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REVIEW ARTICLE

Romiplostim:A Novel c-Mpl/CD110 Receptor Ligand for the Management of Idiopathic Thrombocytopenic Purpura

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ABSTRACT

Idiopathic Thrombocytopenic Purpura (ITP) is an autoimmune disease characterised predominantly by an antibody mediated destruction of platelets. Few treatment options are available for ITP like corticosteroids, IV immunoglobulins, splenectomy, rituximab, danazole and cyclophosphamide. All these treatment options work by reducing the destruction of platelets by antibody. However recent evidence suggests that production of platelet is reduced in 75% of thrombocytopenic patient with ITP. Romiplostim is a novel thrombopoetin receptor agonist approved by USFDA in August 2008 that increases the platelet count in ITP patients unresponsive to corticosteroids, immunoglobulins or splenectomy. Romiplostim is not currently available in India. The addition of Romiplostim in the treatment options for ITP will surely improve the outcomes in ITP patients.

Key Words

Romiplostim, Thrombopoetin receptor agonist, Idiopathic Thrombocytopenic Purpura

Key Message

Romiplostim is novel thrombopoesis stimulating peptibody found to be potentially useful in treatment of ITP where corticosteroids, immunoglobulins or splenectomy has failed. There are less incidences of development of autoantibodies against Romiplopstim as found in other agents of its class.

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Introduction

Idiopathic thrombocytopenic purpura, also known as primary immune thrombocytopenic purpura, is an autoimmune disorder that is characterized predominantly by antibody-mediated platelet destruction [1],[2].The clinical manifestations of ITP are highly variable and range from complete lack of symptoms to frank haemorrhage from any site, the most serious of which is intracranial[3].

Corticosteroids, intravenous immunoglobulins, splenectomy, rituximab, danazol and cyclophosphamide are the available current therapy which primarily focuses on reduction of this platelet destruction [4]. If the patient's situation is not life threatening, corticosteroids are the standard initial treatment[5]. Intravenous immunoglobulins are generally recommended for patients with critical bleeding and for those who are unresponsive to corticosteroids[5]. The platelet count can also be supported by the administration of anti-D immunoglobulin, which is active in 70–75% of Rh-positive patients in the pre splenectomy setting[6]. Splenectomy is traditionally considered to be the second line of treatment in adults with ITP, in whom achieving a normal safe platelet count with initial corticosteroid therapy has failed. For those who are refractory to or who relapse after splenectomy, there is a long list of available approaches like rituximab, danazol and

immunosuppressive agents, but immunosuppressive agents are associated with an increased risk of infection[7].

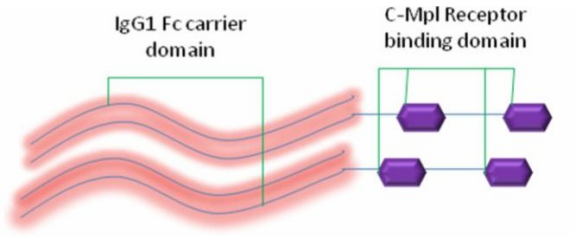
However, recent evidence suggests that decreased platelet production might also have a role in ITP [8]. For example, kinetic studies have shown that platelet production is not increased in over 75% of thrombocytopenic patients with chronic ITP, which is contrary to expectations, [9],[10] and thrombopoietin concentrations are normal or near normal in patients with this disease[11],[12],[13] Moreover, anti platelet antibodies inhibit the in-vitro growth of megakaryocyte precursor cells and bone marrow megakaryocytes in ITP, which can be apoptotic[14]. Often, current therapies aimed at the reduction of platelet destruction are either ineffective or poorly tolerated. Therefore, treatments targeting the increasing platelet production alone, or in combination with existing therapies, provide an opportunity to improve outcomes in patients with this chronic disease [15].

After the identification and isolation of thrombopoietin and the thrombopoietin receptor c-Mpl in the process of platelet production in 1994, two agents, recombinant human thrombopoietin rHuTPO and rHu- MGDF, underwent study in a multitude of settings. Although initially promising action, the development of these agents was halted in 1998 after the emergence of neutralizing antibodies that cross-reacted with endogenous TPO, producing consistent thrombocytopaenia in healthy subjects[16].

