improving the dose-density of the ICE program, was assessed.

patients and methods

patients

Forty-one transplant-eligible patients with relapsed and primary refractory aggressive non-Hodgkin’s lymphoma were enrolled on an Institutional Review Board (IRB) approved protocol 96-17 at Memorial Sloan-Kettering Cancer Center (MSKCC) between 8/96 and 9/98, after obtaining informed consent and in concordance with the Helsinki protocol. This study was conducted in a double-blind manner: all MSKCC staff and all Amgen personnel directly involved in patient care and data assessment were blinded with respect to study drug assignment. A data safety monitoring board at Amgen reviewed safety data on an on-going basis. Efficacy was monitored at a planned interim analysis.

patient eligibility

Eligible patients were in first relapse or had primary refractory disease to an anthracycline-based chemotherapy regimen. Patients with parenchymal brain disease at relapse were excluded. The diagnosis of relapsed disease was established by biopsy. Pathologic diagnoses were reviewed by the hematopathology staff at MSKCC and eligible diagnoses in the WHO/REAL classification included:

(i) follicular lymphoma, grade III (FCL, Grade III);
(ii) diffuse large B cell lymphoma (DLBCL);
(iii) transformed DLBCL;
(iv) anaplastic large cell lymphoma (ALCL);
(v) mantle cell lymphoma (MCL);
(vi) peripheral T cell (PTCL).

All procedures were in accordance with the Helsinki protocol.

A minimum platelet count of 40 × 10⁹/l was required. Patients who received warfarin, heparin (other than flushes for central venous catheters), ticlopidine hydrochloride or aspirin within 7 days of enrollment were excluded. Patients with a history of thromboembolic disease within the last 12 months, with the exception of catheter related thromboses, were also excluded, as were patients with concurrent idiopathic thrombocytopenic purpura. Patients had normal baseline cardiac function based upon echocardiogram or gated blood pool scan with an ejection fraction of >50% as measured since last chemotherapy.

Patients were precluded from concurrent enrollment on any other protocol using an investigational or non-approved drug or biologic and from the use of hematopoietic growth factors other than filgrastim within 2 weeks of study entry.

pretreatment evaluation

Patients had an evaluation of extent of disease with a CT scan of the chest, abdomen and pelvis, gallium scanning with SPECT imaging, bone marrow aspiration and biopsy. Lumbar punctures were performed in the case of bone marrow, testicular, paranasal sinus involvement or the presence of multiple extranodal sites of disease. A serum sample was obtained to serve as the baseline for determination of antibodies to PEG-rHuMGDF.

ICE chemotherapy

ICE chemotherapy was administered as previously described [4]. ICE was intended to be delivered at 14-day intervals provided there was no evidence of clinical progression. There were no dose reductions; instead, treatment was delayed until the absolute neutrophil count was >1000/µl and the platelet count was >50 000/µl.

study design

The study was designed as a two-stage, placebo-controlled randomized phase I/II trial. The first stage (Phase I) was designed to choose a CED of PEG-rHu-MGDF. After a planned interim analysis to choose the CED, the second stage was a placebo-controlled randomized phase II design.

patient randomization

In Stage I, patients were randomized to receive filgrastim in combination with placebo, PEG-rHuMGDF at 2.5 µg/kg/day or PEG-rHuMGDF at
5.0 μg/kg/day. Dosing was based on pre-clinical primate models and contemporaneous phase I/II data [Amgen, on file]. Patients received study drug (either placebo or one of two doses of PEG-rHuMGDF) following each cycle of ICE chemotherapy on days 4–10. Filgrastim was administered days 6–13 at a dose of 5 μg/kg/day following ICE cycles 1 and 2 and at a dose of 10 μg/kg/day following mobilization cycle 3.

During Stage II, the CED was chosen to be 5 μg/kg/day based on an apparent difference in stem cell mobilization. Patients were randomized to receive filgrastim in combination with placebo or PEG-rHuMGDF at 5 μg/kg/day on days 4–10. Filgrastim was administered at the same dose and schedule as in Stage I.

