

EFFECTS OF POLYETHYLENE GLYCOL-CONJUGATED RECOMBINANT HUMAN MEGAKARYOCYTE GROWTH AND DEVELOPMENT FACTOR ON PLATELET COUNTS AFTER CHEMOTHERAPY FOR LUNG CANCER

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ABSTRACT

Background Polyethylene glycol (PEG)-conjugated recombinant human megakaryocyte growth and development factor (MGDF, also known as PEG-rHuMGDF), a recombinant molecule related to thrombopoietin, specifically stimulates megakaryopoiesis and platelet production and reduces the severity of thrombocytopenia in animals receiving myelosuppressive chemotherapy.

Methods We conducted a randomized, double-blind, placebo-controlled dose-escalation study of MGDF in 53 patients with lung cancer who were treated with carboplatin and paclitaxel. The patients were randomly assigned in blocks of 4 in a 1:3 ratio to receive either placebo or MGDF (0.03, 0.1, 0.3, 1.0, 3.0, or 5.0 μg per kilogram of body weight per day), injected subcutaneously. No other marrow-active cytokines were given.

Results In the 38 patients who received MGDF after chemotherapy, the median nadir platelet count was 188,000 per cubic millimeter (range, 68,000 to 373,000), as compared with 111,000 per cubic millimeter (range, 21,000 to 307,000) in 12 patients receiving placebo ($P=0.013$). The platelet count recovered to base-line levels in 14 days in the treated patients as compared with more than 21 days in those receiving placebo ($P<0.001$). Among all 40 patients treated with MGDF, 1 had deep venous thrombosis and pulmonary embolism, and another had superficial thrombophlebitis.

Conclusions MGDF has potent stimulatory effects on platelet production in patients with chemotherapy-induced thrombocytopenia. (N Engl J Med 1997; 336:404-9.)

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THROMBOCYTOPENIA is a complication of chemotherapy that can increase the risk of hemorrhage,¹ necessitate platelet transfusions, and limit the doses of myelotoxic agents. Platelet transfusions can prevent bleeding, but infectious and allergic complications² and refractoriness due to alloimmunization reduce their usefulness.¹ For these reasons, a specific stimulator of platelet production could have important clinical applications.

Thrombopoietin, the recently isolated and cloned ligand for the cytokine receptor Mpl,³⁻¹⁰ is the key hormone regulating the development of megakaryo-

cytes.³⁻¹² When hematopoietic progenitor cells are incubated with thrombopoietin, they develop into megakaryocytes, which release morphologically and functionally normal platelets.¹³ Thus, thrombopoietin supports all stages of platelet production in vitro. Evidence of the in vivo role of thrombopoietin includes the inverse relation between plasma thrombopoietin levels and platelet or megakaryocyte mass,^{11,12} the reduced numbers of megakaryocytes and platelets (to 15 percent of the normal counts) in mice lacking the gene for Mpl^{14,15} or thrombopoietin,¹⁶ and potent thrombocytopenic activity.^{3,4,7,17-23}

Polyethylene glycol-conjugated recombinant human megakaryocyte growth and development factor (MGDF, also known as PEG-rHuMGDF) is a polypeptide related to thrombopoietin that contains the receptor-binding N-terminal domain of thrombopoietin. The polypeptide has 163 amino acids and is conjugated with polyethylene glycol on the N terminal by reductive alkylation. MGDF is a potent stimulator of megakaryocyte maturation and platelet production in vitro,^{11,13} and it increases platelet production and platelet counts in normal animals.^{17,20} It is approximately 10 times more potent in vivo than the unconjugated polypeptide,^{18,24} and it reduces the severity of thrombocytopenia in animal models of myelosuppression.^{18,21-23}

We conducted a clinical trial of the safety and biologic effects of various doses of MGDF in patients receiving chemotherapy with carboplatin and paclitaxel for non-small-cell lung cancer. These chemotherapeutic agents have well-characterized profiles of efficacy^{25,26} and toxicity²⁷ and present a low risk of complicating infection.

