REGULAR ARTICLE

blood advances

Assessment of romiplostim immunogenicity in pediatric patients in clinical trials and in a global postmarketing registry

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Key Points

- Development of antibodies to romiplostim or thrombopoietin occurs infrequently in pediatric patients with immune thrombocytopenia who are treated with romiplostim.
- Presence of anti-romiplostim or anti-thrombopoietin antibodies is not associated with loss of platelet response or other clinical sequelae.

Development of first-generation thrombopoietins (TPOs) was halted due to antibodies that neutralized endogenous TPO, causing protracted thrombocytopenia in some patients. The second-generation TPO receptor agonist romiplostim, having no homology to TPO, was developed to circumvent potential immunogenicity. We examined the development of binding and neutralizing antibodies to romiplostim and TPO among pediatric patients with primary immune thrombocytopenia (ITP) in 5 clinical trials and a global postmarketing registry. In the trials, 25 of 280 (8.9%) patients developed anti-romiplostim binding antibodies. The first positive result was detected 67 weeks (median) after romiplostim treatment was initiated. The median romiplostim dose was 8 μg/kg, and the median platelet count was 87×10^9/L. Most patients who developed anti-romiplostim binding antibodies (18 of 25 [72%]) had ≥90% of platelet assessments showing a response. Anti-romiplostim neutralizing antibodies developed in 8 of 280 (2.9%) patients. The development of anti-romiplostim neutralizing antibodies was unrelated to the romiplostim dose, and most patients who developed the antibodies (7 of 8 [88%]) had platelet response. Nine of 279 (3.2%) patients developed anti-TPO binding antibodies, and 1 (0.4%) developed transient anti-TPO neutralizing antibodies. In 8 patients who developed anti-romiplostim neutralizing antibodies, no TPO cross-reactivity was observed. In the postmarketing registry, 3 of 19 (15.8%) patients developed anti-romiplostim binding antibodies; 1 (5.3%) patient developed anti-romiplostim neutralizing antibodies. These results suggest that immunogenicity to romiplostim occurs infrequently in pediatric patients with ITP and is generally not associated with loss of platelet response or other negative clinical sequelae.

Introduction

Primary immune thrombocytopenia (ITP) is a chronic autoimmune bleeding disorder that is characterized by a low platelet count resulting from inadequate platelet production combined with increased platelet destruction mediated by antibodies that bind to platelet antigens.[1-3] ITP incidence in pediatric patients (age <18 years) is 8.8 per 100 000 person-years.[4] Although ITP resolves spontaneously in most pediatric patients,[5] persistent and especially chronic ITP may lead to severe bleeding.[2] Corticosteroids, IV

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Qualified researchers may request data from Amgen clinical studies. Complete details are available at the https://wwwext.amgen.com/science/clinical-trials/clinical-data-transparency-practices/clinical-trial-data-sharing-request/.

The full-text version of this article contains a data supplement.

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immunoglobulin (IVIg), and IV anti-D are effective first-line options for pediatric patients who require drug treatment[2,6,7]; however, pediatric patients with persistent or chronic ITP may require other modalities to treat or prevent bleeding.

The first-generation thrombopoietins (TPOs) include recombinant human TPO (rhTPO) and pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF or MGDF).[8] Clinical development of these 2 agents was stopped on the discovery that antibodies to MGDF also neutralized native TPO, leading to thrombocytopenia.[9,10] Romiplostim is a second-generation TPO receptor agonist that has no amino acid sequence homology to endogenous TPO. Romiplostim is approved for treatment of adult patients[11-13] and pediatric patients (≥1 year of age)[11,12] with chronic ITP who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy.

Even though romiplostim has no amino acid sequence homology to endogenous TPO, administration of romiplostim to patients poses the risk of formation of low-affinity conformational antibodies that can cross-react with and neutralize endogenous TPO, leading to loss of response.[14] We conducted a retrospective analysis of immunogenicity results from 5 completed clinical trials that evaluated romiplostim treatment in pediatric patients with ITP, as well as immunogenicity results from a global postmarketing registry that contained data from spontaneously submitted requests for immunogenicity testing among pediatric patients who showed loss of response to romiplostim treatment. The clinical trials are registered at www.clinicaltrials.gov as NCT00515203, NCT00116688, NCT01444417, NCT01071954, and NCT02279173.

Materials and methods

Data sources for clinical trials

The retrospective analysis included data from pediatric patients (<18 years old at screening) with ITP who had initially received romiplostim in 5 Amgen-sponsored clinical trials[15-19] (supplemental Table S1). Patients had received weekly subcutaneous injections of romiplostim or placebo, as reported in each study (supplemental Table S1). Immunogenicity was assessed at baseline and scheduled intervals, typically before and after treatment in shorter trials, and every 12 to 24 weeks in longer trials. Procedures in each trial were in accordance with the ethical standards of the institutional review board or independent ethics committee of each participating institution and with the Declaration of Helsinki of 1975. For each trial, patients or their legally acceptable representative (parent or guardian) had provided written informed consent.

Data source for the patient registry

In clinical practice, when a platelet response to romiplostim is not maintained in a patient, the recommendation is to search for causative factors, including development of anti-drug antibodies. Blood samples are submitted to the manufacturer for detection of anti-romiplostim and anti-TPO antibodies. In February 2009, the manufacturer implemented a prospective postmarketing registry study (Amgen Study 20080091) that included adult and pediatric patients. Per the protocol of the global postmarketing registry study, antibody results were to be reported to the requesting physician and recorded in the global postmarketing registry. Patients who tested positive for anti-romiplostim neutralizing antibodies were to be followed up every 3 months for up to 12 months, unless the result became negative for anti-romiplostim neutralizing antibodies, with patients allowed to continue romiplostim treatment. Patients who tested positive for anti-TPO neutralizing antibodies were to be followed up quarterly until the anti-TPO neutralizing antibodies decreased to undetectable levels or the levels stabilized over at least a 12-month follow-up period. The treating physician was to be informed of the detection of the anti-TPO neutralizing antibodies within 10 business days and was asked to discontinue romiplostim. During the follow-up period for patients with a positive neutralizing antibody to romiplostim or TPO, antibody levels, clinical history, treatment, platelet levels, and other clinical sequelae were to be evaluated. Data for pediatric patients (<18 years of age) who had immunogenicity results from May 2009 through May 2016 were included in the current analysis.