**stem cell mobilization**

A dialysis quality catheter was placed during the third cycle of ICE. Leukapheresis was performed when the white blood cell count (WBC) recovered to >5 × 10⁹/l or when the peripheral blood CD34⁺ count was >50 cells/mm³, as determined by flow cytometry. All seven doses of study drug were administered. Administration of filgrastim 10 μg/kg/day began day 6 and continued until leukapheresis was complete. Leukapheresis continued until >6 × 10⁹/kg CD34⁺ cells were collected, or a maximum of five leukaphereses were performed, whichever occurred first. Patients in whom <2.0 × 10⁹/kg CD34⁺ cells were collected were considered mobilization failures. These patients were observed until absolute neutrophil count (ANC) and platelet counts recovered or day 14 of cycle 3, whichever occurred last. For each apheresis, 10 l of blood were processed over 2.5–3 hours. Before cryopreservation, mononuclear cells in each leukapheresis collection were analyzed for CD34 expression using flow cytometry as previously described [18].

**standardized transfusion management**

Prophylactic transfusion of irradiated platelets was administered for platelet counts below 10 × 10⁹/l. Platelet transfusions were permitted for:

(i) the prevention of bleeding during invasive procedures, such as insertion of central venous access catheters, if the platelet count was less than 50 × 10⁹/l;
(ii) in thrombocytopenic patients to control clinically significant hemorrhage (e.g., hemorrhage requiring red cell transfusions or hemorrhage in critical anatomical locations);
(iii) if otherwise clinically indicated (e.g., sepsis).

Irradiated red blood cell products were administered if hemoglobin fell below 7.0 g/dl, in an otherwise asymptomatic patient. Otherwise, red blood cell transfusions were administered when clinically indicated.

**evaluation during and at end of treatment**

Physical examination and performance status assessment was performed prior to each chemotherapy treatment. Complete blood counts with differentials were collected three times per week. Continuous records of adverse events during transfusion were recorded. CT scans of the chest, abdomen and pelvis were repeated 2–4 weeks after cycle no. 3 of ICE to evaluate the extent of disease and responsiveness to chemotherapy. Gallium scanning and bone marrow biopsies were repeated after the third cycle of ICE if the initial studies were positive.

The incidence of thrombocytopenia, duration of thrombocytopenia, platelet nadir, days and units of platelets transfused, and the number of patients who recovered platelet counts by day 14 of each cycle of ICE chemotherapy was evaluated. The CD34⁺ cell yield from apheresis products was evaluated in all patients who completed three cycles of ICE chemotherapy.

Following completion of the study, serum was obtained for evaluation of antibodies directed at PEG-rHuMGDF at least 4 weeks following completion of ICE no. 3.

**criteria for response to ice chemotherapy**

A modification of the international lymphoma study group response criteria was utilized [19], requiring all patients achieving a complete response by CT criteria to also have normalized their gallium scan.

**biostatistical considerations: general study design**

This was a randomized, double-blinded, placebo-controlled, parallel cohort, dose-finding clinical trial of PEG-rHuMGDF in patients with histologically proven non-Hodgkin’s lymphoma (NHL). The relative safety and efficacy of two different dose levels of PEG-rHuMGDF were studied and a CED was selected. In the subset of patients who underwent leukapheresis, the number of CD34⁺ cells in the leukapheresis product was estimated.

Per design, an interim analysis of the dose-finding stage of the study was conducted after 18 patients were enrolled and had either completed three cycles of ICE or were withdrawn from the study. The clinically effective dose was the dose of PEG-rHuMGDF in which >67% of patients recovered platelet count to ≥50 × 10⁹/l by day 14 of each cycle and in which <33% of patients in a cohort experienced a dose-limiting toxicity. Patients were included in this analysis if they received at least one dose of study drug. All statistical comparisons are descriptive in nature and are considered hypothesis seeking. The primary endpoint of the study was the number of cycles during which thrombocytopenia (i.e., platelet count <20 × 10⁹/l) was experienced by subjects during the ICE regimen. Other measures of platelet response included day 14 platelet recovery (i.e., unsupported platelet count ≥50 × 10⁹/l by day 14 of each cycle), duration of thrombocytopenia, severity of thrombocytopenia and incidence of platelet transfusions.