METHODS

Patients

Adults with advanced (stage III or IV) non-small-cell lung cancer were enrolled if they were eligible for the study and gave informed consent. This study was approved by the internal review

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boards of the participating institutions. The eligibility criteria included adequate performance status (a Karnofsky score of at least 60) and adequate bone marrow function (neutrophil count, at least 1500 per cubic millimeter; platelet count, from 150,000 to 450,000 per cubic millimeter; and hemoglobin, at least 10 g per deciliter [133 μmol per liter]), and liver function (serum bilirubin, below 2.0 mg per deciliter [34 μmol per liter]). Patients were excluded from the study if they had a history of arterial or venous thrombosis, ischemic vascular disease, brain metastases, or had received extensive radiotherapy (to more than 30 percent of bone marrow volume) or any cytotoxic chemotherapy.

Study Design

Patients were randomly assigned in blocks of 4 in a 1:3 ratio to receive either placebo or MGDF at a specified dose (0.03, 0.1, 0.3, 1.0, 3.0, or 5.0 μg per kilogram of body weight per day). When all four patients in a cohort completed 21 days of study, the dose could be increased in the subsequent cohort. The study was designed to identify the dose of MGDF that would maintain a platelet count after chemotherapy of at least 80 percent of the base-line count, with grade III or IV (as defined by the World Health Organization²⁸) drug-related adverse events occurring in less than one third of patients. The personnel at the sites and the study monitors were not aware of the drug assignments.

In the preliminary phase of the study, either the study drug or placebo was injected subcutaneously each day for up to 10 days, followed by a 4-day observation period before chemotherapy. Beginning on the day after chemotherapy, the study drug or placebo was administered daily for a maximum of 16 consecutive days, or until the platelet count increased to at least 600,000 per cubic millimeter. Shorter schedules of administration (seven days and three days) were also tested.

MGDF (Amgen, Thousand Oaks, Calif.) was more than 95 percent pure as determined by reverse-phase high-performance liquid chromatography and was negative for endotoxin. The placebo vials contained vehicle only.

Cancer Chemotherapy

Chemotherapy was administered on day 1 after premedication with dexamethasone, antihistamines, and antiemetics. The dose of carboplatin (Paraplatin) was adjusted on the basis of the measured 24-hour creatinine clearance to give a predicted area under the curve of the serum concentration plotted against time of 9 mg per milliliter times the number of minutes.²⁷ Paclitaxel (Taxol) was administered immediately after carboplatin at a dose of 175 mg per square meter of body-surface area over a three-hour period. Filgrastim (recombinant human granulocyte colony-stimulating factor [G-CSF]) was not administered. A second, identical cycle of chemotherapy was administered on day 22 to patients whose symptoms were stable or improved.

Study Evaluations

After each injection of MGDF or placebo, the patients were monitored for at least two hours. The platelet count, mean platelet volume, white-cell count, and differential count were measured daily. Serum biochemistry and plasma coagulation (prothrombin time, partial-thromboplastin time, fibrinogen, and fibrin-split products) were measured before, during, and after the administration of MGDF or placebo. Platelet counts were obtained on days 8 and 15 of cycle 2. Antibodies to MGDF were assessed in a blinded manner by a specific radioimmunoassay that detects them at a dilution of at least 1:40,000 (≤ 15 ng per milliliter) and does not react with antibodies to other hematopoietic cytokines.

Statistical Analysis

Descriptive statistical analyses were performed. Data are expressed as medians and ranges unless otherwise specified. The

patients treated with MGDF were compared with those receiving placebo by Fisher's exact test in the case of categorical data (with adjustment for multiple comparisons if necessary), the Wilcoxon rank-sum test in the case of continuous data, and Kaplan-Meier analysis and the log-rank test for data on the recovery of platelets.²⁹

RESULTS

Patients

A total of 53 patients were enrolled in the study, 40 in the MGDF group and 13 in the placebo group. MGDF and placebo were administered to three patients each before chemotherapy. Among these six patients studied in the preliminary phase, three (two in the MGDF group and one in the placebo group) did not continue the study into the post-chemotherapy phase. The remaining three patients (one assigned to 0.03 μg of MGDF per kilogram per day and two assigned to placebo) were studied in both phases. An additional 47 patients (37 in the MGDF group and 10 in the placebo group) received the study drug only after chemotherapy. There were no significant differences between the groups with regard to the stage of lung cancer, sex ratio, age, Karnofsky performance status, or base-line platelet count (Table 1).

