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An open-label, unit dose-finding study of AMG 531, a novel thrombopoiesis-stimulating peptibody, in patients with immune thrombocytopenic purpura

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Abstract

The objective of this open label, phase 1–2, multicentre trial was to evaluate the safety of AMG 531, a novel thrombopoiesis-stimulating peptibody, and its effect on platelet counts in adults with immune thrombocytopenic purpura. Four patients were assigned to each of four unit-dose cohorts: 30, 100, 300 or 500 μg, administered subcutaneously on days 1 and 15 (or day 22 if the day 15 platelet count was $>50 \times 10^9$/l). Safety was assessed by adverse event (AE) monitoring, clinical laboratory studies and antibody assays. Platelet response was defined as a platelet count double the baseline value and between 50 and 450×10^9/l. Sixteen patients (10 women) were enrolled. The 500-μg cohort was discontinued because the first patient's platelet count became unacceptably high. AEs were generally expected and mild or moderate; the most frequent was headache (eight of 16 patients). Two patients experienced serious AEs related to AMG 531 (severe headache and elevated serum lactic dehydrogenase; thrombocytopenia). Platelet responses occurred with all doses and with a dose equivalent to ≥1 μg/kg in eight of 11 patients. In summary, patients tolerated AMG 531 well at the doses tested. No anti-AMG or antithrombopoietin antibodies were detected. Doses equivalent to ≥1 μg/kg increased platelet counts.

Keywords: immune thrombocytopenia, thrombopoietin, AMG 531.

Introduction

Immune thrombocytopenic purpura (ITP) is an autoimmune disorder usually characterised by platelet destruction caused by antiplatelet autoantibodies (Bottiger & Westerholm, 1972; McMillan, 1981; Kelton & Gibbons, 1982; George *et al*, 1995). ITP may also be associated with impaired thrombopoiesis, as demonstrated by data showing that platelet production is either below normal or normal in two-thirds of ITP patients (Heyns *et al*, 1982, 1986; Stoll *et al*, 1985; Ballem *et al*, 1987), rather than above normal as might be expected given low platelet counts.

As ITP in adults is usually a chronic condition with few spontaneous remissions, the goal of treatment is not to cure but to maintain a haemostatically safe platelet level. Treatment is required for those with persistently low counts, recurrent, and spontaneous bleeding episodes and in those undergoing a procedure likely to cause blood loss. There is a lack of good quality comparative clinical trial data to help guide treatment, but the reviews from the American Society of Haematology and the British Committee for Standards in Haematology provide good solid guidelines to aid decision making (George *et al*, 1996; Provan *et al*, 2003).

Currently available treatments for ITP are generally directed towards increasing platelet counts through modulation of the immune system. Treatment with standard doses of corticosteroids, splenectomy or both is generally successful for approximately 60% of patients (Berchtold & McMillan, 1989). Intravenous immunoglobulin and anti-D antibody (WinRho SDF, Wabi Biopharmaceuticals, Boca Raton, FL, USA) are also used if the count needs to be increased rapidly, and both have been used for maintenance therapy. Some 20% of patients will be refractory to first-line therapy, and over a third will relapse following an initial response. A variety of therapies have been tried in these patients, including pulsed dexamethasone, rituximab, vinca alkaloids, azathioprine, cyclophosphamide, danazol, dapsone, ciclosporin A, mycophenolate mofetil, combination chemotherapy and staphylococcal

A immunoadsorption. However, response rates are variable and side effects are often substantial (Berchtold & McMillan, 1989; Siegel *et al*, 1989; George *et al*, 1995; McMillan, 1997; Provan & Newland, 2002).