As there were no significant differences in efficacy between the 2.5 or 5 mcg/kg groups (see results), for efficacy analyses of the entire program all PEG-rHuMGDF treated patients were pooled (those receiving either 2.5 or 5 μg/kg) and compared with placebo treated patients. However, since this was an early phase I/II study, comparisons were considered hypothesis seeking, rather than confirmatory.

In the subset of patients who underwent leukapheresis, the number of CD34⁺ cells in the leukapheresis product was quantified. The CD34⁺ cell content for each PEG-rHuMGDF dose cohort was compared with placebo using the Wilcoxon rank sum test. Incidence of mobilization failure, percentage of patients who reached the target of 6 × 10⁶ CD34⁺ cells/kg, and the number of aphereses required to reach the target in the three treatment groups was assessed using Fisher’s Exact Test.

Overall survival (OS) was calculated from the start of therapy until death from any cause. Event-free survival was computed from the start of treatment until one of the following events occurred: progression of disease, relapse or death. Survival curves were generated using the method of Kaplan and Meier [20]. Univariate analyses were performed using the log-rank test [21]. Categorical variables were compared using the Fisher exact test. Survival analyses, univariate analyses and comparisons of categorical variables were performed using SPSS version 11 (SPSS, Chicago, IL), with log rank comparisons of placebo versus PEG-rHuMGDF cohorts.

**results**

**patient characteristics**

Forty-one patients with primary refractory or first relapsed aggressive NHL were enrolled on this IRB approved clinical trial; three patients experienced chemotherapy-related toxicity prior to receiving study drug and were not considered evaluable. Patient characteristics prior to the initiation of ICE chemotherapy are listed in Table 1. There were 24 male patients and 14 female patients. The median age was 44 years;
21% of patients were greater than 60 years of age. Twenty-nine per cent of the patients had a poor performance status, as defined by a Karnofsky performance status less than 80 per cent. Stage IV disease or multiple extranodal sites of involvement pre-ICE was present in 37 and 32% of patients, respectively. DLBCL was the most common histology accounting for 63% of patients. Thirty-nine per cent of the patients had refractory disease (defined as failure to achieve a CR or progression on therapy); the remaining 61% were in first relapse.

During the dose finding stage, 22 patients were initially randomized, including six patients on placebo, eight patients on PEG-rHuMGDF 2.5 μg/kg/day, and eight patients PEG-rHuMGDF 5 μg/kg/day. Two patients in the PEG-rHuMGDF 2.5 μg/kg/day experienced toxicity during ICE cycle no. 1 (ifosfamide encephalopathy, rapid atrial fibrillation) before receiving treatment on study and were not included in the hematological analysis. During the second phase, 10 patients were assigned placebo and nine patients PEG-rHuMGDF 5 μg/kg/day; one patient in the PEG-rHuMGDF 5 μg/kg/day experienced progressive disease immediately following ICE no. 1 (before receiving blinded study drug) and was not included in the hematological analysis.

### ICE response

Median exposure to ICE was comparable among the three treatment groups (data not shown). Thirty-three of the 38 evaluable patients received all three cycles of ICE chemotherapy. Placebo-treated patients achieved a response rate of 75% and PEG-rHuMGDF-treated patients a response rate of 82%. Seventeen patients achieved a complete remission and 13 patients a partial remission for overall response rate of 79% that is somewhat better than our historical response rates. Patients receiving PEG-rHuMGDF were more likely to have received ICE on schedule (£31 days) than patients on placebo (77 versus 44%; P = 0.05, Fisher’s exact test).