Hematologic Effects

Platelet counts rose in two of the three patients assigned to MGDF before chemotherapy (to 849,000 platelets per cubic millimeter in the patient assigned to the 0.03- μg dose and to 1,010,000 platelets

TABLE 1. BASE-LINE CHARACTERISTICS OF THE 53 PATIENTS WITH NON-SMALL-CELL LUNG CANCER ACCORDING TO STUDY GROUP.

| CHARACTERISTIC | PLACEBO (N = 13) | MGDF (N = 40) | P VALUE |
|---|---------------------|------------------|---------|
| Cancer stage — no. of patients (%) [*] | | | 0.54 |
| III | 5 (38) | 20 (50) | |
| IV | 8 (62) | 20 (50) | |
| Karnofsky performance score — no. of patients (%) [†] | | | 0.89 |
| 60 | 0 | 2 (5) | |
| 70 | 2 (15) | 4 (10) | |
| 80 | 5 (38) | 12 (30) | |
| 90 | 6 (46) | 21 (52) | |
| 100 | 0 | 1 (2) | |
| Age — yr | | | 0.24 |
| Median | 66 | 58 | |
| Range | 48–73 | 32–78 | |
| Sex — no. of patients (%) | | | 1.00 |
| Male | 9 (69) | 27 (68) | |
| Female | 4 (31) | 13 (32) | |
| Platelet count — cells $\times 10^{-3}/\text{mm}^3$ | | | 0.45 |
| Median | 297 | 386 | |
| Range | 226–521 | 184–543 | |

^{*}Stages were as described by Feld et al.³⁰

[†]Karnofsky scores were as described by Feld et al.³⁰

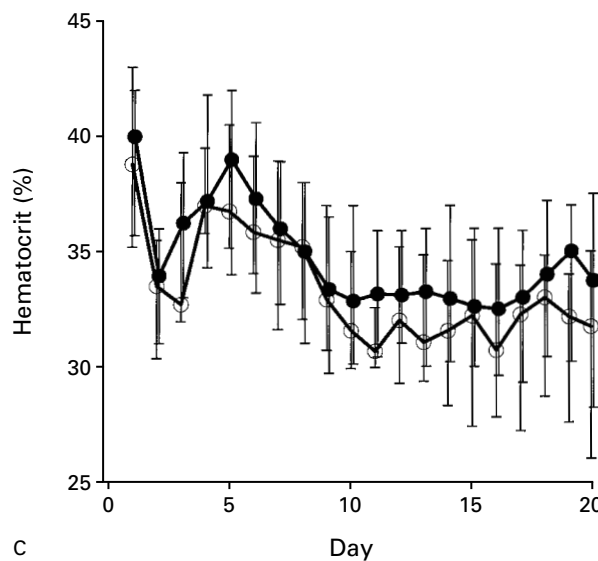
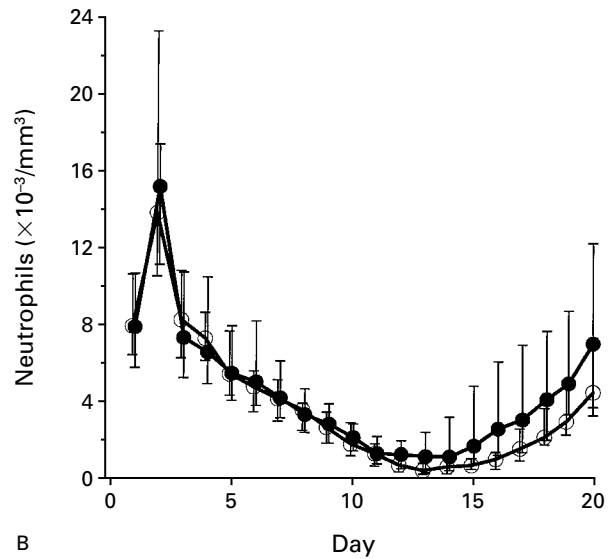
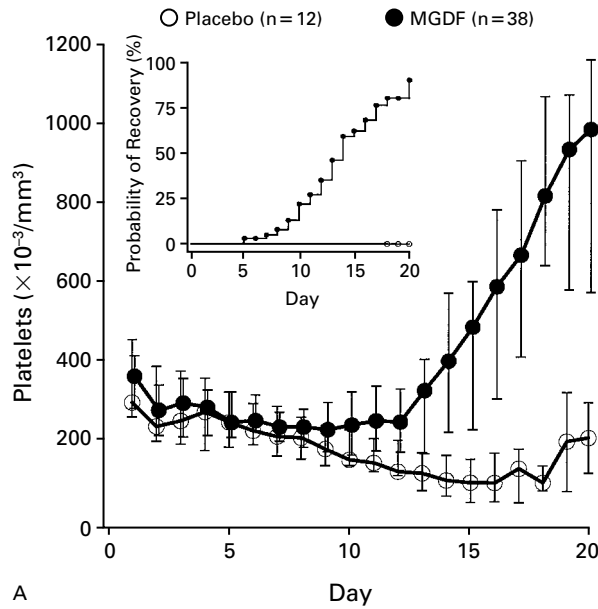