Romiplostim is a novel thrombopoiesis stimulating peptibody that binds to and activates the human thrombopoietin receptor despite having no sequence homology with human thrombopoietin[16],[17]. Due to the different sequences of amino acids with respect to endogenous TPO in romiplostim, there is no risk of producing neutralizing antibodies that cross-reacted with endogenous TPO[18]. FDA approved romiplostim in 2008 for the treatment of thrombocytopenia in patients with chronic immune (idiopathic) thrombocytopenic purpura, who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy[19]. It is available only through a restricted distribution program called the NEXUS (Network of Experts Understanding and Supporting *Nplate* and Patients) Program. Only prescribers and patients registered with the program are able to prescribe, administer and receive the product [19].

Chemistry

Romiplostim is a peptibody of approximately 60 kDa [20]. It is engineered mainly to prevent the problem of cross reacting antibodies[17]. It consists of a disulphide-bonded human IgG1 heavy chain and kappa light chain constant regions (an Fc fragment) with two identical peptide sequences linked covalently at residue 228 of the heavy chain with the use of polyglycine [Table/Fig 1] [20]. The Mpl receptor binding domain stimulates megakaryopoesis in the same way as thrombopoietin and the carrier Fc component of the molecule binds to the FcRn salvage receptor and undergoes endothelial recirculation, resulting in a substantially longer half time than the peptide alone. The Fc portion increases the half life of the compound to around 20 hours [20]. The Mpl receptor binding was selected by screening the libraries of peptides having no similarity with that of thrombopoietin, but with a tertiary structure that helps to bind with the receptor [21]. This non similarity between romiplostim and thrombopoetin helps in getting the non production of cross reacting antibodies.



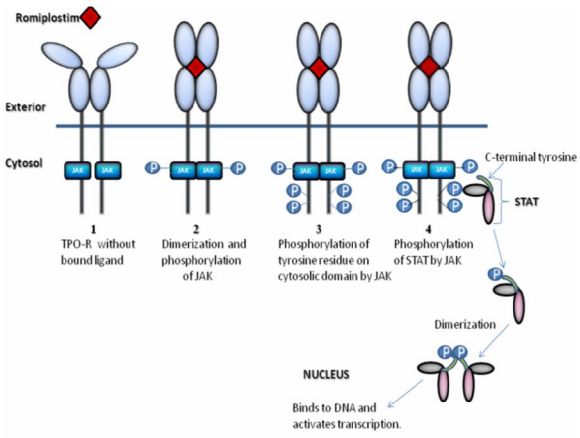
(Table/Fig 1) Diagrammatic representation Romiplostim

Mechanism of action

Romiplostim stimulates platelet production by a mechanism similar to that of endogenous TPO; despite having different sequences of amino acids than TPO[16],[17]. So, to understand the mechanism of action of romiplostim, it is

necessary to understand the physiology of TPO. Thrombopoietin (TPO) is a growth factor that regulates thrombopoiesis by promoting the viability and growth of megakaryocyte colony-forming cells. *In vitro*, TPO stimulates both early megakaryocyte development such as megakaryocytic colony forming units (MK-CFU), as well as late maturation such as the number, size and ploidy of megakaryocytes[22].

TPO acts through a TPO receptor (TPO-R), also referred to as c-Mpl or CD110, which is a member of the type 1 cytokine receptor superfamily[23]. TPO-R is located on haematopoietic stem cells, megakaryocytic progenitor cells (MK-CFU) and platelets[16]. TPO –R stimulation activates the JAK-STAT pathway and it ultimately stimulates the differentiation of megakaryocytes from the earlier progenitor cells, which leads to increased platelet production [Table/Fig 2] [24].