Immunogenicity testing

Immunogenicity to romiplostim and TPO was assessed in a stepwise process, with all assay validation and threshold (or cutoff point) determinations established according to published industry white papers at the time of assay validation.[14,20-22] In brief, the samples were screened for binding antibodies by surface plasmon resonance immunoassay (SPRIA). Determining whether a serum sample from a patient treated with romiplostim has anti-drug antibodies requires that the analytical method validate an assay cutoff point that enables discrimination of samples that are positive and those that are negative for anti-drug antibodies. At the time the immunogenicity assay was validated using SPRIA and a bioassay platform, Amgen followed the published guidance for industry at that time.[21,22] The screening assay cutoff point was statistically derived using the mean +3 standard deviation and removal of assay values that were outliers. For data that were not normally distributed, the Box-Cox procedure[23] was used to decide an appropriate transformation to normality. The upper limit on the range of the expected values for the population was determined by calculating the upper bound of a 1-sided 95% prediction interval for the distribution of the assay values from 141 patients with ITP who were treatment naive for romiplostim. Any sample testing at or above threshold was considered reactive, providing a 5% false-reactive rate. Reactive samples required further testing in the confirmatory step, which included spiking in excess drug and assessing the percentage reduction in binding. At the time of SPRIA validation, most companies, including Amgen, implemented the ≥50% reduction (a cutoff point that is more likely to detect an effective anti-drug antibody response) in the confirmatory step as the criteria to detect a sample that was positive for binding anti-drug antibodies.[14] Detection limits were 400 ng/mL for anti-romiplostim antibodies and 200 ng/mL for anti-TPO antibodies. If the addition of romiplostim or TPO reduced binding by ≥50% in the test for drug-specific antibodies, then binding was considered to be drug specific.

Samples confirmed positive for binding antibodies were tested for neutralizing antibodies by using a murine cell line transfected with the human TPO receptor gene that proliferates when cultured with TPO or romiplostim. The immunogenicity assays and statistical methods used to evaluate the results have been described in detail.[14] In brief, in the bioassay for neutralizing antibodies, cutoff points were statistically derived from the 99% lower bound of the least squares mean established using serum from 100 patients with ITP. Total assay variance calculated by analysis of variance incorporated patient, day, and plate differences into the calculation of

prediction limits, with inhibition of romiplostim-induced proliferation by ≥16.0% or inhibition of TPO-induced proliferation by ≥25.9% considered a sign of neutralizing activity. To confirm that the neutralizing activity was related to immunoglobulin, samples that tested above the assay cutoff points were diluted and then treated with a protein G + protein L bead mixture (protein G/L) to remove all human immunoglobulins, including IgG. After treatment, the samples were tested in corresponding romiplostim or TPO assays. Samples with neutralizing activity that then increased romiplostim-induced or TPO-induced cell proliferation by ≥23.7% after incubation with protein G/L were considered to be positive for neutralizing antibodies.

Statistical methods

Incidences of binding and neutralizing antibodies were determined for each data source. For clinical trials, occurrence of antibodies was classified as transient if the patient had no sample or had a negative baseline result and a negative result at the patient's last time point tested or was classified as persistent if the patient had a positive result at the last time point tested.

In the clinical trials, immunogenicity data were evaluated for pediatric patients with ITP who had been treated with romiplostim at any time and 4 subjects who had received placebo only. Baseline demographic and clinical characteristics were determined in patients who had received placebo or romiplostim only and in those who had received both placebo and romiplostim. Baseline demographic and clinical characteristics were summarized for patients with vs without development of postbaseline anti-romiplostim binding antibodies, with 95% confidence intervals (CIs) used to evaluate characteristics that differed between the cohorts. For patients who developed antibodies in a clinical trial, data listings were generated for the outcomes of romiplostim dose, platelet counts, treatment-emergent adverse events (AEs) of bleeding or hypersensitivity, platelet response, and occurrence of subsequent antibodies after detection of first antibodies, as detailed in Table 1. Mean (quartile 1, quartile 3 [Q1, Q3]) platelet count and median (Q1, Q3) weighted romiplostim dose were evaluated for patients with positive transient or persistent anti-romiplostim or anti-TPO antibodies at week 1, a week before and a week after an antibody-positive result, and at the end of treatment.

Table 1. Data listings for patients who developed anti-romiplostim or anti-TPO antibodies in clinical trials

	Outcome
1	Romiplostim dose and cumulative romiplostim dose
2	Platelet counts over time
3	Treatment-emergent AEs of bleeding (defined as 1 or more events from MedDRA hemorrhages [Standardized MedDRA Query]) or hypersensitivity (defined as 1 or more events from the Standardized MedDRA Queries for anaphylactic reaction, anaphylactic-anaphylactoid shock conditions, angioedema or hypersensitivity) that started or worsened within 30 d after antibody detection (i.e., within 30 d of the collection date of a sample that tested positive for antibodies).
4	Proportion of weekly platelet responses (≥50 × 10^9/L without rescue medication use in the prior 4 wk) after detection of anti-romiplostim or anti-TPO binding or neutralizing antibodies.
5	Occurrence of subsequent antibodies after detection of the first antibodies.

MedDRA, Medical Dictionary for Regulatory Activities.

For the postmarketing registry, immunogenicity data were evaluated for pediatric patients for whom spontaneous requests for antibody testing of blood samples were submitted during the period of interest. The focus was on patients in whom anti-romiplostim or anti-TPO antibodies were detected.

Results

Patients

Pediatric patients in clinical trials. Data from pediatric patients with ITP who had been treated with romiplostim in Amgen-sponsored clinical trials[15-19] were available for this analysis (supplemental Table S1). For patients who continued from the parent studies[15-17] to the extension studies,[18,19] data from both study phases were included in the analysis, but each patient was counted only once. Seventy-one patients had ITP duration (calculated from time of ITP diagnosis to time of enrollment of the first ITP study) between >6 and ≤12 months and 211 patients had ITP duration of >12 months (supplemental Table S2). This report presents immunogenicity results for the 282 patients, with a cutoff date of 15 August 2019.

