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

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Summary

Antibodies to first-generation recombinant thrombopoietin (TPO) neutralized endogenous TPO and caused thrombocytopenia in some healthy subjects and chemotherapy patients. The second-generation TPO receptor agonist romiplostim, having no sequence homology to TPO, was developed to avoid immunogenicity. This analysis examined development of binding and neutralising antibodies to romiplostim or TPO among adults with immune thrombocytopenia (ITP) in 13 clinical trials and a global postmarketing registry. 60/961 (6·2%) patients from clinical trials developed anti-romiplostim-binding antibodies post-baseline. The first positive binding antibody was detected 14 weeks (median) after starting romiplostim, at median romiplostim dose of 2 μg/kg and median platelet count of 29.5×10^9/l; most subjects had ≥98·5% of platelet assessments showing response. Neutralising antibodies to romiplostim developed in 0·4% of patients, but were unrelated to romiplostim dose and did not affect platelet count. Thirty-three patients (3·4%) developed anti-TPO-binding antibodies; none developed anti-TPO-neutralising antibodies. In the global postmarketing registry, 9/184 (4·9%) patients with spontaneously submitted samples had binding antibodies. One patient with loss of response had anti-romiplostim-neutralising antibodies (negative at follow-up). Collectively, anti-romiplostim-binding antibodies developed infrequently. In the few patients who developed neutralising antibodies to romiplostim, there was no cross-reactivity with TPO and no associated loss of platelet response.

Keywords: thrombopoietin, romiplostim, immune thrombocytopenia, immunogenicity, TPO receptor agonist.

Chronic immune thrombocytopenia (ITP) is an autoimmune disorder characterised by low circulating platelet counts. The pathophysiology of primary ITP is thought to be caused by increased platelet destruction, mediated by antibodies that bind to platelet antigens, combined with inadequate platelet production that is probably also immunologically mediated.[1,2] Corticosteroids are the standard initial treatment for adults with ITP, but relapse is common, with 70–90% of adults responding to initial corticosteroid treatment but approximately 50% of adults with newly-diagnosed ITP losing platelet response at 6 months after initial treatment.[3] Thrombopoietin (TPO) is the major regulator of platelet production, and acts by increasing platelet production by stimulating megakaryocyte colony-forming cells and increasing the number, size and ploidy of megakaryocytes.[4] One first-generation recombinant human TPO molecule was shown to increase platelet counts in adults, but clinical investigation was stopped when it was discovered that antibodies developed against this molecule and also neutralised native TPO, leading to thrombocytopenia.[5,6]

The second-generation TPO receptor agonist, romiplostim, is a fragment crystallisable (Fc)-peptide fusion protein (peptibody) composed of a human immunoglobulin G1 (IgG1) Fc domain, with each single-chain subunit covalently linked at the C-terminus to a peptide chain containing two TPO mimetic receptor-binding peptides (TMP).[7] Romiplostim has been shown to increase platelet counts in both adults and children with ITP who have had an insufficient

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response to corticosteroids, immunoglobulins or splenectomy.[8] Although romiplostim has no amino acid sequence homology to native TPO, a theoretical risk exists for the formation of antibodies against romiplostim that could also bind native TPO, potentially leading to loss of response. To examine this possibility, we conducted a retrospective analysis of immunogenicity results from adults with ITP in 13 completed prospective clinical trials of romiplostim, as well as requests for immunogenicity testing among romiplostim-treated patients with loss of response that were spontaneously submitted from 18 countries to a global postmarketing registry.

Materials and methods

Data source

The retrospective analysis included adults aged ≥18 years with ITP who received romiplostim in 13 completed prospective romiplostim clinical trials (Table I).[9–19] In each trial, romiplostim was administered subcutaneously once weekly, at a starting dose of 1 μg/kg in most cases, and titrated between 0 and 10 μg/kg (0 and 15 μg/kg in three trials[11,12]) to maintain platelet counts of 50–200 × 10^9/l (up to 450 × 10^9/l in early trials[9]). For the early trial by Newland *et al.*,[15] romiplostim was administered to four dose cohorts of 30, 100, 300 and 500 μg. Immunogenicity was assessed at baseline and at scheduled intervals, typically before and after treatment in shorter trials and every 12–24 weeks in longer trials. Procedures in each trial were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975.