(Table/Fig 2) Molecular mechanism of Romiplostim

Pharmacokinetics

Systemic exposure (C_0 and AUC_{0-t}) to romiplostin after intravenous administration increases more than proportionally with dose. This correlates with the fact of target mediated disposition. Romiplostim binds to c-Mpl on platelets and other cells in the thrombopoiesis linage such as megakaryocytes, and is subsequently internalized and degraded inside these cells. The mean elimination half life is short and increases with the dose (1.5, 2.4 and 13.8 hours for doses of 0.3,1 and 10 mcg/kg, respectively). In the long term extension study in patients with chronic ITP who received a weekly treatment of Romiplostim subcutaneously over the dose range of 3 to 15 mcg/kg, peak serum concentrations were observed around 7 to 50 hrs post dose (median:3.5 days). Serum concentrations varied among the patients and did not correlate with the dose administered [19], [25]. The elimination of Romiplostim is governed by c-Mpl receptors. Thus, patients with higher platelet counts are associated with lower serum concentrations and vice versa. In another ITP clinical study, no accumulation in serum romiplostim concentrations was observed after six weekly doses of 3 mcg/kg of romiplostim[19], [21]. PK/PD relationships between the Romiplostim dose and platelet counts in ITP patients showed a linear tendency between the baseline normalized platelet count ratio and the dose administered. ITP patients are more sensitive to Romiplostim than the healthy subjects [15], [26], [27]. Also, the subcutaneous route of Romiplostim is a convenient, effective and well tolerated treatment option for ITP[28].

Clinical studies

Two clinical studies provided the major data assessing the effects of Romiplostim among patients with chronic ITP[29]. Study 1 enrolled patients who had not undergone splenectomy and Study 2 enrolled patients who were refractory to splenectomy. Both the studies used randomized (2:1; active: placebo), double-blind, placebo controlled designs with the enrollment of patients who were thrombocytopaenic despite prior therapy with at least one prior ITP medication. Patients were exposed to the study drug for six months with weekly measurement of platelet counts. At the end of the study, patients were observed for another 12 weeks without administration of the study drug.

The primary endpoint was "durable platelet response," defined as at least six weekly platelet counts ≥ 50,000/mcL during the last eight weeks of the drug treatment study, in the absence of "rescue medications" at any time during the 24 week treatment period. The major secondary endpoints involved various comparisons of platelet count "responses" (defined as any weekly platelet count ≥ 50,000/mcL) and comparison of the use of thrombocytopaenia

"rescue medications." During the first 12 weeks of the study, investigators could decrease or eliminate the use of any concomitant ITP medications, based upon the observed platelet counts. The baseline characteristics of enrolled subjects were similar between the randomized groups, with most subjects having received multiple prior ITP medications. Within the dataset pool of both studies, 11 patients had received a single prior ITP medication (2 in the placebo group and 9 in the Romiplostim group). In both studies, statistically significant differences were observed for the primary and secondary endpoints, as shown [Table/Fig 3] [15],[18],[30].

(Table/Fig 3) Study 1 and Study 2 proving efficacy of Romiplostim

Outcome	Study 2 (splenectomy)		Study 1 (no splenectomy)		p-value*
	Placebo n = 21	Romiplo-stim n = 42	Placebo n = 21	Romiplo-stim n = 41	
Durable platelet response, n (primary EP)	0 (0%)	16 (38%)	1 (5%)	25 (61%)	< 0.01
Overall platelet response, n	0 (0%)	33 (79%)	3 (14%)	36 (88%)	< 0.01
Weeks with platelet response, mean (SD)	0.2 (0.5)	12.3 (7.9)	1.3 (3.5)	15.2 (7.5)	< 0.01
Subjects requiring rescue medication, n	12 (57%)	11 (26%)	13 (62%)	7 (17%)	< 0.01
Subjects with durable platelet response with "stable dose", n	0 (0%)	13 (31%)	0 (0%)	21 (51%)	< 0.01

*p-value was similar for each study; "stable dose" was defined as a dose maintained within ± 1 mcg/kg during the last 8 weeks of treatment. (30)

Other notable efficacy findings included the number of subjects who were able to discontinue all baseline concomitant ITP medications, as shown in [Table/Fig 4]. Overall, an increase in platelet counts from the baseline by ≥ 20,000/mcL at any time point in either study (exclusive of eight weeks following a rescue medication), was achieved by approximately 90% of all subjects receiving Romiplostim and approximately by 30% of subjects receiving placebo. After a median of approximately 39 weeks of Romiplostim therapy in the long term extension study, patients continued to maintain platelet count responses in a pattern similar to those achieved during the 24 weeks of the two phase 3 clinical studies.

Thus, Romiplostim was well tolerated and increased and maintained platelet counts in splenectomised and non-splenectomised patients with ITP. Many patients were able to reduce or discontinue other ITP medications[15],[18].