Pediatric patients in the postmarketing registry study. A total of 217 patients (184 adults, 19 pediatric patients, and 14 patients with age missing in the data) for whom spontaneous requests for antibody testing of blood samples were submitted from May 2009 through May 2016 had immunogenicity results in the manufacturer's postmarketing registry study. This report presents immunogenicity results for the 19 pediatric patients.

Antibodies to romiplostim in clinical trials

Anti-romiplostim binding antibodies. At baseline, samples from 269 patients were collected and tested for anti-romiplostim binding antibodies (Figure 1); 2 (0.7%) patients tested positive for the antibodies. Postbaseline samples were collected from 280 patients and tested for anti-romiplostim binding antibodies; 25 (8.9%) patients tested positive for the antibodies, of whom 14 (5.0%) had transient and 11 (3.9%) had persistent antibodies. Baseline characteristics examined were similar between the cohorts (Table 2), with overlapping 95% CIs for percentages and interquartile ranges for cohort medians.

For the 25 patients who developed postbaseline anti-romiplostim binding antibodies (either transient or persistent), median time to the first positive result was 67 weeks (Table 3). The median romiplostim dose at which antibodies were first detected was 8 μg/kg, the median cumulative romiplostim dose was 389 μg/kg, and the median platelet count was 87 × 10^9/L. Three of the 25 patients had treatment-emergent AEs (beginning or worsening within 30 days after antibody detection) of bleeding (2 patients) or hypersensitivity (1 patient) after detection of the antibodies (Table 3). Of the 24 patients who had weekly platelet counts, 23 (96%) had weekly platelet counts ≥50 × 10^9/L (platelet response) at 1 or more measurements and 18 (75%) had ≥90% of platelet assessments showing a response after the first detection of anti-romiplostim binding antibodies, with no rescue medication within 4 weeks after antibody detection. Patients 19 and 21, who had experienced bleeding AEs, had platelet assessments showing no response after the first detection of anti-romiplostim binding antibodies (2% and 45%, respectively). The baseline characteristics examined were similar in

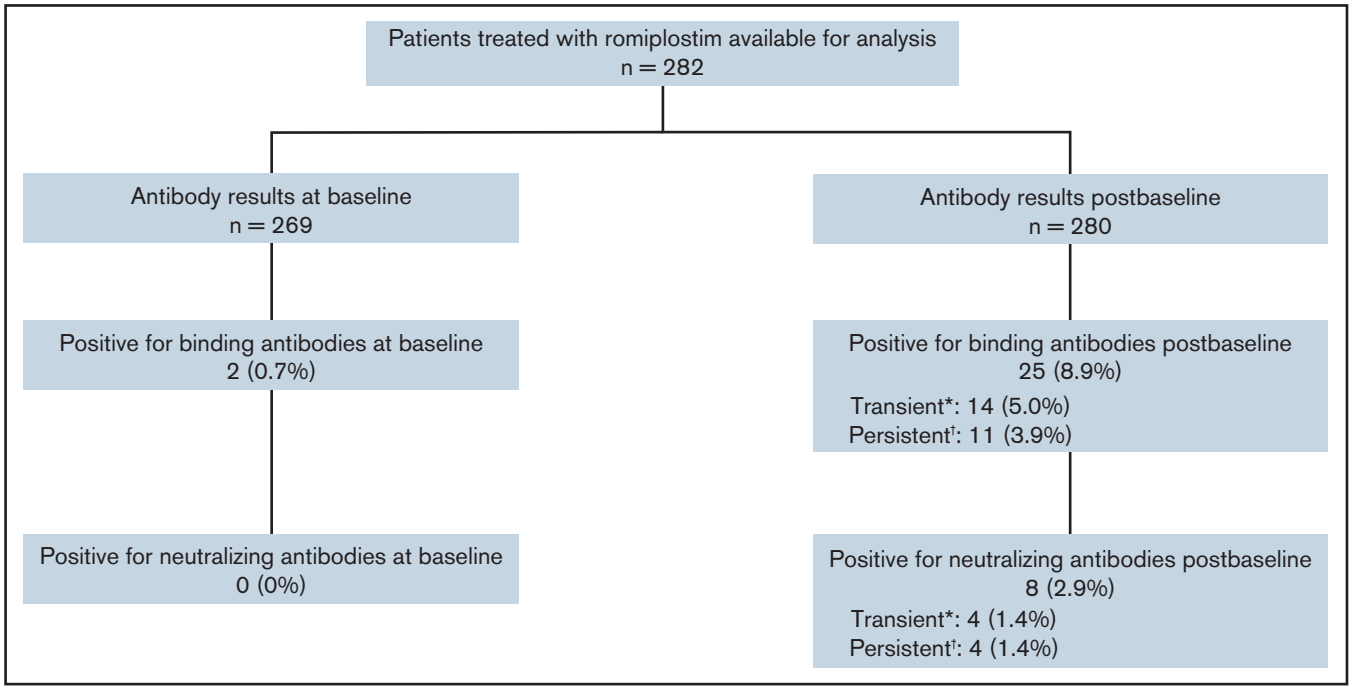

Figure 1. Anti-romiplostim binding and neutralizing antibodies among patients enrolled in clinical trials. Pediatric patients (age <18 years at screening) with ITP who received 1 or more doses of romiplostim in any of the ITP studies (NCT00515203, NCT00116688 [extension], NCT01444417, NCT01071954 [extension], and NCT02279173) are included in the analysis. Patient samples taken at baseline and at scheduled intervals, typically before and after treatment in shorter trials and every 12 to 24 weeks in longer trials, were screened for anti-romiplostim binding antibodies first and then those that tested positive for binding antibodies were tested for anti-romiplostim neutralizing antibodies. *Negative result at the patient's last time point tested. †Positive result at the patient's last time point tested.

patients who developed transient or persistent anti-romiplostim binding antibodies (supplemental Table S3).

Anti-romiplostim neutralizing antibodies. No patients tested positive for anti-romiplostim neutralizing antibodies at baseline (Figure 1). Postbaseline, anti-romiplostim neutralizing antibodies were detected in 8 of 280 (2.9%) patients; 4 (1.4%) had transient and 4 (1.4%) had persistent anti-romiplostim neutralizing antibodies.