Rather than modulating the immune system, another therapeutic approach could be to directly stimulate platelet production. Megakaryocytopoiesis is controlled by signalling through the Mpl receptor, a member of the haematopoietic receptor superfamily (Methia *et al*, 1993). The ligand for this receptor, thrombopoietin (TPO), has been established as the primary growth factor in the regulation of platelet production (Debili *et al*, 1995; Sheridan *et al*, 1997; Nichol, 1998). AMG 531, a novel thrombopoiesis-stimulating peptibody, consists of a peptide-binding domain, which stimulates megakaryocytopoiesis in the same way as TPO, and a carrier Fc domain (Wang *et al*, 2004). AMG 531 activates the Mpl receptor to stimulate the growth and maturation of megakaryocytes, and this effect ultimately results in increased production of platelets.

In a double-blind, randomised, placebo-controlled study in healthy volunteers, AMG 531 was well tolerated and produced a dose-dependent increase in platelet counts after a single intravenous or subcutaneous administration of doses ranging from 0·3 to10 μg/kg (Wang *et al*, 2004). The present study was designed to evaluate the safety of unit dosing of AMG 531 and platelet response in patients with ITP.

Methods

Study design

This study was a phase 1–2, open-label, dose-escalation, multicentre trial conducted in Europe. The protocol was reviewed and approved by an independent ethics committee at each investigational centre before any study procedures were initiated. Thrombocytopenic patients with ITP were assigned to one of four sequential dose cohorts: AMG 531, 30, 100, 300 or 500 μg. AMG 531 was supplied as a sterile, frozen liquid in 5·0 mg/ml, single-entry, 1·0 ml glass vials. Frozen liquid was thawed at room temperature before dose administration.

The study consisted of a 2- to 4-week pretreatment period, a 3-week treatment period, and an 8-week post-treatment observation period. Two administrations of the assigned dose were to be given by subcutaneous injection, the first on day 1 of the study and the second on day 15 or 22 if the patient's platelet count was higher than 50×10^9/l on day 15. Rescue medications, including corticosteroids, intravenous immunoglobulin and anti-D immunoglobulin, were permitted if a patient's platelet count fell below 10×10^9/l or if bleeding occurred.

The primary objective of the study was to evaluate the safety of AMG 531. Adverse events (AEs) were monitored throughout the study. Complete blood counts were performed during the pretreatment period; on days 1, 3, 5, 8, 10, 12, 15, 17, 19 and 22 during treatment; and at least weekly during the post-treatment observation period. Blood chemistries were performed before treatment; on days 1, 8 and 15 during treatment; and on day 29 after treatment. Coagulation panels were performed before treatment and on days 8 and 22 during treatment. Development of anti-AMG 531 or anti-TPO antibodies was assessed with a neutralising bioassay (Aledort *et al*, 2004; Wang *et al*, 2004) pretreatment and on days 29 and 78 after treatment.

The secondary objective of the study was to evaluate the efficacy of AMG 531 in elevating platelet counts to a targeted therapeutic level (platelet response). A platelet response was defined as a platelet count that was double the baseline level and between 50 and 450×10^9/l.

Patient eligibility

Eligible patients were adults diagnosed with ITP according to American Society of Hematology guidelines (George *et al*, 1996). Two of three platelet counts taken during the screening and pretreatment period had to be $<30 \times 10^9$/l in patients not receiving ITP therapy or $<50 \times 10^9$/l in patients receiving a constant dose schedule of corticosteroids. Patients with a history of arterial or untreated venous thrombotic disease were excluded from the study, as were those with three or more of the following predisposing factors for thromboembolic events: diabetes, use of oral contraceptives by patients who smoked, hypercholesterolaemia or use of medication for hypertension. Also excluded were patients with active malignancy or bone marrow stem cell disorder and those who had received any treatment for ITP, except for a constant dose schedule of corticosteroids, within 4 weeks prior to the screening visit. All patients gave written, informed consent before any study-specific screening procedures were performed.

Statistical evaluation

Descriptive statistics were used to summarise AEs for each dose cohort and across all cohorts. No formal comparisons were made between cohorts. All other safety assessments were summarised in patient listings. The proportion of patients for whom a platelet response was achieved after each dose was summarised for each cohort and across all cohorts. The proportions of patients with an increase in platelets $\geq 20 \times 10^9$/l over baseline, a platelet count $\geq 100 \times 10^9$/l or a peak platelet count $\geq 450 \times 10^9$/l were also summarised. Platelet count data after the use of rescue medication were not included in these analyses.

