Megakaryocyte Growth and Development Factor: A Review of Early Clinical Studies

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ABSTRACT
Megakaryocyte growth and development factor (MGDF), a Mpl ligand, recently entered clinical trials worldwide and has been demonstrated to have potent biological activity. MGDF administration causes a dose-dependent increase in platelet count but no effect on white cell count or hematocrit. These platelets are morphologically and functionally normal. When administered following moderately myelosuppressive chemotherapy, MGDF significantly enhances platelet recovery, although scheduling in relation to chemotherapy may be important in optimizing the full effects. MGDF mobilizes progenitor cells of multiple hematopoietic lineages, and may enhance the effects of filgrastim on peripheral blood progenitor cell levels after chemotherapy. MGDF is well tolerated and does not cause toxicity similar to that observed with other thrombopoietic cytokines. Numerous studies are under way to help determine the precise role of MGDF in clinical practice. The Oncologist 1997;2:311-318

INTRODUCTION
The term thrombopoietin (TPO) was first coined in 1958 by Keleman to describe a humoral substance which appeared to regulate platelet production [1]. Keleman found that the urine, serum and plasma of animals and patients with severe thrombocytopenia could increase platelet numbers when injected into normal animals [2]. The successful cloning of TPO followed a long and fruitless attempt to purify the molecule from plasma, serum, urine and conditioned culture media with various cell lines [3-6].

The initial breakthrough in the search for TPO came with the discovery of a murine myeloproliferative oncogene, v-mpl [7]. That this was a finding of great serendipity was realized with the recognition that the cellular homologue of v-mpl, termed c-mpl, encoded the cytoplasmic portion of a membrane protein expressed on hematopoietic cells, predominantly those of megakaryocytic lineage [8, 9]. A critical observation was that the addition of c-mpl antisense oligonucleotides to cultured CD34+ cells selectively prevented the generation of megakaryocyte colonies [9]. The flurry of activity searching for the ligand to c-mpl came to a climax in 1994, with the publication of the sequence of the cDNA [10-15]. Mpl ligand has subsequently been shown to be the central physiological regulator of megakaryocytopoiesis and platelet production, and is now referred to in its natural form as thrombopoietin [16].

Human TPO is a 60-70 kDa, glycosylated protein which is primarily produced in the liver and kidneys [10, 17]. It consists of 332 amino acids, is highly conserved between species, and has 23% homology with human erythropoietin (EPO) [18]. Megakaryocyte growth and development factor (MGDF) is a truncated protein, with homology with the EPO-like aminoterminus of human TPO [10, 19]. It has identical biological properties to TPO in vitro [19-21] and in vivo [22, 23], suggesting that the EPO-like domain contains all the required elements to bind and activate c-mpl. However, MGDF has a short circulatory half-life, which can be markedly increased when coupled to polyethylene glycol [24]. The pegylated form (PEG-rHuMGDF) is approximately ten times more potent in vivo than the unconjugated polypeptide [24]. Clinical trials with PEG-rHuMGDF commenced in Australia and the United States in May 1995.

PRECLINICAL STUDIES
Mpl ligand acts as a megakaryocyte growth and differentiation/maturatation factor [20, 25-30]. The pivotal role of the
cytokine in platelet production is demonstrated by the marked reduction in platelet levels in c-mpl-deficient mice to 5% to 15% of littermate controls. Because platelet counts in the deficient animals are detectable, it is obvious that other thrombopoietic cytokines, such as interleukin 3 (IL-3), IL-6, IL-11 and stem cell factor, play a minor role in platelet production.

Further evidence of the regulatory role of Mpl ligand in thrombopoiesis is demonstrated by the finding of an inverse relationship between platelet count and serum Mpl ligand in animals and humans [20, 34-37]. As platelet counts recover, TPO levels decrease in parallel. Interestingly, production of TPO appears to be regulated by platelet mass, rather than at the transcriptional level [38-40], and it is unlikely that the rate of TPO gene expression is altered in response to physiological stimuli [16]. Platelet c-mpl has a high affinity for the ligand [41, 42], and once bound, the ligand is internalized and degraded [43].

Mpl ligand increases platelet sensitivity to a number of agonists in vitro [44-49], but no alteration in platelet function is detectable following in vivo administration [23].

The in vivo physiological effects of Mpl ligand have been studied in a number of animal models. In mice, daily administration of MGDF produced a profound increase in marrow and splenic megakaryocyte progenitors (CFU-Mk) and a three- to fourfold increase in platelet numbers [22, 24, 50-52]. This effect was lineage-specific, with no noticeable effect on red or white cell counts. Similarly, in nonhuman primates platelet levels up to 10 times baseline were observed after 10 days of daily administration of MGDF [23, 50, 53, 54]. In baboons, PEG-rHuMGDF produced dose-dependent increases in the circulating platelet count over a dose range from 0.05 to 2.5 µg/kg/day [23, 53]. Log-dose dependent increases in bone marrow megakaryocyte numbers, volume and ploidy were recorded [53]. Toxicity in these animal studies was minimal, and few side effects could be ascribed to the cytokine.