### Event-free survival and overall survival analyses

At a median follow-up of 8.5 years, the Kaplan–Meier estimate of the proportion of patients alive and event-free is 47 and 31%, respectively (Figure 1). Overall survival (OS) for the PEG-rHuMGDF-treated patients versus the placebo-treated patients was 59 versus 31% (P = 0.06), respectively; event-free survival was 36 versus 21% (P = 0.39), respectively (Figures 2 and 3). For the patients who received high dose therapy and ASCT (n = 31) the proportion of patients alive and event free is 55 and 38%, respectively. Among transplanted PEG-rHuMGDF-treated patients versus placebo-treated

---

**Table 1. Patient characteristics for 38 patients treated on study**

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>(n = 38)</th>
<th>Placebo</th>
<th>MGDF 2.5 mcg/kg/day</th>
<th>MGDF 5 mcg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapse</td>
<td>23</td>
<td>60.5%</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Refractory</td>
<td>15</td>
<td>39.5%</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
<td>63%</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>37%</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Age, median 44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–59</td>
<td>30</td>
<td>79%</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>60–68</td>
<td>8</td>
<td>21%</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Performance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥80 %</td>
<td>27</td>
<td>71%</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>≤70 %</td>
<td>11</td>
<td>29%</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>13</td>
<td>34%</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>III</td>
<td>11</td>
<td>29%</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>IV</td>
<td>14</td>
<td>37%</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>LDH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;200</td>
<td>16</td>
<td>42%</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>≤200</td>
<td>22</td>
<td>58%</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>ENS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>17</td>
<td>45%</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>24%</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>≥2</td>
<td>12</td>
<td>32%</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLBCL</td>
<td>24</td>
<td>63%</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>ALCL</td>
<td>3</td>
<td>8%</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>MCL</td>
<td>1</td>
<td>3%</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>PTCL</td>
<td>5</td>
<td>13%</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>tDLCL</td>
<td>3</td>
<td>8%</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>FCL grade 3</td>
<td>2</td>
<td>5%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>5</td>
<td>13%</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>32</td>
<td>84%</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>saaIPI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low risk (0–1)</td>
<td>21</td>
<td>55%</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>High risk (2–3)</td>
<td>17</td>
<td>45%</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

LDH = lactate dehydrogenase, ENS = extranodal sites, IHC = immunohistochemistry, saaIPI=second-line age-adjusted IPI.
In the setting of relapsed or primary refractory aggressive NHL, we previously demonstrated that the age-adjusted second-line international prognostic index (sIPI) predicts survival with a second-line intent to treat ASCT program [18, 22]. Likewise, in this study the sIPI defined low risk (0–1 factors) and high risk (2–3 factors) that predicted event-free (41 versus 14%) and overall survival (67 versus 14%; \( P = 0.002 \) and \( P < 0.001 \)).

However, in addition to the sIPI, patients receiving all three cycles of ICE chemotherapy on schedule had a significant improvement in OS and EFS. For patients treated with three cycles of ICE on schedule (planned 28-day 63 days between cycles 1 and 3) versus those patients with delays in therapy (>31 days), OS was 67 versus 22% \( (P = 0.055) \) and EFS was 45 versus 11% \( (P = 0.05; \) Figures 4 and 5). For transplanted patients, the OS and EFS for patients treated on schedule versus delayed was 73 versus 13% \( (P = 0.004) \) and 49 versus 13% \( (P = 0.04) \), respectively. Delays in ICE delivery were secondary to thrombocytopenia 83% of the time, with a 10-day difference in median cycle delivery (28 versus 38 days). Peg-rHuMGDF was associated with a significant increase in the rate of therapy being delivered on time—77 versus 44% (Fisher's exact test, \( P = 0.05) \); stated differently, patients on placebo were 4.4 times (range 1.1–17.8) more likely to be delayed than patients receiving Peg-rHuMGDF.