Results

Patient enrollment and disposition

Sixteen patients were enrolled in the study and received at least one dose of AMG 531. Fourteen patients received both of the planned injections, with an interval between injections of

approximately 2 weeks in 10 patients and approximately 3 weeks in four patients. When the platelet count of the first patient who received the 500-μg dose increased to 1062×10^9/l following the first dose, this cohort was discontinued, and subsequent patients were assigned to the 300-μg cohort. Thus, four patients were assigned to the 30-μg cohort; four to the 100-μg cohort; seven to the 300-μg cohort; and one to the 500-μg cohort.

Two patients were withdrawn from treatment after the first of the planned injections because of unacceptably high platelet counts: 1536×10^9/l in a patient in the 300-μg cohort and, as noted above, 1062×10^9/l in the patient who received the 500-μg dose. Both patients remained in the study to the end of the observation period. The patient in the 500-μg cohort was included in the analyses of demography, baseline characteristics, and safety, but not in the analyses of efficacy.

Study population

Ten of the 16 patients were women, and 15 of 16 were white; they ranged in age from 20 to 84 years (Table I). All patients had thrombocytopenia, with a median baseline platelet count of $14{\cdot}5 \times 10^9$/l. The median duration of ITP was 8·0 years. Three patients used prednisone during the study, and 13 had undergone splenectomy before study entry.

Safety evaluation

All 16 patients reported at least one AE (Table II); these were generally mild or moderate in severity. Two of four patients with serious AEs had events that were reported as related to AMG 531 treatment (worsening of thrombocytopenia post-treatment with the 300-μg dose, and headache and a transient increase in lactic dehydrogenase with the 500-μg dose). No thrombotic events were reported, including the patients who were withdrawn from treatment because of elevated platelet counts. No deaths and no discontinuations due to AEs occurred during the study.

Table III summarises the AEs with an incidence ≥10% across all dose cohorts. The most frequent AE was headache, which was reported by eight of the 16 patients. AEs were reported infrequently with the 30-μg dose; no dose-related trends were observed in the higher-dose cohorts.

Haemoglobin concentration, white blood cell count, blood chemistry levels, and coagulation values remained stable during the study. No untoward trends in laboratory values were observed. No anti-AMG 531 or anti-TPO antibodies were detected.

Platelet counts

The effect of AMG 531 on platelet counts, by cohort and overall, is shown in Table IV. The protocol-defined therapeutic

Table I. Patients' demographic and baseline characteristics.

	AMG 531 dose				
	30 μg ($n = 4$)	100 μg ($n = 4$)	300 μg ($n = 7$)	500 μg ($n = 1$)	Total ($n = 16$)
Sex, *n* (%)					
Female	3 (75)	2 (50)	4 (57)	1 (100)	10 (63)
Male	1 (25)	2 (50)	3 (43)	0 (0)	6 (38)
Race, *n* (%)					
White	4 (100)	4 (100)	6 (86)	1 (100)	15 (94)
Black	0 (0)	0 (0)	1 (14)	0 (0)	1 (6)
Age (years)					
Median	67·0	38·5	60·0	45·0	50·0
Range	38–69	23–51	20–84	–	20–84
Weight (kg)					
Median	78·8	77·0	68·0	55·0	73·5
Range	74–93	62–91	48–93	–	48–93
Pretreatment platelet count ($\times 10^9$/l)					
Median	10·8	14·6	15·5	28·8	14·5
Range	8·5–22·0	6·0–30·8	6·0–25·3	–	6·0–30·8
Duration of ITP (years)					
Median	17·0	3·5	7·8	13·3	8·0
Range	7·1–27·5	1·9–26·0	4·5–8·3	–	1·9–27·5
Concurrent prednisone, *n* (%)	0 (0)	1 (25)	1 (14)	1 (100)	3 (19)
Prior splenectomy, *n* (%)	3 (75)	2 (50)	7 (100)	1 (100)	13 (81)

ITP, immune thrombocytopenic purpura.