The ability of Mpl ligand to alleviate thrombocytopenia has been studied in detail. In mice given ablative doses of chemotherapy either alone or with radiation, daily injections of MGDF resulted in abrogation of severe thrombocytopenia and a significant reduction in mortality [22, 24]. No effect was observed on other cell lineages. However, when MGDF or TPO were combined with G-CSF, neutrophil recovery was also markedly enhanced [55-57]. In murine models of progenitor cell transplantation, the period of severe thrombocytopenia after transplant was substantially reduced by MGDF [58-60]. In primates, treatment-induced thrombocytopenia was prevented with administration of MGDF [54, 55, 61, 62] or TPO [63]. In these studies, both the platelet nadir and duration of thrombocytopenia were abrogated.

**CLINICAL STUDIES WITH MGDF**

**Methods**

We assessed the safety and biological activities of PEG-rHuMGDF in a series of phase 1 studies. The initial trials were randomized, double-blinded and placebo controlled. In the first study [64-66], 17 patients with advanced cancer received either PEG-rHuMGDF (13 patients) or placebo (4 patients) alone prior to chemotherapy. PEG-rHuMGDF was administered by daily s.c. injection in sequential cohorts of 0.03 (n = 3), 0.1 (n = 3), 0.3 (n = 4) and 1.0 (n = 3) µg/kg/day. The placebo and lower three dose schedules were given for 10 days, whereas the highest dose was given for six, seven, and nine days because of early thrombocytosis.

In the second study [67], PEG-rHuMGDF or placebo (3:1 ratio) was given with filgrastim after carboplatin 600 mg/m² and cyclophosphamide 1,200 mg/m² to 41 patients (Table 1, Table 2). Fifteen patients who had participated in the pre-chemotherapy trial received the same study drug and dose after chemotherapy. Study drug was given daily for up to 21 days at doses of 0.03, 0.1, 0.3 and 1.0 µg/kg/day commencing on the day after chemotherapy. In the cohorts receiving 1, 3 or 5 µg/kg/day, the duration of PEG-rHuMGDF administration was shortened to seven days because of asymptomatic thrombocytosis in the previous dose levels. Filgrastim 5 µg/kg/day was given until neutrophil recovery. In the absence of intolerable toxicity or disease progression, patients were able to receive the chemotherapy in 28-day intervals. After the second and subsequent cycles of chemotherapy, PEG-rHuMGDF was not given. The functional characteristics of PEG-rHuMGDF produced platelets, and the mobilization of peripheral blood progenitor cells was assessed in detail in both studies.

**Platelet Counts**

**Pre-chemotherapy**

A dose-dependent increase in platelet counts was observed following the administration of PEG-rHuMGDF (Fig. 1), although considerable individual variation in response was seen. Patients receiving 0.3 and 1 µg/kg PEG-rHuMGDF had increases in platelet counts ranging from 51% to 584%. However, an increase in bone marrow megakaryocytes by up to 1.8-fold was observed in all PEG-rHuMGDF cohorts. The platelet numbers began to increase from day 6 and continued to rise despite cessation of PEG-rHuMGDF. The peak count was reached between days 12 and 18, and platelets returned to normal between days 22 and 30. The rise in platelet counts in the lower dose cohorts was considerably less pronounced. No effects were observed on absolute neutrophil count or hematocrit.
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These observations are similar to those of Vadhan-Raj et al. [68], who gave a single dose of glycosylated TPO alone prior to chemotherapy. They reported elevated platelet counts of up to 212% and the kinetics of thrombocytosis were consistent with the findings in our study.

Post-chemotherapy

The doses of chemotherapy delivered in our study were moderately myelosuppressive. Administration of PEG-rHuMGDF significantly enhanced platelet recovery when given in “effective” doses (0.3 to 5 µg/kg/day). In these patients (n = 25), the platelet nadir occurred significantly earlier than in the placebo group (Fig. 2A), analogous to the effect of G-CSF on neutrophil recovery following chemotherapy [69]. PEG-rHuMGDF did not influence the depth of the platelet nadir, but rather shortened the time to recovery of pre-treatment platelet count (median 17 days versus 22 days for the placebo group) (Fig. 2B). PEG-rHuMGDF did not influence neutrophil recovery or red cell toxicity. Antibodies to PEG-MGDF were not detected in any patients.