**Hematological response**

_**Analysis of initial randomized dose-finding phase of study.**_ Both Peg-rHuMGDF doses met the CED criteria: >67% of patients recovered platelet count to \( \geq 50 \times 10^9/l \) by day 14 of each cycle and <33% of patients experienced a dose-liming toxicity. During the dose-finding phase of the study, no patients receiving PEG-rHuMGDF required a platelet transfusion, while

**Prognostic factors analysis for EFS and OS**

In the setting of relapsed or primary refractory aggressive NHL, we previously demonstrated that the age-adjusted second-line

**Figure 1.** Overall and event-free survival for all patients \( (n = 41) \).

**Figure 2.** Overall survival for Peg-rHuMGDF (combined 2.5 and 5.0 mcg/kg/day 4–12, \( n = 22 \)) plus G-CSF versus placebo + filgrastim supported patients \( (n = 16) \). At 8.5 years median follow-up, KM estimates are 59 versus 31%, respectively \( (P = 0.06) \).

**Figure 3.** Event-free survival for Peg-rHuMGDF (combined 2.5 and 5.0 mcg/kg/day 4–12, \( n = 22 \)) plus G-CSF versus placebo + filgrastim supported patients \( (n = 16) \). At 8.5 years median follow-up, KM estimates are 36 versus 21%, respectively \( (P = 0.39) \).
33% of the patients receiving placebo were transfused platelets. PEG-rHuMGDF at a dose of 5 μg/kg/day was chosen as the CED because of a better CD34+ cell yield (data not shown). However, platelet recovery was the same in the cohorts receiving 2.5 and 5 mcg/kg of PEG-rHuMGDF; thus, the data from all PEG-rHuMGDF supported cycles of ICE was pooled to provide a more robust exploratory analysis.

**combined efficacy analysis of placebo versus PEG-rHuMGDF.** Although this was a phase I/II dose finding study, a number of parameters demonstrated clinically meaningful differences between patients receiving PEG-rHuMGDF versus placebo. There were 16 patients treated in the placebo cohort and 22 patients treated with PEG-rHuMGDF. The incidence of grade IV thrombocytopenia was 35% in the placebo cohort in comparison to 15% in the PEG-rHuMGDF supported ICE cycles (P = 0.02). Importantly, the 16 patients who received placebo had a median platelet nadir of 20 000/μl versus 49 000/μl for the 22 patients who received PEG-rHuMGDF (P = 0.008). This translated to only 42% of ICE chemotherapy administered on schedule for the placebo supported cycles versus 75% in the PEG-rHuMGDF supported cycles (P = 0.008). Finally, 23% of cycles of ICE chemotherapy were followed by platelet transfusions in patients who received placebo versus only 8% in the PEG-rHuMGDF supported cycles of ICE (P = 0.04). There was no difference in the number of CD34+ cells/kg collected, number of apheresis procedures or mobilization failures between placebo + filgrastim treated patients versus the PEG-rHuMGDF + filgrastim treated patients.

**post-ASCT hematological evaluation**

Sixteen patients are evaluable for long-term hematological recovery post-ASCT. Eleven patients received PEG-rHuMGDF and filgrastim mobilized PBPCs and five patients received filgrastim alone. The median WBC, Hgb and Platelet count at 12, 24 and 36 months post-ASCT were similar in both cohorts of patients.

**safety evaluations**

*adverse events.* Adverse events were reported in 100% (23) of PEG-rHuMGDF-treated subjects, and 94% (15 of 16) of placebo-treated subjects. The most common events were a variety of symptoms (nausea, fever, constipation and rigors), which were consistent with the clinical course of the underlying disease and chemotherapy administered during the study. Hemorrhagic events occurred largely in the context of thrombocytopenia and all three thrombotic events were associated with vascular access catheters.

Five events (rigors, arthralgia, back pain, headache and hypertension) were reported at least 10% more frequently in the PEG-rHuMGDF group than in the placebo group. However, 14 events were reported at least 10% more frequently in the placebo group compared with the PEG-rHuMGDF group. These included fever, skeletal pain, abdominal pain, anxiety, access pain, and dyspnea, all of which affected at least 25% of placebo subjects (data not shown).