In clinical practice, when a patient fails to maintain a platelet response to romiplostim, a search is recommended for causative factors, including the development of neutralising antibodies. Blood samples submitted spontaneously to the manufacturer for the detection of antibodies to romiplostim and native TPO were recorded in the global postmarketing registry. Antibody testing results were shared with treating physicians.

Immunogenicity testing

Immunogenicity assays were validated and described in detail previously.[20] A stepwise process was followed to assess immunogenicity to romiplostim or TPO (Fig 1). In step 1, the sample was screened for binding antibodies by surface plasmon resonance immunoassay (SPRIA). Detection limits were 400 ng/ml for anti-romiplostim antibodies (detection sensitivity was 133 ng/ml for anti-romiplostim antibodies and 23 ng/ml for anti-TMP antibodies) and 200 ng/ml for anti-TPO antibodies. In step 2, samples with detected binding antibodies were assessed by SPRIA for drug-specific antibodies. If the addition of romiplostim or TPO reduced binding by ≥50%, then the sample was considered positive for drug-specific binding antibodies. In step 3, samples confirmed positive for binding antibodies were tested for neutralising antibodies by using a murine cell line transfected with the human TPO receptor gene, which proliferates when cultured with romiplostim or TPO. Inhibition of TPO-

Table I. Clinical trials of romiplostim in adults with ITP included in the analysis.

References	Study number	NCT registration*	Study design	Control	Number that received romiplostim
Bussel *et al.*[9]	20000137	00111475	Phase 1	None	24
Bussel *et al.*[9]	20000137	00111475	Phase 2	Placebo	17
Newland *et al.*[15]	20010218	00117143	Phase 1–2	None	16
Shirasugi *et al.*[17]	20050162	00305435	Phase 2	None	12
Newland *et al.*[16]	20080435	01143038	Phase 2	None	75
Kuter *et al.*[12]	20030105	00102323	Phase 3	Placebo	42
Kuter *et al.*[12]	20030212	00102336	Phase 3	Placebo	42
Kuter *et al.*[14]	20060131	00415532	Phase 3	Standard care	156
Shirasugi *et al.*[19]	20060216	00603642	Phase 3	Placebo	22
Janssens *et al.*[11]	20040209	00508820	Phase 3	None	406
Janssens *et al.*[10]	20080009	00907478	Phase 4	None	169
Kuter *et al.*[13]	20030213	00116688	Extension	None	291†
Shirasugi *et al.*[18]	20060113	00440037	Extension	None	44‡
				Total	1046

ITP, immune thrombocytopenia.

*Registration number at www.clinicaltrials.gov

†In the open-label extension study 20030213, n = 238 patients were treated with romiplostim in previous studies, and n = 53 patients received placebo or standard-of-care treatment in previous studies and were treated with romiplostim for the first time in this study.

‡In the open-label extension study 20060113, n = 11 patients were treated with romiplostim in study 20050162, n = 21 patients were treated with romiplostim in study 20060216 and n = 12 patients received placebo in study 20060216 and were treated for the first time with romiplostim in this study.

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induced proliferation by at least 25·9%, or inhibition of romiplostim-induced proliferation by at least 16·0%, was a sign of neutralising activity. Samples with neutralising activity were then treated with a Protein G+Protein L bead mixture (Protein G/L) to remove all human immunoglobulins, including IgG. Samples with neutralising activity that then increased TPO-induced or romiplostim-induced cell proliferation by at least 23·7% following incubation with Protein G/L were considered positive for neutralising antibodies. Statistical methods were described in detail previously.[20]