Figure 1. Median Platelet Count (Panel A), Neutrophil Count (Panel B), and Hematocrit (Panel C) on Each Study Day in the 50 Patients Given MGDF or Placebo after Chemotherapy with Carboplatin and Paclitaxel.

The bars indicate interquartile ranges. The inset in Panel A shows the Kaplan–Meier estimate of the probability of returning to the base-line platelet count after chemotherapy.

per cubic millimeter in the patient assigned to the 0.1- μg dose). These two patients were therefore not included in the post-chemotherapy phase of the study.

The nadir of the platelet count after chemotherapy in the patients given placebo was lower than in the patients given MGDF, at all the doses tested (Fig. 1A and Table 2). The median nadir platelet count was 188,000 per cubic millimeter (range, 68,000 to 373,000) in the MGDF group, as compared with 111,000 per cubic millimeter (range, 21,000 to 307,000) in the placebo group ($P=0.013$). One patient who was given placebo received a transfusion of platelets for hemoptysis and thrombocytopenia (platelet count, 21,000 per cubic millimeter). The nadir of the platelet count occurred earlier in the MGDF group than in the placebo group (median interval to the nadir, 7 vs. 15 days; $P<0.001$).

The platelet counts returned to base line more rapidly after treatment with MGDF than after the administration of placebo (Fig. 1A, inset). The median time needed to return to the base-line platelet count was more than 21 days in the placebo group and was 14 days in the MGDF group ($P<0.001$).

In the patients treated with MGDF, the platelet count rose progressively over the 20 days of observation, reaching a median peak of 692,000 per cubic millimeter (range, 231,000 to 1,890,000) by day 20. The platelet count increased to more than 1 million per cubic millimeter in 10 patients treated with MGDF. In the patients receiving at least 1.0 μg of MGDF per kilogram per day, the proportion who had platelet counts exceeding 1 million per cubic millimeter appeared to decrease with shorter schedules of administration (Table 2). In the placebo

TABLE 2. DOSES AND SCHEDULES OF THE STUDY DRUG AND RESPONSES OF THE PLATELET COUNT IN 50 PATIENTS AFTER CHEMOTHERAPY.

| STUDY DRUG AND DOSE | NO. OF PATIENTS | PLATELET COUNT ($\times 10^{-3}/\text{mm}^3$) | | | NO. OF PATIENTS WITH >1 MILLION PLATELETS/ mm^3 | DAY OF NADIR COUNT | DAY OF PEAK COUNT | DAY OF RETURN TO BASE-LINE COUNT* |
|------------------------------|-----------------|---|---------------|-----------------|--|--------------------|-------------------|-----------------------------------|
| | | BASE LINE | NADIR | PEAK† | | | | |
| | | median (range) | | | | | | |
| Placebo | 12 | 310 (226–521) | 111 (21–307) | 330 (236–574) | 0 | 15 (13–21) | 1 (1–21) | >21 |
| MGDF‡ | | | | | | | | |
| All doses | 38 | 386 (184–543) | 188 (68–373) | 692 (231–1890) | 10 | 7 (2–16) | 18 (1–20) | 14 |
| 0.03 μg | 5 | 364 (274–518) | 224 (68–279) | 538 (274–583) | 0 | 6 (3–15) | 2 (1–20) | 17 |
| 0.1 μg | 3 | 478 (424–484) | 285 (230–307) | 673 (493–675) | 0 | 4 (2–15) | 18 (17–20) | 16 |
| 0.3 μg | 3 | 411 (266–413) | 163 (122–346) | 757 (411–985) | 0 | 6 (5–12) | 18 (1–20) | 11 |
| 1.0 μg | 7 | 391 (216–543) | 186 (89–246) | 1269 (449–1650) | 4 | 9 (6–14) | 18 (1–20) | 13 |
| 1.0 μg for 7 days | 3 | 402 (313–490) | 252 (137–373) | 802 (313–1070) | 1 | 8 (2–15) | 17 (1–18) | 14 |
| 3.0 μg for 7 days | 6 | 357 (213–452) | 180 (68–326) | 987 (231–1890) | 3 | 3 (2–16) | 17 (7–18) | 14 |
| 3.0 μg for 3 days | 5 | 328 (184–379) | 184 (93–274) | 841 (405–985) | 0 | 6 (5–13) | 19 (2–20) | 13 |
| 5.0 μg for 3 days | 6 | 366 (233–509) | 157 (106–287) | 797 (476–1157) | 2 | 9 (3–12) | 19 (14–20) | 13 |