Table II. Overall summary of adverse events (AEs).

	AMG 531 dose				
	30 μg ($n = 4$), *n*	100 μg ($n = 4$), *n*	300 μg ($n = 7$) *n*	500 μg ($n = 1$) *n*	Total ($n = 16$), *n* (%)
All AEs	4	4	7	1	16 (100)
Severe	0	0	2	1	3 (19)
Serious	0	0	3	1	4 (25)
All treatment-related AEs	1	3	3	1	8 (50)
Severe	0	0	1	1	2 (13)
Serious*	0	0	1	1	2 (13)
Discontinuations due to AEs	0	0	0	0	0 (0)
Deaths during study†	0	0	0	0	0 (0)

Investigators used their clinical judgment to assess the severity of an AE. A serious AE was defined as one suggesting a significant hazard or side effect (i.e. an AE that was fatal or life-threatening, required or prolonged hospitalisation or resulted in significant disability or congenital anomaly).
*300-μg cohort, thrombocytopenia and headache; 500-μg cohort, transient increase in lactic dehydrogenase.
†Includes deaths during the 8-week observational period after the last administration of AMG 531.

Table III. Most frequent adverse events (incidence 10% or higher across all doses).

	AMG 531 dose				Total ($n = 16$), *n* (%)
	30 μg ($n = 4$), *n*	100 μg ($n = 4$), *n*	300 μg ($n = 7$), *n*	500 μg ($n = 1$), *n*	
Headache	0	3	4	1	8 (50)
Arthralgia	0	2	3	0	5 (31)
Fatigue	0	1	3	0	4 (25)
Contusion	0	2	2	0	4 (25)
Epistaxis	1	1	2	0	4 (25)
Petechiae	0	2	1	1	4 (25)
Ecchymosis	1	1	1	0	3 (19)
Injection site haemorrhage	1	1	1	0	3 (19)
Peripheral oedema	0	0	3	0	3 (19)
Nasopharyngitis	0	1	2	0	3 (19)
Diarrhoea	1	0	1	0	2 (13)
Mouth haemorrhage	0	0	2	0	2 (13)
Oral mucosal petchiae	0	1	1	0	2 (13)
Back pain	1	0	0	1	2 (13)
Musculoskeletal pain in extremity	0	0	2	0	2 (13)
Haematoma	0	0	1	1	2 (13)
Breast mass (female)*	0	0	1	0	1 (10)
Menorrhagia*	0	1	0	0	1 (10)

*Percentages based on number of female patients for whom data were available for safety evaluation: 30 μg, $n = 3$; 100 μg, $n = 2$; 300 μg, $n = 4$; 500 μg, $n = 1$; total, $n = 10$.

target for platelet response (a level that was double the baseline value and between 50 and 450×10^9/l) was achieved in one of four patients in the 30-μg cohort, in all four in the 100-μg cohort, and in four of seven in the 300-μg cohort after the first injection, the second injection or both injections. Overall, the platelet counts of 12 of 15 patients (80%) increased to $\geq 20 \times 10^9$/l above baseline, and the platelet counts of eight of 15 patients (53%) were $\geq 100 \times 10^9$/l. Mean increases in platelet counts of $>20 \times 10^9$/l were observed by 5 d after the first dose in the 100-μg cohort ($26{\cdot}3 \times 10^9$/l) and in the 300-μg cohort ($43{\cdot}3 \times 10^9$/l). A median of 10 d (range, 5–13 d) elapsed between the day of the first dose and the day of the peak platelet count (Table V). Figure 1 shows the peak platelet counts by unit dose administered.

Achievement of the platelet response therapeutic target was further evaluated after conversion of the unit dose to a weight-

Table IV. Effect of AMG 531 on platelet counts.