Statistical methods

Patient incidences of binding antibodies and neutralising antibodies were determined for each data source. For clinical trials, baseline demographic and clinical characteristics were summarised for patients with *versus* without development of anti-romiplostim-binding antibodies post-baseline; 95% confidence intervals (CIs) examined which characteristics differed between the cohorts. For each patient who developed anti-romiplostim-binding antibodies in a clinical trial, a data listing included romiplostim doses and platelet counts over time, treatment-emergent adverse events of bleeding [defined as at least one event from the Medical Dictionary for Regulatory Activities (MedDRA) haemorrhages (Standardised MedDRA Query)] or hypersensitivity (defined as at least one event from the Standardised MedDRA Queries for anaphylactic reaction, anaphylactic-anaphylactoid shock conditions, angioedema or hypersensitivity) within 30 days after antibody detection, the proportion of weekly platelet responses ($\geq 50 \times 10^9$/l without rescue medication use in the prior 8 weeks) after antibody detection and occurrence of subsequent antibodies. The global postmarketing registry was to focus on patients for whom positive neutralising antibodies (to romiplostim or TPO) were detected; data listings were not available for spontaneously submitted samples.

Results

Antibodies to romiplostim or TPO in clinical trials

A total of 1,046 romiplostim-treated patients from 13 completed clinical trials were available for analysis (Fig 2). At baseline, 958 patient samples were collected and tested. 35 patients (3·7%) tested positive for romiplostim-binding antibodies at baseline. Post-baseline, 961 patient samples were collected and tested. 80 patients (8·3%) tested positive for romiplostim-binding antibodies post-baseline, including 20 patients (2·1%) who also had positive results at baseline and 60 patients (6·2%) with positive results only post-baseline.

Characteristics of patients who did and did not develop romiplostim-binding antibodies post-baseline are summarised in Table II. The development of anti-romiplostim-binding antibodies post-baseline was more frequent in patients with ITP duration of more than 3 years at baseline, prior splenectomy, history of allergies, lower baseline TPO levels, lower baseline platelet counts and a higher number of previous treatments for ITP. However, only a history of allergies had non-overlapping 95% CIs at baseline to indicate a statistically significant difference between patients who did or did not develop anti-romiplostim-binding antibodies post-baseline. The 95% CIs for percentages and interquartile ranges for medians overlapped between the cohorts for all other characteristics that were examined, suggesting that baseline characteristics including age, sex, race, prior medical history, prior treatments, baseline platelet counts and baseline TPO levels or TPO-binding antibodies did not differ with statistical significance between patients who developed

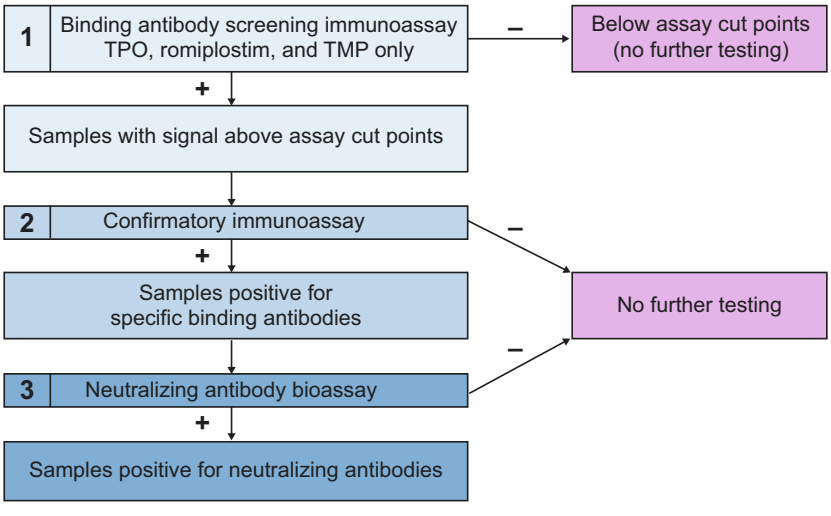

Fig 1. Strategy for assessment of immunogenicity. [Colour figure can be viewed at wileyonlinelibrary.com]