The median time to the first positive result of anti-romiplostim neutralizing antibodies was 64 weeks (Table 4), with a median romiplostim dose of 7 μg/kg, a median cumulative romiplostim dose of 354 μg/kg, and a median platelet count of 61 $\times$ 10^9/L. Seven patients continued to receive romiplostim after anti-romiplostim neutralizing antibodies were detected; of those, 6 had a subsequent platelet response at 1 or more weekly measurements. All 6 had ≥50% of platelet assessments showing a platelet response after the first detection of anti-romiplostim neutralizing antibodies, with no rescue medication within 4 weeks after antibody detection. In 3 of the 6 patients, platelet counts remained ≥50 $\times$ 10^9/L for all the study weeks evaluated after the first detection of anti-romiplostim neutralizing antibodies until the end of study. After the first detection of anti-romiplostim antibodies, the remaining 3 patients had three or fewer of more than 20 measurements taken with platelet counts of <50 $\times$ 10^9/L; in all 3 patients, platelet counts recovered to ≥50 $\times$ 10^9/L in the subsequent weeks and remained above that level until the end of treatment. Overall, 7 of 8 patients (88%) who developed anti-romiplostim neutralizing antibodies had a platelet response. Bleeding and hypersensitivity treatment-emergent AEs occurred in 2 of 8 patients who developed postbaseline anti-romiplostim neutralizing antibodies (Table 4). Patient 19, who had continued to receive romiplostim after anti-romiplostim neutralizing antibodies were detected and had no subsequent platelet response after detection of antibodies, experienced both bleeding and hypersensitivity AEs. Patient 25 experienced bleeding AEs.

Antibodies to TPO in clinical trials

Anti-TPO binding antibodies. At baseline, 2 of 269 (0.7%) patients who had received romiplostim tested positive for anti-TPO binding antibodies, and none tested positive for anti-TPO neutralizing antibodies. Postbaseline, 1 patient (patient 28) who had received placebo tested positive for transient anti-TPO binding antibodies. Nine of 279 patients (3.2%) who had received romiplostim tested positive for anti-TPO binding antibodies; 8 (2.9%) had transient antibodies, and 1 (0.4%) had persistent antibodies. Patient 10, who tested positive for anti-TPO binding antibodies had tested positive for anti-romiplostim binding and neutralizing antibodies. Baseline characteristics examined were similar between the cohorts, with overlapping 95% CIs for percentages and interquartile ranges for medians between the cohorts (supplemental Table S4).

For the 10 patients who developed postbaseline anti-TPO binding antibodies, the median time to the first positive result, median romiplostim dose, median cumulative romiplostim dose, and median platelet count are summarized in supplemental Table S5. Bleeding occurred in 3 patients after detection of anti-TPO binding antibodies. No treatment-emergent AEs of hypersensitivity were reported. No treatment-emergent AEs occurred in patient 10, the only patient

Table 2. Characteristics of pediatric patients with ITP with postbaseline anti-romiplostim binding antibody results in clinical trials

	Developed postbaseline anti-romiplostim binding antibodies			
	Yes (n = 25)		No (n = 259)	
Characteristic	n (%) or median	(95% CI) or [Q1, Q3]	n (%) or median	(95% CI) or [Q1, Q3]
Female	14 (56.0)	(34.9, 75.6)	128 (49.4)	(43.2, 55.7)
Age, y	10.0	[6.0, 14.0]	10.0	[6.0, 13.0]
Race, White	19 (76.0)	(54.9, 90.6)	196 (75.7)	(70.0, 80.8)
Baseline platelet count ($\times 10^9$/L), median	18.0	[14.3, 22.0]	14.0	[7.5, 23.7]
ITP duration (>3 y)*	9 (36.0)	(18.0, 57.5)	93 (35.9)	(30.1, 42.1)
Prior splenectomy	3 (12.0)	(2.5, 31.2)	17 (6.6)	(3.9, 10.3)
Baseline anti-romiplostim binding antibodies	0 (0)	(0.0, 13.7)	2 (0.8)	(0.1, 2.8)
Baseline anti-romiplostim neutralizing antibodies	0 (0)	(0.0, 13.7)	0 (0)	(0, 1.4)
Previous ITP treatments, median n	2.0	[2.0, 3.0]	2.0	[2.0, 3.0]
Prior rituximab use	5 (20.0)	(6.8, 40.7)	54 (20.8)	(16.1, 26.3)
Prior corticosteroid use	23 (92.0)	(74.0, 99.0)	225 (86.9)	(82.1, 90.7)
Medical history				
Abdominal pain	0 (0)	(0, 13.7)	12 (4.6)	(2.4, 8.0)
Allergies	4 (16.0)	(4.5, 36.1)	31 (12.0)	(8.3, 16.6)
Autoimmune neutropenia	0 (0)	(0, 13.7)	0 (0)	(0, 1.4)
Autoimmune thyroiditis	0 (0)	(0, 13.7)	1 (0.4)	(0, 2.1)
Chest pain	0 (0)	(0, 13.7)	0 (0)	(0, 1.4)
Immunodeficiency	0 (0)	(0, 13.7)	3 (1.2)	(0.2, 3.3)
Kidney disorder	1 (4.0)	(0.1, 20.4)	2 (0.8)	(0.1, 2.8)
Liver disorder	0 (0)	(0, 13.7)	1 (0.4)	(0, 2.1)
Musculoskeletal pain	1 (4.0)	(0.1, 20.4)	12 (4.6)	(2.4, 8.0)
Oropharyngeal pain	0 (0)	(0, 13.7)	4 (1.5)	(0.4, 3.9)
Pain	0 (0)	(0, 13.7)	3 (1.2)	(0.2, 3.3)
Thyroid disease	1 (4.0)	(0.1, 20.4)	2 (0.8)	(0.1, 2.8)

Baseline demographic and clinical characteristics were determined for the 284 patients (n = 25 and n = 259) who had received placebo or romiplostim only and patients who received both placebo and romiplostim.

*ITP duration was calculated from the time of ITP diagnosis to the time of enrollment in the first ITP study.

who tested positive for anti-romiplostim binding and neutralizing antibodies and anti-TPO binding antibodies. Nine of the 10 patients who had received romiplostim had platelet responses at 1 or more weekly measurements. Most of these patients had ≥92% of platelet assessments showing a response after the first detection of anti-TPO binding antibodies, with no rescue medication within 4 weeks after antibody detection. Patient 28 who had received placebo and tested positive for transient anti-TPO binding antibodies showed no platelet response at any of the weekly measurements.