	AMG 531 dose			Total
	30 μg ($n = 4$)	100 μg ($n = 4$)	300 μg ($n = 7$)	($n = 16$), n (%)
Protocol-defined platelet response				
Doubling of baseline platelet count, ≥50 and ≤450 × 10^9/l				
First dose	1/4	4/4	4/7	9/15
Second dose	1/4	3/4	3/6	7/14
Both doses	1/4	3/4	3/6	7/14
Neither dose	3/4	0/4	3/7	6/15
Platelet count, ≥20 × 10^9/l above baseline				
First dose	2/4	4/4	5/7	11/15
Second dose	1/4	3/4	4/6	8/14
Both doses	1/4	3/4	3/6	7/14
Neither dose	2/4	0/4	3/7	3/15
Platelet count, ≥100 × 10^9/l				
First dose	1/4	3/4	3/7	7/15
Second dose	0/4	1/4	2/6	3/14
Both doses	0/4	1/4	1/6	2/14
Neither dose	3/4	1/4	3/7	7/15
Peak platelet count, ≥450 × 10^9/l				
First dose	0/4	0/4	1/7	1/15
Second dose	0/4	0/4	0/6	0/14
Both doses	0/4	0/4	0/6	0/14
Neither dose	4/4	4/4	6/7	14/15

Platelet counts associated with rescue medication were excluded.

Table V. Time from first dose to peak platelet count.

	AMG 531 dose			
	30 μg ($n = 4$)	100 μg ($n = 4$)	300 μg ($n = 7$)	Total ($n = 15$)
Median (d)	10·5	9·0	12·0	10·0
Range (d)	8–11	5–10	8–13	5–13

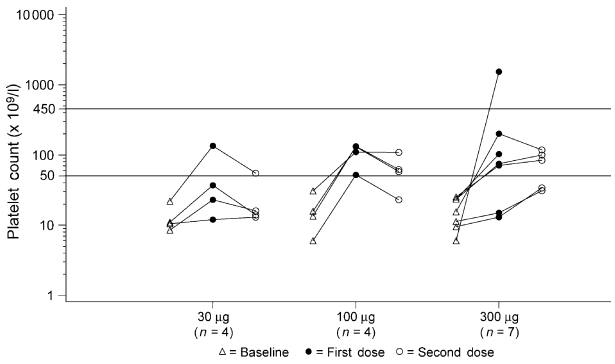

Fig 1. Peak platelet counts by unit dose administered. Platelet counts associated with rescue medication were excluded. The horizontol lines show the targeted platelet range.

based (μg/kg) dose. All patients in the 30-μg cohort received a weight-adjusted dose lower than 1 μg/kg, and no patient in any cohort received a dose higher than 10 μg/kg. The targeted therapeutic response was achieved with the first injection, the second injection or both injections of AMG 531 in eight of 11 patients who received dose-equivalents ≥1 μg/kg. Figure 2 shows peak platelet counts by weight-adjusted dose administered.

Discussion

The results reported in this study support the concept that stimulation of platelet production in ITP is possible in a significant proportion of patients with the condition, and confirmed that the treatment appears to be well tolerated. This opens up a new therapeutic approach in ITP that deserves further exploration.

AMG 531 was well tolerated at all doses tested, and no unexpected safety issues or concerns were identified. The most frequently reported AE was headache. No deaths occurred, and no patient discontinued the study because of AEs. Apart from the anticipated changes in platelet count, no clinically significant changes were detected in any of the blood studies taken during monitoring. No anti-AMG 531 or anti-TPO antibodies