*Medians estimated by Kaplan–Meier analysis are shown.

†Values shown are the peak platelet counts reached before day 22 of the cycle.

‡Doses of MGDF are given in micrograms per kilogram of body weight per day. Doses for which no duration of treatment is specified were given for a maximum of 16 consecutive days or until the platelet count increased to at least 600,000 per cubic millimeter.

group, the platelet count was highest on day 1. There was no dose-related effect on either the neutrophil count (median nadir in the placebo group, 250 per cubic millimeter; in the MGDF group, 67 per cubic millimeter; $P=0.075$) (Fig. 1B) or the hematocrit (Fig. 1C).

Safety

Among the 53 enrolled patients, the adverse events observed were consistent with the effects of the underlying lung cancer and of the chemotherapy (Table 3). The most common adverse events were disturbances of the gastrointestinal system, primarily nausea, which were reported by 78 percent of the MGDF group and 77 percent of the placebo group, and disturbances of the musculoskeletal system, primarily arthralgia, which were reported by 62 percent and 69 percent of the respective groups. No immediate local or systemic reactions were noted after the injections of MGDF, nor did MGDF affect body weight, vital signs, serum chemistry, prothrombin time, partial-thromboplastin time, fibrin-split products, or fibrinogen levels.

In one patient treated after chemotherapy with 3 μg of MGDF per kilogram per day for seven days, deep venous thrombosis and pulmonary embolism developed on day 15, when the platelet count was 243,000 per cubic millimeter (subsequent maximal count, 772,000 per cubic millimeter, on day 18). The thrombosis resolved when heparin was given. Superficial thrombophlebitis of the saphenous vein developed on day 12 (platelet count, 468,000 per cubic millimeter) in a patient treated with 1 μg of

MGDF per kilogram per day. The condition resolved with rest and aspirin. No episodes of superficial or deep venous thrombosis were observed in the placebo group. Another patient had a diffuse, pruritic grade 2 maculopapular rash that resolved rapidly after treatment with antibiotics and MGDF was discontinued.

Serum samples obtained before and after the administration of the study drug were tested for antibodies to MGDF. None were positive.

In 8 patients given placebo and 23 patients given MGDF, platelet counts were measured during the second cycle of chemotherapy, when neither MGDF nor placebo was administered. On day 8 of that cycle, the median platelet counts were 290,000 per cubic millimeter (range, 169,000 to 354,000) in the patients assigned to placebo and 332,000 per cubic millimeter (range, 134,000 to 741,000) in the patients assigned to MGDF ($P=0.35$). On day 15 these counts were 101,000 per cubic millimeter (range, 42,000 to 241,000) and 90,000 per cubic millimeter (range, 30,000 to 297,000), respectively ($P=0.65$).