Anti-TPO neutralizing antibodies. No patients tested positive for anti-TPO neutralizing antibodies at baseline. Patient 34, who was 1 of 279 patients (0.4%) who had received romiplostim, tested positive for transient anti-TPO neutralizing antibodies by postbaseline week 100. The patient had received romiplostim (3 μg/kg weekly dose, 364 μg/kg cumulative dose) and had tested positive for anti-TPO binding antibodies, but had not tested positive for anti-romiplostim binding or neutralizing antibodies. At the time of detection of anti-TPO neutralizing antibodies, the patient had a platelet count of 176 $\times$ 10^9/L. No treatment-emergent AEs of bleeding or hypersensitivity occurred in this patient. The patient continued to receive romiplostim and had ≥85% of platelet assessments showing a platelet response after the first detection of anti-TPO neutralizing antibodies, with no rescue medication 4 weeks after antibody detection.

Platelet count and romiplostim exposure over time for patients with positive transient or persistent anti-romiplostim or anti-TPO antibodies

The therapeutic benefit of improved median platelet counts continued after development of transient binding antibodies to romiplostim (Figure 2A). An initial decline in median platelet count after detection of persistent anti-romiplostim binding antibodies was observed, but this effect was followed by an increase in median platelet count before the end of treatment. After detection of transient anti-TPO binding antibodies, median platelet count decreased but remained >50 $\times$ 10^9/L (Figure 2B). An initial decrease followed by an increase in platelet count before the end of treatment was observed after detection of persistent anti-TPO binding antibodies, which is similar to the median platelet count response after detection of persistent anti-romiplostim binding antibodies. For patients with transient and persistent anti-romiplostim neutralizing antibodies and the

Table 3. Listing of pediatric patients with ITP who developed postbaseline anti-romiplostim binding antibodies in clinical trials

	At first detection of anti-romiplostim binding antibodies					After detection of first anti-romiplostim binding antibodies
Patient no. (n = 25)	Treatment week	Romiplostim dose (μg/kg)	Cumulative romiplostim dose (μg/kg)	Platelet count ($\times 10^9$/L)	AEs*	Platelet response† n1/N1 (%)
Transient antibodies‡ (n = 14)						
1	12	5	49	189	–	117/117 (100)
2	107	6	594	94	Hypersensitivity: rash	360/360 (100)
3	139	10	1370	79	–	291/293 (99)
4	52	8	344	173	–	75/75 (100)
5	75	–	675	–	–	–
6	12	1	12	143	–	156/156 (100)
7	104	10	864	27	–	28/39 (72)
8	96	10	858	32	–	63/63 (100)
9	51	9	394	178	–	105/105 (100)
10	51	10	345	111	–	84/84 (100)
11	52	10	469	77	–	16/17 (94)
12	52	5	242	387	–	48/48 (100)
13	51	10	320	146	–	28/31 (90)
14	148	4	300	48	–	12/12 (100)
Persistent antibodies (n = 11)§						
15	64	7	291	201	–	–
16	104	1	102	98	–	5/5 (100)
17	25	8	108	35	–	143/144 (99)
18	67	–	596	16	–	0/1 (0)
19	12	8	47	25	Bleeding: contusion, petechiae	1/57 (2)
20	148	10	1341	38	–	11/11 (100)
21	76	8	520	192	Bleeding: petechiae	23/51 (45)
22	100	10	940	178	–	57/57 (100)
23	52	10	447	38	–	89/108 (82)
24	76	7	389	52	–	37/37 (100)
25	76	5	295	61	–	81/81 (100)
Median	67	8	389	87	–	100%
Range	12-148	1-10	12-1370	16-387	–	0%-100%

MedDRA, Medical Dictionary for Regulatory Activities.

*Bleeding AEs (defined as at least 1 event from MedDRA hemorrhages [Standardized MedDRA Query]) or hypersensitivity AEs (defined as at least 1 event from the Standardized MedDRA Queries for anaphylactic reaction, anaphylactic-anaphylactoid shock conditions, angioedema, or hypersensitivity) that started or worsened within 30 days after antibody detection (within 30 days of the date of collection of a sample that tested positive for antibodies).

†Platelet response was defined as a weekly platelet count $\geq 50 \times 10^9$/L during the treatment period without a rescue medication in the past 4 weeks (n1)/total number of weekly platelet counts (N1).

‡Negative result at the patient's last time point tested.

§Positive result at the patient's last time point tested.

Table 4. Characteristics of pediatric patients with ITP who developed postbaseline anti-romiplostim neutralizing antibodies in clinical trials

	At first detection of anti-romiplostim neutralizing antibodies					After first detection of anti-romiplostim antibodies	
Patient no. (n = 8)	Treatment week	Platelet count ($\times 10^9$/L)	Romiplostim dose (μg/kg)	Cumulative romiplostim dose (μg/kg)	AEs*	Received romiplostim	Platelet response n1/N1 (%)†
Transient antibodies (n = 4)‡							
10	99	36	6	745	–	Yes	20/39 (51)
13§	51	146	10	320	–	Yes	26/31 (84)
19§	12	25	8	47	Bleeding: contusion, petechiae at week 12‖	Yes	0/57 (0)
24§	76	52	7	389	–	Yes	25/37 (68)
Persistent antibodies (n = 4)¶							
5	75	–	–	675	–	No	–
11	52	77	10	469	–	Yes	11/17 (65)
12#	52	387	5	242	–	Yes	42/48 (88)
25#	76	61	5	295	Bleeding (epistaxis at week 101 and 124; contusion at week 148)	Yes	78/81 (96)
Median	64	61	7	354	–	–	100%
Range	12-99	25-387	5-10	47-745	–	–	2%-100%

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*Bleeding AEs (defined as at least 1 event from MedDRA hemorrhages [Standardized MedDRA Query]) or hypersensitivity AEs (defined as at least 1 event from the Standardized MedDRA Queries for anaphylactic reaction, anaphylactic-anaphylactoid shock conditions, angioedema, or hypersensitivity) that started or worsened within 30 days after antibody detection (within 30 days of the date of collection of a sample that tested positive for antibodies).