No pattern of distribution of adverse events, severe events, or serious events suggested a relationship with PEG-rHuMGDF treatment.

**Figure 4.** Overall survival for all patients completing three cycles of ICE chemotherapy stratified by dose intensity maintained (ICE × 3 cycles administered ≤ 31 days) versus diminished dose intensity (ICE × 3 administered >31 days). At 8.5 years median follow-up, KM estimates are 67 versus 22%, respectively (P = 0.06).

**Figure 5.** Event-free survival for all patients completing three cycles of ICE chemotherapy stratified by dose intensity maintained (ICE × 3 cycles administered ≤ 31 days) versus diminished dose intensity (ICE × 3 administered >31 days). At 8.5 years median follow-up, KM estimates are 45 versus 11%, respectively (P = 0.05).
antibody evaluations. All patients were analysed for the presence of antibodies to Peg-rHuMGDF at least 4 weeks after the last administration. There were no patients that demonstrated detectable anti-MGDF antibodies in this study.

discussion
We have established that ICE is a highly active pre-transplant cytoreductive regimen for patient with both NHL and Hodgkin’s lymphoma [4, 23]. In NHL, there is a 66–72% overall response rate. Patients achieving a CR have a better outcome than those achieving a PR [4, 22]. It is possible that outcomes are compromised by limitations to dose intensity as a consequence of ICE chemotherapy’s myelotoxicity, despite the use of filgrastim. Thrombocytopenia is the overwhelming cause of dose delay. Thus, ICE chemotherapy was a logical regimen in which to explore the efficacy of a thrombopoietic agent. PEG-rHuMGDF has excellent preclinical efficacy data in murine and primate models [24–27]. In addition, the pharmacokinetic data and the favorable clinical safety profile of PEG-rHuMGDF at the time made it a logical agent to test in combination with ICE chemotherapy. In this study we sought to establish and test a CED of PEG-rHuMDF, comparing the efficacy of daily SC doses of 2.5 and 5.0 µg/kg/day, in the setting of multiple cycles of ICE chemotherapy with filgrastim. At these doses there were shorter intervals between therapy, improved nadir platelet counts and reduced transfusion support without adversely impacting on stem cell mobilization. Unlike murine models [28], we did not see evidence of PEG-rHuMGDF improving peripheral CD34+ cell counts beyond the already efficacious filgrastim.

We found that PEG-rHuMGDF was well tolerated with non-hematological side effects comparable to placebo. The response rate to ICE was similar in all cohorts with placebo patients having a response rate of 75% and PEG-rHuMGDF patients a response rate of 82%. Baseline histology, disease status, prior chemotherapy and/or radiotherapy, remission status, age, gender, ethnicity, Karnofsky performance status, was well balanced between patient cohorts.

In the phase I dose finding component, 39% of cycles of ICE chemotherapy were delayed in the placebo patients versus 17% in the PEG-rHuMGDF supported ICE cycles. Grade III/IV thrombocytopenia was evident in 61 and 33% of patients receiving placebo versus 19 and 5% in the PEG-rHuMGDF patients. Finally, 32% of placebo patients received platelet transfusions versus none of the PEG-rHuMGDF patients. There was no statistical difference between the two PEG-rHuMGDF cohorts. However, because the number of CD34+ cells/kg mobilized were superior in the 5 mcg cohort compared to the 2.5 mcg cohort of PEG-rHuMGDF, the study was amended and the 2.5 mcg/kg cohort was dropped. Nevertheless, we combined all PEG-rHuMGDF supported cycles of ICE chemotherapy to evaluate its effects on platelets because of the lack of a difference in the 2.5 and 5 mcg/kg cohorts in this regard.