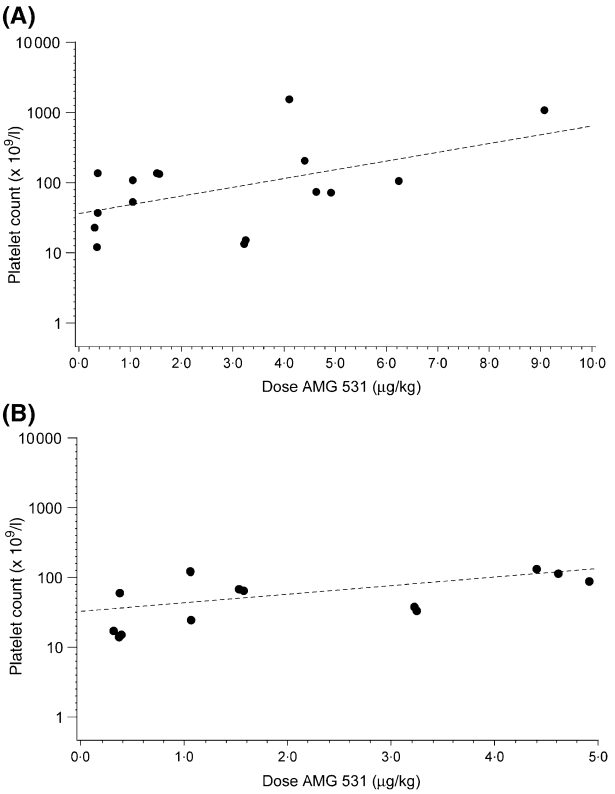

Fig 2. Peak platelet counts by weight-adjusted dose administered. (a) first dose; (b) second dose. Platelet counts associated with rescue medication were excluded.

were detected throughout the study. AMG 531 achieved the targeted platelet response in eight of the 11 patients who received doses equivalent to 1 μg/mg or more.

The standard long-term therapy for ITP involves increasing platelet counts by altering the patient's immune system, with the goal of reducing autoantibody production or blocking the monocyte/macrophage system. AMG 531 was developed to provide an alternative to immunomodulatory therapy, one that is directed at platelet production. This recombinant peptibody has a method of action similar to that of endogenous TPO, in that it stimulates megakaryocytopoiesis and thrombopoiesis by binding to the TPO receptor Mpl. *In vitro* studies have shown that the combination of AMG 531 and endogenous TPO exerts an enhanced effect on megakaryocyte colony growth *in vitro* (Broudy & Lin, 2004). Additionally, a randomised, double-blind, placebo-controlled study of the pharmacokinetics and pharmacodynamics of AMG 531 in healthy volunteers showed that a single intravenous or subcutaneous injection of AMG 531 brought about a dose-dependent increase in platelet counts (Wang *et al*, 2004). AMG 531 was effective in achieving a dose-dependent increase in platelet counts. One patient in this study demonstrated a worsening of thrombocytopenia following the initial response. This may reflect the increased numbers of the TPO receptor Mpl on the expanded megakaryocyte mass being less responsive to endogenous TPO, and is a further indication for limiting the dose of AMG 531.

In the current study, no anti-AMG 531 or anti-TPO antibodies were detected in the patients with ITP who were treated with AMG 531. This finding is particularly important given the results of previous studies (Li *et al*, 2001; Basser *et al*, 2002) with a pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF). Those studies found that some patients produced antibodies to PEG-rHuMGDF and that these antibodies also neutralised endogenous TPO. These effects led to the development of thrombocytopenia (Li *et al*, 2001) or pancytopenia (Basser *et al*, 2002) in a small number of patients and resulted in cessation of the clinical development of PEG-rHuMGDF (Broudy & Lin, 2004). Although both PEG-rHuMGDF and AMG 531 initiate signalling by binding to the Mpl receptor, PEG-rHuMGDF contains the first 163 amino acids of endogenous TPO; this sequence homology presumably leads to the production of anti-TPO antibodies. Unlike PEG-rHuMGDF, AMG 531 was designed to have no sequence homology to endogenous TPO.

In summary, the results of this study indicate that AMG 531 causes a dose-dependent increase in platelet counts among adult thrombocytopenic patients with ITP. AMG 531 was generally well tolerated, and no anti-TPO or anti-AMG 531 antibodies were detected. Future studies will investigate long-term, weekly, weight-based dosing to evaluate the durability of the platelet response among patients with ITP.

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