†Platelet response was defined as a weekly platelet count $\geq 50 \times 10^9$/L during the treatment period without a rescue medication in the past 4 weeks (n1)/total number of weekly platelet counts (N1).

‡Negative result at the patient's last time point tested.

§Patients who had additional positive tests for transient anti-romiplostim antibodies: patient 13 at week 76; patient 19 at weeks 18 and 21; and patient 24 at weeks 104, 145, 167, 180, and 194.

‖Patient 19 experienced additional AEs of bleeding (contusion) and hypersensitivity (drug eruption and rash) at week 18 and bleeding (blood urine, contusion, petechiae) and hypersensitivity (drug eruption, rash) at week 21.

¶Positive result at the patient's last time point tested.

#Patients who had additional positive tests for persistent anti-romiplostim antibodies: patient 12 at week 100; patient 25 at weeks 101, 124, 148, and 157.

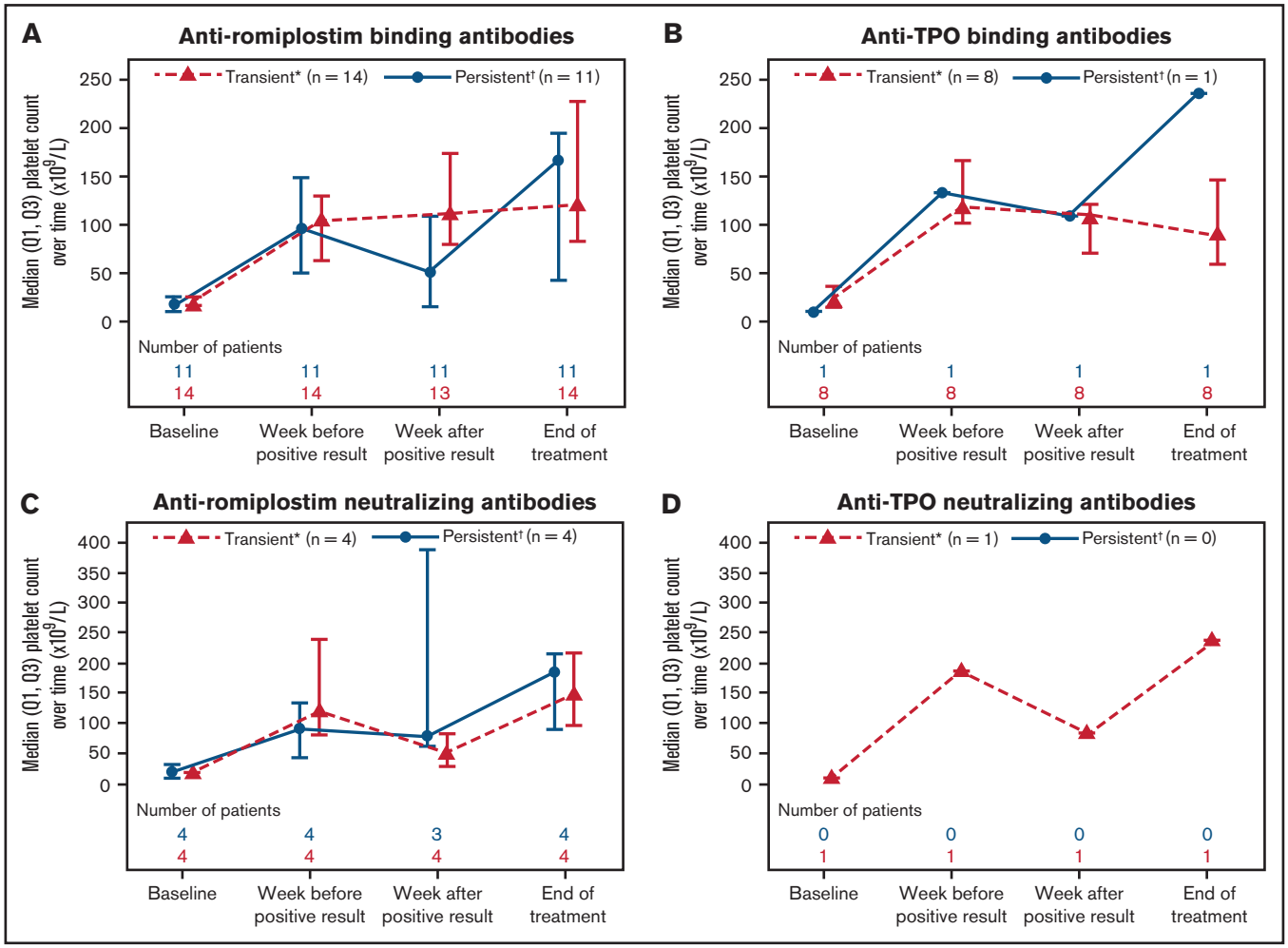

Figure 2. Platelet count over time in patients with positive binding or neutralizing transient vs persistent anti-romiplostim antibodies (A, C) or anti-TPO antibodies (B,D). The platelet count was evaluated for patients with positive transient or persistent anti-romiplostim or anti-TPO antibodies at week 1, a week before and a week after an antibody-positive result, and at the end of treatment. *Transient: negative result at the patient's last time point tested. †Persistent: positive result at the patient's last time point tested.

1 patient with transient anti-TPO neutralizing antibodies, platelet response was similar to that which occurs after detection of persistent anti-romiplostim or persistent anti-TPO binding antibodies, with an initial decrease followed by an increase in median platelet counts before the end of treatment (Figure 2C-D).

Transient and persistent anti-romiplostim binding antibodies, transient anti-TPO binding antibodies, and transient anti-romiplostim neutralizing antibodies were identified when the romiplostim dose was close to the median dose of 8 μg/kg (Figure 3A-C), and all of the antibodies were persistently identified at lower romiplostim doses, in the 3 to 5 μg/kg range (Figure 3D).

Antibodies to romiplostim or TPO in the postmarketing registry

Of the 19 pediatric patients for whom spontaneous requests for antibody testing of blood samples were submitted from May 2009 through May 2016, 3 (15.8%) tested positive for anti-romiplostim binding antibodies; 1 (5.3%) of these patients tested positive for anti-romiplostim neutralizing antibodies. No patients tested positive for anti-TPO binding or neutralizing antibodies. The 1 patient who tested positive for anti-romiplostim neutralizing antibodies experienced decreased therapeutic response to romiplostim; however, no further details are available.