(Table/Fig 4) Incidence of Concurrent ITP Medication Discontinuation from Baseline

	Study 2 (splenectomy)		Study 1 (no splenectomy)	
	Placebo	Romiplostim	Placebo	Romiplostim
Number of subjects receiving con ITP med at baseline	6	12	10	11
Subjects with con ITP meds discontinued at week 13	0/6	5/12	3/10	2/11
Subjects with con ITP meds discontinued at week 25	0/6	8/12	3/10	4/11

Indication

- Currently, Romiplostim is indicated for the treatment of thrombocytopaenic patients with chronic ITP.
- In patients of ITP who are nonsplenectomized but have inadequate response or intolerance to corticosteroids and/or immunoglobulins.
- In patients of ITP who are splenectomized but have insufficient response to spelenectomy [30].
- Romiplostim should be used only in patients with ITP where there is an increased risk of bleeding and no attempt should be made to normalize platelet counts of normal individuals[29],[31].

Dosage Regimen

The initial dose for Romiplostim is 1 mcg/kg, based on the actual body weight. Then the dose is adjusted on a weekly basis by increments of 1 mcg/kg until the patient achieves a platelet count ≥ 50 x 10^9/L or as necessary to reduce the risk for bleeding. The maximum weekly dose is 10mcg/kg. During Romiplostim therapy, CBCs, including platelet count and peripheral blood smears should be assessed weekly until a stable platelet count (≥ 50 x 10^9/L for at least 4 weeks without dose adjustment) has been achieved. Monitoring of CBCs including platelet counts and peripheral blood smears should continue for one month thereafter.

- If the platelet count is < 50 x 10^9/L, increase the dose by 1 mcg/kg.
- If the platelet count is > 200 x 10^9/L for 2 consecutive weeks, reduce the dose by 1 mcg/kg.
- If the platelet count is > 400 x 10^9/L, do not dose. Continue to assess the platelet count weekly. After the platelet count has fallen to

< 200 x 10^9/L, Romiplostim should be resumed at a dose reduced by 1 mcg/kg.

Romiplostim should be discontinued if the platelet count does not increase to a level sufficient to avoid clinically important bleeding after 4 weeks of Romiplostim therapy at the maximum weekly dose of 10mcg/kg. Monitoring of CBCs including platelet counts, should continue weekly for at least 2 weeks following the discontinuation of Romiplostim [19].

Adverse Drug Reactions

Adverse drug reactions of Romiplostim (incidence <5%) as per study reports 1 and 2 were dizziness, insomnia, myalgia and abdominal pain .Headache and arthralgia were reported in both Romiplostin as well as in the placebo arm [19]

In the phase-1 study, the most frequently reported adverse events are confusion, ecchymosis or both, which occurred in 67% of 24 patients [17].

Safety Concerns Over The Following Side Effects Are Yet To Be Determined

- Reticulin formation and risk for marrow fibrosis

In the phase 3 study, one event (serious) was reported for a patient who received Romiplostim. This subject was a 40 year old man participating in Study 2 who had a history of "reticulin fibrosis" at the time of enrollment for the study [31].

- Risk for malignancy or progression of malignancy

In the two phase 3 studies, only seven adverse events for "neoplasia" were reported, five in the placebo group (n = 41) and two in the Romiplostim group (n = 84). The two neoplasms in the Romiplostim group consisted of a basal cell carcinoma in one subject and a B cell lymphoma in another subject [31].

- Thrombotic risk

Within the pool of the two phase 3 studies, only three thrombotic events were reported.

Overall, within the safety dataset of 204 patients with chronic ITP, a total of 14 patients experienced thrombotic events following Romiplostim initiation, inclusive of the uncontrolled exposure [31].

- Alteration of intrinsic TPO/worsening of thrombocytopaenia after cessation of Romiplostim therapy

In the phase 1 and 2 studies, (n = 57 patients receiving Romiplostim), four subjects experienced a decrease in platelet counts below the pretreatment baseline levels. All counts approximated baseline levels within 14 days of the thrombocytopaenia onset [31].

- Immunogenicity

Overall, 17/204 (8%) of patients exposed to Romiplostim developed binding antibodies against the drug and 9/204 (4%) developed binding antibodies against TPO. No patient developed neutralizing antibodies to TPO. One patient with chronic ITP developed neutralizing antibodies to Romiplostim in the open-label, extension study [31].