A hint to the importance of scheduling of PEG-rHuMGDF was given by the observation that faster platelet recovery occurred in five patients who received PEG-rHuMGDF 0.3 or 1.0 µg/kg/day both before and after chemotherapy, compared with that of nine patients given 1.0 µg/kg/day only after chemotherapy. In addition, recovery of platelet

| Table 1. Baseline characteristics of 41 patients with advanced cancer administered placebo plus filgrastim or PEG-rHuMGDF plus filgrastim after chemotherapy |
|---------------------------------|-----------------|-----------------|
| Placebo                        | PEG-rHuMGDF     |
| Number of patients             | 10              | 31              |
| Median age (range)             | 59 (36-69)      | 57 (20-74)      |
| Male/Female                    | 7/3             | 22/9            |
| Median Karnofsky performance status (range) | 85 (70-100) | 90 (60-100) |
| Tumor type                     |                 |                 |
| Non-small cell lung            | 3               | 12              |
| Carcinoma of unknown primary   | 2               | 4               |
| Gastric                        | 2               | 2               |
| Kidney                         | 1               | 2               |
| Small cell lung                | 1               | 1               |
| Carcinoid                      | 1               | 0               |
| Other*                         | 0               | 10              |
| Prior chemotherapy             | 1               | 6               |
| Prior radiotherapy             | 1               | 5               |
| Median (range) baseline platelet count \(\times 10^9/\text{l}\) | 270 (139-370) | 273 (148-487) |

*aIncludes ovary, thyroid, esophagus, liver, colon, melanoma, bladder, pancreas. Reproduced with permission [67].

| Table 2. Non-hematological adverse events in 41 patients who received study drug |
|---------------------------------|-----------------|
| Placebo + filgrastim (n = 10)   | PEG-rHuMGDFb + filgrastim (n = 31) |
| **Local**                       |                 |
| Injection site reaction         | 0 (0)*          | 2 (6)           |
| **Systemic**                    |                 |
| Lethargy/drowsiness             | 1 (10)          | 17 (55)         |
| Hot flushes/fever†              | 6 (60) [1]†     | 8 (26)          |
| Bone pain                       | 3 (30)          | 8 (26)          |
| **Neurological**                |                 |
| Headache                        | 6 (60)          | 13 (42)         |
| Dizziness                       | 3 (30)          | 12 (38)         |
| **Respiratory**                 |                 |
| Cough                           | 1 (10)          | 8 (26)          |
| Dyspnea                         | 3 (30)          | 12 (38) [3]     |
| **Gastrointestinal**            |                 |
| Nausea and/or vomiting          | 9 (90) [1]      | 22 (71) [4]     |
| Diarrhea                        | 3 (30)          | 15 (48) [2]     |
| Mucositis                       | 5 (50)          | 8 (26)          |
| **Metabolic**                   |                 |
| Hypokalemia                     | 2 (20)          | 5 (16)          |
| **Thrombo-embolic**             |                 |
| Thrombophlebitis                | 0 (0)           | 1 (3)           |
| Pulmonary embolism              | 0 (0)           | 1 (3) [1]†      |

*There was no difference in the frequency of any toxicity between patients given placebo and those given PEG-rHuMGDF (Fisher’s exact test).
†Number of patients (percentage).
‡Not related to neutropenia.
§Numbers in square brackets represent number of events that were grade 3.
¶This event was grade 4.

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count to normal following the second cycle of chemotherapy was significantly reduced in those patients who had received PEG-rHuMGDF after the first cycle compared to the placebo group (21 versus 24 days; \( p = 0.013 \)). This difference was greater in patients who experienced a shorter interval between cessation of PEG-rHuMGDF and commencement of the second cycle of chemotherapy. These observations have led to further studies investigating the potential benefit in administering PEG-rHuMGDF prior to chemotherapy.

The results from our studies contrast to those of Fanucchi et al., who administered PEG-rHuMGDF alone after mildly myelosuppressive chemotherapy (paclitaxel and carboplatin) [70]. Fifty-three patients with non-small cell lung cancer were treated. At all dose levels of PEG-rHuMGDF, the nadir of the platelet count was higher and the time to recovery of baseline platelet count shorter than that observed with placebo. No dose response to PEG-rHuMGDF was observed.

**Platelet Function**

We assessed platelet function during PEG-rHuMGDF administration by measures of platelet aggregation and release of adenosine triphosphate (ATP) in vitro in response to a variety of standard agonists, and analyzed platelet surface activation markers, as described elsewhere [65]. The previously reported observations that recombinant forms of Mpl ligand increase sensitivity of platelets to aggregating agents were not borne out by our study. There were no significant changes in aggregation response or ATP release between baseline measurements and repeated testing during and after PEG-rHuMGDF administration when given alone [65], or when given with filgrastim after chemotherapy [67]. Furthermore, there were no changes in coagulation parameters. The platelets produced by PEG-rHuMGDF were morphologically normal by light- and electron-microscopy, and platelet activation markers did not change over the duration of the study. In addition, in a patient given aspirin because of asymptomatic thrombocytosis (platelet peaking at \( 1,876 \times 10^9 /l \)), platelets responded with the expected inhibition of aggregation response and ATP release.