Discussion

Romiplostim has no homology to TPO; however, concerns about its potential immunogenicity have remained an issue. This analysis of data from 5 clinical trials in which pediatric patients with ITP were treated with romiplostim showed that anti-romiplostim binding antibodies developed infrequently, with most antibodies being transient. A few patients (2.9%) who tested positive for anti-romiplostim binding antibodies developed anti-romiplostim neutralizing antibodies. Of note, anti-romiplostim antibodies did not bind to TPO. Data from a postmarketing registry also showed that, when patients responded suboptimally to romiplostim treatment, >80% did not develop anti-romiplostim antibodies.

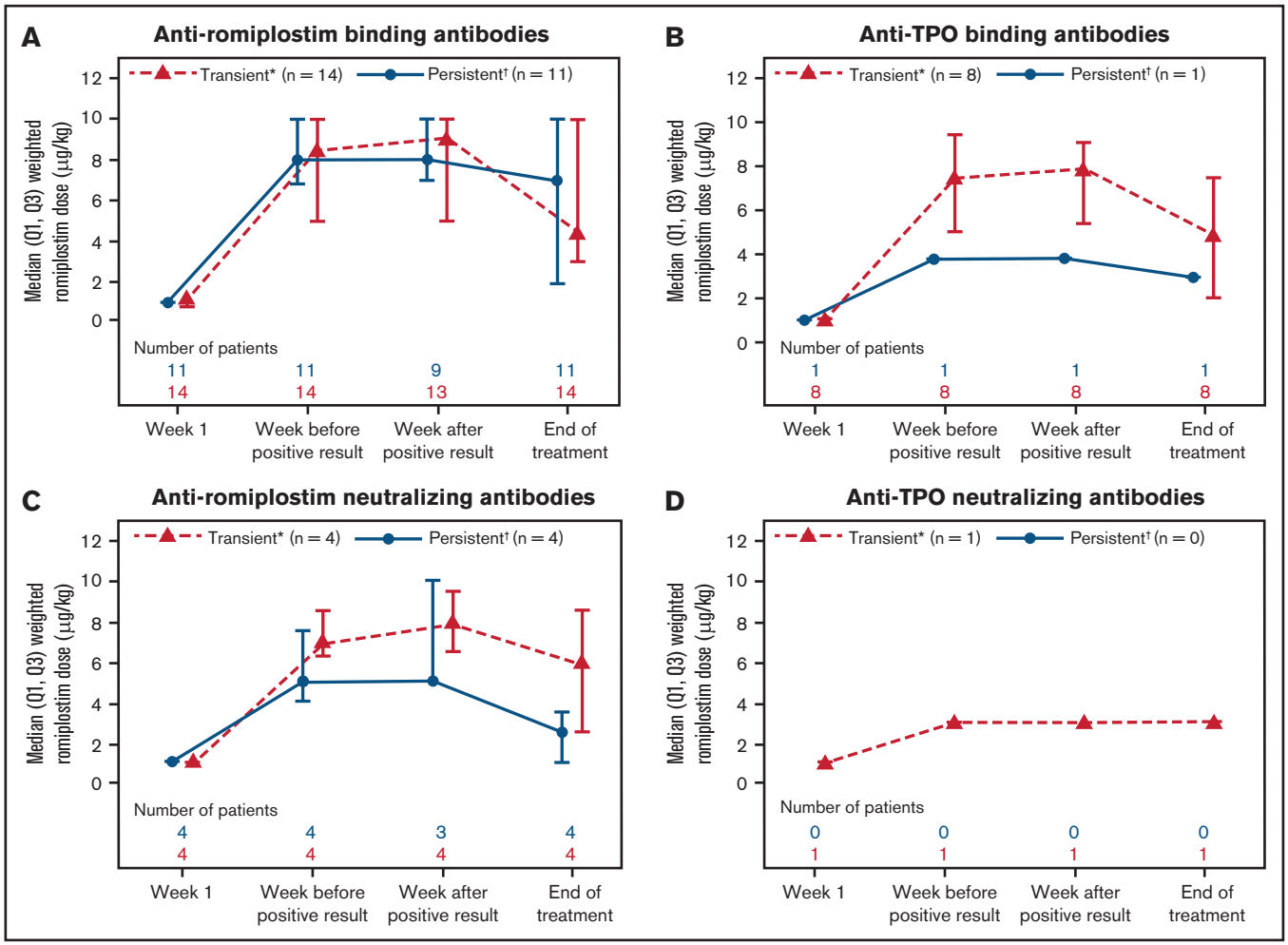

Figure 3. Romiplostim exposure over time in patients with positive binding or neutralizing transient vs persistent anti-romiplostim antibodies (A,C) or anti-TPO antibodies (B,D). The romiplostim dose was evaluated in patients with positive transient or persistent anti-romiplostim or anti-TPO antibodies at week 1, a week before and a week after an antibody-positive result, and at the end of treatment. *Transient: negative result at the patient's last time point tested. †Persistent: positive result at the patient's last time point tested.

An initial decrease in platelet response was observed when immunogenicity to romiplostim or TPO was detected, but this did not decrease to clinically significant levels. Most patients continued to receive treatment after detection of anti-romiplostim antibodies, with platelet levels subsequently increasing for the duration of treatment. Loss of platelet response after antibody detection was observed only in patient 19, who developed anti-romiplostim binding and neutralizing antibodies and continued to receive romiplostim after detection of antibodies. However, patient 10, who developed anti-romiplostim binding and neutralizing antibodies and anti-TPO binding antibodies, had a platelet response after antibody detection. These findings suggest that the presence of anti-romiplostim neutralizing antibodies is not necessarily associated with a clinical effect in pediatric patients, as measured by the lack of an observed effect on platelet response. No obvious pattern of romiplostim dose increase was observed when immunogenicity to romiplostim or TPO was detected. In addition, no association between anti-romiplostim or anti-TPO antibody occurrence and AE development was observed.