Conclusion

Romiplostim is a novel drug that can be used effectively in ITP where corticosteroids, immunoglobulins and splenectomy are not successful. It marks an era of a new biological therapy directed towards the production of thrombocytes in ITP patients. It proves to be beneficial in preventing an emergency situation like bleeding in patients with ITP.

Abbreviations

ITP: - Idiopathic thrombocytopenic purpura
TPO: - Thrombopoietin
c-Mpl: - Murine myeloproliferative leukaemia proto-oncogene
rHuTPO: - Recombinant human thrombopoietin
rHu- MGDF: - Recombinant human megakaryocyte growth and differentiation factor

MK-CFU: - Megakaryocytic-colony forming units
SD:-Standard deviation
CBC: -Complete blood count
AUC: - Area under curve

References

[1]. George JN, El-Harake MA, Raskob GE. Chronic idiopathic thrombocytopenic purpura. N Engl J Med 1994; Nov 3; 331(18): 1207–11.

[2]. Karpatkin S. Autoimmune (idiopathic) thrombocytopenic purpura. Lancet 1997; May 24; 349(9064): 1531–6.

[3]. Cohen YC, Djulbegovic B, Shamai-Lubovitz O, Mozes B. The bleeding risk and natural history of idiopathic thrombocytopenic purpura in patients with persistent low platelet counts. Arch Intern Med 2000; Jun 12; 160 (11): 1630-8.

[4]. George JN, Kojouri K, Perdue JJ, Vesely SK. Management of patients with chronic, refractory idiopathic thrombocytopenic purpura. Semin Hematol 2000; Jul; 37(3): 290–8.

[5]. George JN, Woolf SH, Raskob GE, Wasser JS, Aledort LM, Ballem PJ et al. Idiopathic thrombocytopenic purpura: a practice guideline developed by explicit methods for the American Society of Hematology. Blood 1996; Jul 1 ; 88(1): 3–40.

[6]. Scaradavou A, Woo B, Woloski BM, Cunningham-Rundles S, Ettinger LJ, Aledort LM et al. Intravenous anti-D treatment of immune thrombocytopenic purpura: experience in 272 patients. Blood 1997; Apr 15; 89 (8): 2689-700.

[7]. Stasi R, Provan D. Management of immune thrombocytopenic purpura in adults. Mayo Clin Proc 2004; Apr; 79 (4): 504-22

[8]. McMillan R, Wang L, Tomer A, Nichol J, Pistillo J. Suppression of in vitro megakaryocyte production by antiplatelet autoantibodies from adult patients with chronic ITP. Blood 2004; Oct 23; 103(4): 1364–9.

[9]. Heyns AD, Lotter MG, Badenhorst PN, Kock FD, Pieters H, Herbst C et al. Kinetics and sites of destruction of 111Indium-oxine-labeled platelets in idiopathic thrombocytopenic purpura: a quantitative study. Am J Hematol 1982; 12(2): 167–77.

[10]. Ballem PJ, Segal GM, Stratton JR, Gernsheimer T, Adamson JW, Slichter SJ. Mechanisms of thrombocytopenia in chronic autoimmune thrombocytopenic purpura. Evidence of both impaired platelet production and increased platelet clearance. J Clin Invest 1987; Jul; 80(1): 33–40.

[11]. Nichol JL. Thrombopoietin levels after chemotherapy and in naturally occurring human diseases. Curr Opin Hematol 1998; May; 5(3): 203–8.

[12]. Kosugi S, Kurata Y, Tomiyama Y, et al. Circulating thrombopoietin level in chronic immune thrombocytopenic purpura. Br J Haematol 1996; 93(3): 704–6.

[13]. Emmons RV, Reid DM, Cohen RL, et al. Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte defi ciency and low when due to increased platelet destruction. Blood 1996; 87: 4068–71.

[14]. Houwerzijl EJ, Blom NR, van der Want JJ, Esselink MT, Koornstra JJ, Smit JW et al. Ultrastructural study shows morphologic features of apoptosis and para-apoptosis in megakaryocytes from patients with idiopathic thrombocytopenic purpura. Blood 2004; Jan 15; 103(2): 500–6.

[15]. Kuter DJ, Bussel JB, Lyons RM, Pullarkat V, Gernsheimer TB, Senecal FM et al. Efficacy of romiplostim in patients with chronic immune thrombocytopenic purpura: a double-blind randomized controlled trial. Lancet 2002; Feb 2; 371: 395–403.