**Peripheral Blood Progenitor Cell (PBPC) Mobilization**

Given the expression of the c-mpl on a number CD34+ cells, the observations of progenitor cell mobilization in preclinical models [71], and the unexpected observations during phase 1 studies of G-CSF [72], we assessed the levels of PBPC, when given alone and when used in combination with filgrastim after chemotherapy.

**Pre-chemotherapy**

In contrast to the lineage-dominant effect of PEG-rHuMGDF on mature cell populations, multilineage mobilization of PBPC occurred in patients given MGDF. However, there was a preferential increase in blood levels of megakaryocyte progenitors (Meg-CFC) compared to myeloid (GM-CFC)
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and erythroid (BFU-E) progenitor cells. Mobilization of Meg-CFC was observed in patients given ≥0.1 µg/kg of PEG-rHuMGDF, but GM-CFC and BFU-E increased only at doses ≥0.3 µg/kg. Furthermore, the degree of mobilization appeared to be related to the dose of PEG-rHuMGDF [66].

An interesting observation was that the kinetics of progenitor cell release from the marrow were unlike that of other lineage-dominant cytokines, such as G-CSF. Following administration of the latter, PBPC levels rise almost immediately, peak at day 5 or 6, and fall when G-CSF is ceased. However, PEG-rHuMGDF resulted in a late and sustained rise in progenitor cells, so that increased levels were first detected only on day 8, and were generally greater on day 12, despite discontinuation of the cytokine several days earlier.

**Post-chemotherapy**

Administration of PEG-rHuMGDF in doses of 0.3 to 5.0 µg/kg combined with filgrastim after chemotherapy significantly enhanced mobilization of PBPC compared to placebo plus filgrastim. Furthermore, higher peak levels of PBPC were observed with increasing dose of PEG-rHuMGDF. In the 5 µg/kg cohort, levels of GM-CFC were up to 1,000-fold greater than in the placebo group (Fig. 3).

**Safety**

**Pre-chemotherapy**

Importantly, PEG-rHuMGDF was associated with minimal toxicity. One episode of mild superficial thrombophlebitis which resolved spontaneously was seen in a patient with a platelet count of 694 × 10^9/l. There were no changes in performance status, vital signs or body weight during the study, and there were no changes in biochemical, renal or liver function tests. Unlike other, less potent thrombopoietic cytokines, there was no evidence of induction of an acute phase response.

**Post-chemotherapy**

Two patients developed thrombo-embolic events after treatment with PEG-rHuMGDF, although in both instances the platelet counts at the time were low and no abnormalities in platelet function or surface marker expression were present. Eleven patients developed a platelet count of greater than 1,000 × 10^9/l in the recovery phase post-chemotherapy without clinical sequelae. There were no significant differences in non-hematological toxicity between the groups who received PEG-rHuMGDF compared to placebo.

The low incidence of adverse effects due to PEG-rHuMGDF was consistent with the report from Fanucchi [70]. Glycosylated TPO was also associated with minimal toxicity in the study of Vadhan-Raj et al. [68].

**CONCLUSION**

MGDF is an important new hematopoietic cytokine which potently stimulates the production of functionally and morphologically normal platelets in a lineage-specific manner. It significantly reduces the time to platelet recovery following myelosuppressive chemotherapy and is able to mobilize large numbers of multilineage peripheral blood progenitor cells, both as a single agent and also when given following chemotherapy in concert with filgrastim. Importantly, MGDF is well tolerated and associated with very little significant toxicity.

The potential uses of MGDF include the treatment of disease- and therapy-related severe thrombocytopenia. The most apparent clinical states in which this might apply are
acute leukemia and bone marrow or peripheral blood progenitor cell transplantation. MGDF may also permit the maintenance of dose intensity in multicycle chemotherapy regimens. The lack of toxicity and potent biological activity of MGDF suggest it will be safe in healthy volunteers to enhance the efficiency of platelet or progenitor cell donation. MGDF also has potential for use in other causes of thrombocytopenia, such as HIV infection, liver disorders, surgery, myelodysplasia and auto-immune platelet destruction.

However, before these clinical directions can be explored, there remain questions with regard to the optimum dose and schedule of MGDF. For example, we are currently investigating the effects of different dose and schedules of PEG-rHuMGDF when administered prior to chemotherapy [73]. Given the rapid introduction of MGDF into clinical trials following its discovery, the results of further studies that help to define the role of MGDF are eagerly awaited.

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