We acknowledge that with regard to assessing the impact of anti-drug antibodies on romiplostim response in the pediatric setting and, more importantly, with regard to assessing impact on the pharmacodynamic marker of platelet response, the number of patients who developed anti-drug antibodies was too limited to establish a correlation with clinical findings. It becomes increasingly difficult to assess impact when the incidences of anti-drug antibodies are at low levels, as observed in our analysis with romiplostim in the pediatric population. Using very sensitive assays (detection limits of 400 ng/mL for anti-romiplostim antibodies and 200 ng/mL for anti-TPO antibodies), we found that only patient 19 had no platelet response after testing positive for anti-romiplostim neutralizing antibodies. Of the 8 patients who tested positive for anti-romiplostim neutralizing antibodies, 7 continued romiplostim after their first positive result with no rescue medication needed within 4 weeks of testing positive for anti-drug antibodies. Of the 7 patients, 6 had a subsequent platelet response at 1 or more weekly measurements. Three of the 6 patients maintained platelet counts $\geq 50 \times 10^9$/L after the first positive results for anti-romiplostim neutralizing

antibodies that continued until the end of treatment. The 3 remaining patients had a decrease in platelet counts to $<50 \times 10^9$/L after the first positive result for anti-romiplostim neutralizing antibodies, but platelet counts recovered to $\geq 50 \times 10^9$/L and remained at $\geq 50 \times 10^9$/L until the end of treatment. Patient 34, who tested positive for neutralizing antibodies to TPO, had a platelet count of 176×10^9/L at the time of testing positive for anti-TPO neutralizing antibodies. No treatment-emergent AEs were reported for this patient, and the patient continued to receive romiplostim and had ≥85% of platelet assessments showing a platelet response after the first detection of anti-TPO neutralizing antibodies, with no rescue medication 4 weeks after antibody detection.

Despite the detection of anti-romiplostim and anti-TPO binding and neutralizing antibodies in our study, there was no correlation between anti-drug antibody development and clinical findings such as loss of platelet response or other negative clinical sequelae. This lack of correlation between anti-drug antibody development and clinical effect is most likely the result of the low immunogenicity rates observed in children with ITP. As such, more studies are needed to evaluate and understand the link between the development of anti-romiplostim or anti-TPO antibodies and clinical effect.

Baseline characteristics were similar between patients with or without postbaseline anti-romiplostim or anti-TPO binding antibodies and were similar between patients who developed transient or persistent anti-romiplostim binding antibodies. As such, the ability to predict which patient was more likely to develop antibodies (either transient or persistent) appears to have limited clinical utility, given that no evidence shows that anti-romiplostim or anti-TPO binding antibodies influence clinical outcomes in pediatric patients.

Earlier studies had reported a higher incidence of immunogenicity in pediatric compared with adult populations, with anti-romiplostim binding antibodies occurring in 8% vs 4% of patients and anti-romiplostim neutralizing antibodies in 3% vs <1%.[11,24] However, in an analysis that evaluated romiplostim immunogenicity in adults with ITP in 13 clinical trials,[20] the reported incidence of anti-romiplostim binding antibodies was 6.2% (60 of 961 patients), similar to the 8.9% incidence observed in our pediatric ITP analysis. The incidence of anti-romiplostim neutralizing antibodies was 0.4% in the adult ITP analysis[20] and 2.9% in our pediatric ITP analysis. The incidence of anti-TPO binding antibodies was 3.4% and 3.2%, respectively. No patients developed anti-TPO neutralizing antibodies in the adult ITP analysis[20] compared with 1 (0.4%) patient in our pediatric ITP analysis. Similar to findings from our previous analysis in adults, platelet counts and AEs provided no evidence that development of anti-romiplostim or anti-TPO antibodies was associated with reduced platelet response or increased romiplostim dose.

The postmarketing registry included data from a select subset of patients who received romiplostim in clinical practice and who had either demonstrated a suboptimal platelet response initially or lost their platelet response with continued treatment. Because the registry was established based on spontaneous postmarketing requests for antibody testing, this data source may not represent the general population of patients who have received romiplostim, but rather those with suboptimal platelet response. However, the referral of patient samples for which antibody positivity was suspected implies that the proportion we observed in this sample is likely to be lower than what would be observed in the general patient population, which would include patients with continued response to romiplostim treatment. Although baseline antibody testing was not performed in any of the 19 postmarketing registry patient samples, a previous report has shown that anti-romiplostim and anti-TPO binding antibodies may be observed in patients before romiplostim exposure (7% and 5%, respectively).[14] In cases where there is an observed loss of efficacy or failure to maintain a platelet response within the recommended dose range in pediatric patients with ITP who are undergoing treatment with romiplostim, clinicians should consider searching for causative factors, including development of anti-romiplostim neutralizing antibodies. To eliminate immunogenicity, clinicians can request antibody testing by submitting samples to the drug manufacturer (Amgen, Thousand Oaks, CA).[11] The availability of data generated from the submitted samples can also extend our understanding of the impact of anti-romiplostim or anti-TPO antibodies on the clinical findings of platelet response and safety.

Limitations must be considered in the interpretation of results from our analysis. As mentioned before, lack of an association between antibody development and clinical effect as observed in our analysis could have been caused simply by the low number of pediatric patients who developed antibodies. Also, platelet count fluctuation in pediatric patients with ITP related to disease state and varying turnover rates in the bone marrow and spleen are likely to complicate interpretation of platelet response rates after development of anti-romiplostim binding or neutralizing antibodies.[20] In addition, prior use of therapies, such as rituximab or corticosteroids, may also influence interpretation of platelet response rate after development of anti-romiplostim binding or neutralizing antibodies, but the studies included in this retrospective analysis were not designed to evaluate this relationship.

In summary, results from an analysis of data from 5 clinical trials in which pediatric patients with ITP were treated with romiplostim and data from a romiplostim postmarketing registry suggest that romiplostim immunogenicity occurs infrequently in pediatric patients with ITP and is generally not associated with a loss of platelet response or other negative clinical sequelae.

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Authorship

Contribution: All authors contributed to the study design, analysis of data, interpretation of results, and review and editing of the manuscript drafts for scientific content and have approved submission of the final manuscript draft.

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Takeda; and has received speaker's bureau fees from BioMarin, Octapharma, and Shire/Takeda.

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