DISCUSSION

MGDF had potent stimulatory effects on the production of platelets in patients treated with carboplatin and paclitaxel for advanced lung cancer. At all doses and schedules of MGDF used, the nadir of the platelet count was higher, and the time to the recovery of the base-line platelet count shorter, than with placebo. The stimulation of platelet production was not accompanied by symptoms, clinical signs, or laboratory evidence of inflammation or activation of the

TABLE 3. FREQUENCY OF CLINICALLY RELEVANT NONHEMATOLOGIC ADVERSE EVENTS IN THE 53 PATIENTS ENROLLED IN THE STUDY.*

| ADVERSE EVENT | PLACEBO (N = 13) | MGDF (N = 40) |
|-----------------------------------|---------------------|------------------|
| | no. of patients (%) | |
| Local | | |
| Reaction at injection site | 0 | 0 |
| Systemic | | |
| Fatigue | 5 (38) | 13 (32) |
| Febrile neutropenia | 1 (8) | 1 (2) |
| Rigors | 1 (8) | 3 (8) |
| Flushing | 2 (15) | 6 (15) |
| Arthralgia, myalgia, or bone pain | 9 (69) | 25 (62) [4] |
| Generalized pain | 2 (15) | 6 (15) |
| Dermatologic | | |
| Generalized rash | 0 | 1 (2) |
| Neurologic | | |
| Headache | 2 (15) | 4 (10) |
| Dizziness | 4 (31) | 5 (12) |
| Insomnia | 4 (31) | 4 (10) |
| Anxiety | 1 (8) | 5 (12) |
| Gastrointestinal | | |
| Nausea | 5 (38) | 24 (60) [2] |
| Vomiting | 2 (15) | 20 (50) [3] |
| Diarrhea | 4 (31) | 5 (12) [1] |
| Abdominal pain | 3 (23) | 5 (12) |
| Respiratory | | |
| Dyspnea | 3 (23) [1] | 2 (5) [1] |
| Hemoptysis | 2 (15) | 1 (2) |
| Chest pain | 3 (23) | 2 (5) |
| Thrombotic | | |
| Thrombophlebitis | 0 | 1 (2) |
| Pulmonary embolism | 0 | 1 (2) [1] |

*Numbers in brackets indicate the numbers of patients with events of grade 3 severity. No grade 4 events were observed. There was no significant difference by Fisher's exact test in the frequency of any adverse event between the patients given placebo and those given MGDF.

acute-phase response, adverse effects common when other investigational cytokines are used to treat thrombocytopenia.³¹⁻³⁵ No substantial effects were observed on neutrophil counts or on the hematocrit.

To facilitate the identification of any toxic effects of MGDF and avert the need to use other hematopoietic growth factors (such as G-CSF), we administered doses of chemotherapy that would cause relatively mild systemic effects and be associated with a low risk of febrile neutropenia (although the dose of carboplatin was higher than those commonly given). These considerations necessarily limited the number of instances of clinically significant thrombocytopenia in the study. Only one patient, in the placebo group, required a transfusion of platelets. The value of MGDF in preventing severe thrombocytopenia and averting platelet transfusions in patients undergoing myeloablative chemotherapy will require further study.

Femoral-vein thrombosis and pulmonary embolism developed in one patient treated with MGDF who had a normal but rising platelet count. A second patient treated with the drug had self-limited,

superficial thrombophlebitis of the saphenous vein and an elevated platelet count. These events may have been related to the treatment, but patients with cancer are predisposed to thrombotic complications,³⁶ and chemotherapy may increase the risk.³⁷

In another phase I study,^{38,39} the aggregation of platelets was studied before and after the administration of MGDF and placebo, because various recombinant Mpl ligands have been reported to sensitize platelets to agonist-induced aggregation in vitro.⁴⁰⁻⁴² No evidence was found of an effect of the clinical administration of MGDF on platelet function. Furthermore, in baboons treated with MGDF the degree of in vivo deposition of platelets on thrombogenic surfaces was proportional to the platelet count.²⁰ It is interesting to note that platelets bear receptors for other cytokines, including G-CSF⁴³ and stem-cell factor,⁴⁴ both of which enhance the agonist-induced aggregation of platelets in vitro.^{43,44} Nevertheless, thrombosis has not been identified as a drug-related adverse event during either extensive clinical trials of filgrastim⁴⁵ or the investigational use of recombinant human stem-cell factor.⁴⁶ These data indicate that the in vitro effects of G-CSF and stem-cell factor on platelets may be of limited clinical relevance. Larger clinical studies will therefore be needed to determine the relation, if any, between thrombosis and treatment with thrombocytopenic agents.

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