[16]. Kuter DJ. New thrombopoietic growth factors. Blood 2007; Jun 1; 109(11): 4607–16

[17]. Bussel JB, Kuter DJ, George JN, Mcmillan R, Aledort LM, Conklin GT et al. AMG 531, a thrombopoiesis-stimulating protein, for chronic ITP. N Engl J Med 2006; Oct 19; 355(16): 1672–81.

[18]. Bussel JB, Kutter DJ, Pullarkat V, Lyons RM, Guo M, Nichol JL. Safety and efficacy of long term treatment with romiplostim in thrombocytopenic patients with chronic ITP. Blood [serial online] 2008 Nov 3 [cited 2009 Jan 28]: Available from: URL: http://bloodjournal.hematologylibrary.org/cgi/content/abstract/blood-2008-04-150078v1

[19]. Food and drug administration. Highlights of prescribing information of romiplostim [Online]. 2008 Aug [cited 2009 Jan 28]; 1,2,6,8. Available from: URL: http://www.fda.gov/cder/foi/label/2008/125268lbl.pdf

[20]. Peetersk K, Stassen JM, Collen D, Geet CV, Freson K. Emerging treatments for thrombocytopenia: Increasing platelet production. Drug discovery today 2008; Sep;13(17/18):798-806

[21]. Wang B, Nichol JL, Sullivan JT. Pharmacodynamics and pharmacokinetics of AMG 531, a novel thrombopoietin receptor. Clin Pharmacol Ther 2004; 76 (6): 628-38

[22]. Stasi R, Evangelista ML, Amadori S. Novel thrombopoietic agents: A review of their use in idiopathic thrombocytopenia purpura. Drugs. 2008; 68(7):901-12.

[23]. Methia N, Louache F, Vainchenker W, Wendling F. Oligodeoxynucleotides antisense to the proto-oncogene c-mpl specifically inhibit in vitro megakaryopoiesis. Blood 1993; Sep 1; 82(5):1395-401.

[24]. Lodish H, Berk A, Kaiser CA, Krieger M, Scott MP, Bretscher A et al. Molecular cell

biology. 6th ed. New York: W. H. Freeman and Company; 2008. P. 672-6.
[25]. Clinical Pharmacology and biopharmaceutics Reviews of romiplostim. Food and drug Administration [online].[cited 2009 Jan 28];Available from: URL: http://www.fda.gov/cder/foi/nda/2008/125268s000_ClinPharmR.pdf
[26]. Kumagi Y, Fujita T, Ozaki M, Sahashi K, Ohkura M, Ohtsu T, et al. Pharmacodynamics and pharmacokinetics of AMJ531, a Thrombopoesis Stimulating peptibody, in Healthy Japanese Subjects: A Randomized, Placebo Controlled study. J Clin Pharmacol 2007; 47:1489-97
[27]. Newland A, Caulier MT, Kappers, Klunne M, et al.An open label, unit dose finding study of AMG 531, a novel thrombopoesis stimulating peptibody in patient with ITP. Br J Haematol.2006; 135:547-553
[28]. George J, Bussel JB, Lyons RM, Pullarkat V, Redner R, Terreu D.et al.Self injection of Romiplostim by patients with chronic immune thrombocytopenic Purpura(ITP). Blood [serial online] 2008 Dec [cited 2009 Jan 29]; 112: Available from : URL: http://www.cancercaresouthtexas.com/docs/SelfInjection%20of%20Romiplostim%20by%20Patients%20with%20Chronic%20Immune%20Throbocytopeni.pdf
[29]. Cines DB, Yasothan U, Kirkpatrick P. Romiplostim. Nature Review Drug Discovery 2008 Nov 7:887-88.
[30]. Oncologic drug advisory committee. FDA advisory committee briefing document on romiplostim [Online]. 2008 Mar [cited 2009 Jan 25]; 3,6-9. Available from: URL: http://www.fda.gov/ohrms/dockets/AC/08/briefing/2008-4345b1-01-FDA.pdf
[31]. Broudy VC. & Lin NL. AMG531 stimulates megakaryopoiesis *in vitro* by binding to Mpl. Cytokine 2004 ;25:52–60.