In thirty-eight patients evaluable for all phases of platelet recovery, we found that the addition of PEG-rHuMGDF to the ICE chemotherapy treatment program allowed for a statistically significant improvement in all of the major platelet count recovery data, including the incidence of grade IV thrombocytopenia, days of thrombocytopenia where the platelet count was <30K (data not shown), and median platelet nadir. Importantly, 38% of patients who received placebo received platelet transfusions versus only 9% of patients receiving PEG-rHuMGDF. There was no difference in any mobilization endpoint between placebo treated patients versus PEG-rHuMGDF treated patients.

Perhaps the most important issue for improvement in EFS is day 14 platelet count recovery and subsequent treatment on time. A number of retrospective analyses suggest that maintenance of dose intensity and dose density in the initial treatment program for a patient with aggressive lymphoma is associated with improved survival [12, 13, 29, 30]. This concept, based on the Norton–Simon gompertzian kinetics model of tumor growth [31], has now found validation in a number of prospective randomized studies in both breast cancer and aggressive lymphoma [14, 32]. This issue has not been directly addressed in the salvage chemotherapy setting. Only 44% of ICE chemotherapy cycles were administered on schedule for the placebo supported cycles versus 77% in the PEG-rHuMGDF supported cycles (P = 0.05). Interestingly, patients receiving ICE chemotherapy as planned (e.g. ‘on-time’, duration 31 days or less) had a significant improvement in EFS (45 versus 11%, P = 0.05) and OS advantage (67 versus 22%, P = 0.06). As expected, this data is only important if chemosensitive disease is established and patients actually receive the consolidation with ASCT. These findings are hypothesis generating, given the small numbers and the variable nature of subset analysis. Nevertheless, they suggest the potential for survival benefit with maintenance of dose-density in the second-line setting; clearly, prospective studies will need to ascertain whether these differences merely reflect better baseline biology and functional status, allowing on time delivery of therapy, or if supportive measures to maintain schedule truly confer a survival advantage.

It is important to acknowledge that this was a phase I–II dose finding study and only 41 patients were enrolled. However, the results are provocative and should have led to a larger phase II/III multi-institutional trial. Unfortunately, further clinical trials with PEG-rHuMGDF were suspended secondary to the induction of anti-platelet neutralizing antibodies seen in a small number of normal platelet donors,[33, 34] No patient in this study developed antibodies to PEG-rHuMGDF. Nonetheless, this trial demonstrates a potential strategy for integrating a thrombopoietic agent into a multi-cycle chemotherapy program with mitigation of platelet related toxicity.

The effect of PEG-rHuMGDF on platelet counts recovery appeared to be more significant following cycles two and three of ICE. This is in accord with the in vitro animal models that suggest the kinetics of the rise in the peripheral platelets is delayed. This has been seen in other clinical trials using a single cycle of PEG-rHuMGDF. Thus, in this multiple cycle regimen, it is possible that some of the benefit following the second cycle of ICE was a result of the drug given after the first cycle of ICE. This would suggest that it might be beneficial to administer a course of PEG-rHuMGDF prior to the first cycle of a multiple cycle regimen.

Ultimately, clinical development of PEG-rHuMGDF ceased with the identification of neutralizing antibodies as a significant
toxicity. A new promising agent, AMG 531, is a novel Mpl ligand that was developed to specifically overcome the problem of cross-reacting antibodies by implementing random peptide sequences to activate the Mpl receptor. This molecule consists of an Mpl binding domain and carrier Fc domain, and it is capable of stimulating megakaryopoiesis similarly to thrombopoietin, but lacks any sequence homology to native thrombopoietin. Preclinical and clinical studies have demonstrated effectiveness and safety [35], with reported studies in healthy volunteers and in ITP [36]. There have been no safety signals to date and trials integrating this novel agent into the ICE regimen for relapsed and refractory NHL are currently underway.

acknowledgements

Dr. Janice Gabriole received research support from federal grant K24CA100287. The Mortimer Lacher Fund of the Lymphoma Foundation: We would like to thank the nurses and patients who made this work possible.

references