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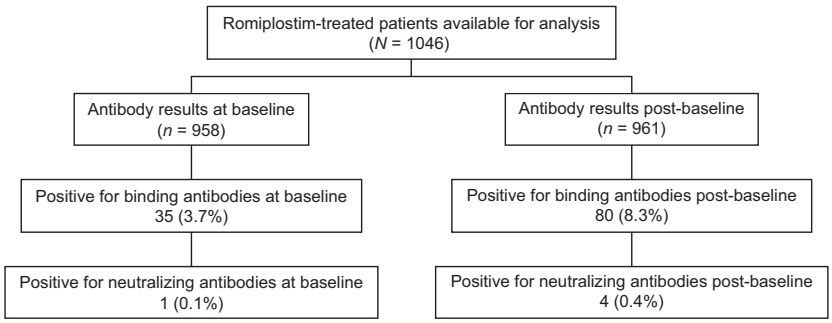

Fig 2. Romiplostim antibody results from clinical trials.

anti-romiplostim-binding antibodies post-baseline and patients who did not.

For the 60 patients who developed post-baseline binding antibodies to romiplostim, the median time of the first positive result was week 14 (range, week 6–123; Table III). When antibodies were first detected, the median dose of romiplostim was 2 μg/kg (range, 1–15) and the median platelet count was 29·5 × 10^9/l (range, 0–483 × 10^9/l). 47 of 60 patients had platelet counts reported after the first positive anti-romiplostim-binding antibody, with a majority of subjects showing platelet response without rescue medication use (median platelet response after first antibody detection 98·5%). (13 of 60 patients did not have follow-up platelet counts after a positive immunogenicity test, nine patients were positive at the end of study assessment and two patients had received rescue medication.) The platelet profile for a sample patient with anti-romiplostim-binding (but not romiplostim-neutralising) antibodies is shown in Fig 3. Treatment-emergent adverse events of bleeding and hypersensitivity occurred in 16 of 60 patients (26·7%) after antibody detection. At their final analysis, 36 patients (60·0%) no longer demonstrated binding antibodies, but 24 (40·0%) continued to have positive binding antibodies. Of these 24 patients, 16 (66·7%) were positive for both anti-romiplostim and anti-TMP antibodies at the first positive test, two (8·3%) were positive only for anti-romiplostim

Table II. Characteristics of adults with ITP with post-baseline antibody results in clinical trials.

	Developed romiplostim-binding antibodies post-baseline			
	Yes (*n* = 60)		No (*n* = 901)*	
Characteristic	*n* (%) or median	(95% CI) or [Q1, Q3]	*n* (%) or median	(95% CI) or [Q1–Q3]
Female	39 (65%)	(52–77%)	562 (62%)	(59–66%)
Median age, years	53	[41, 63]	53	[39, 66]
Race, white	49 (82%)	(70–91%)	789 (88%)	(85–90%)
Median baseline platelet count, ×10^9/l	12·5	[6, 23]	21	[11, 35]
ITP duration (>3 years)	42 (70%)	(57–81%)	517 (57%)	(54–61%)
Prior splenectomy	29 (48%)	(35–62%)	338 (38%)	(34–41%)
Median baseline TPO levels, pg/ml	85	[43, 135]	105	[60, 143]
Baseline TPO-binding antibodies	2 (3%)	(0·4–12%)	21 (2%)	(1–4%)
Baseline TPO-neutralizing antibodies	0 (0%)	(0–6%)	1 (0·1%)	(0·1–0·6%)
Median no. of previous ITP treatments	3	[2, 5]	2	[1, 4]
Prior rituximab use	4 (7%)	(2–16%)	1 (0·1%)	(0–0·6%)
Prior corticosteroid use	4 (7%)	(2–16%)	96 (11%)	(9–13%)
Medical history‡				
Allergies	13 (22%)	(12–34%)	76 (8%)	(7–10%)
Systemic lupus	1 (2%)	(0–9%)	5 (0·6%)	(0·2–1%)
Autoimmune disease/immunodeficiency	0 (0%)	(0–6%)	4 (0·4%)	(0·1–1%)
Bone marrow-associated disorder/pain†	5 (8%)	(3–18%)	28 (3%)	(2–5%)
Liver disorder	4 (7%)	(2–16%)	30 (3%)	(2–5%)
Thyroid disease	2 (3%)	(0·4–11%)	19 (2%)	(1–3%)
Kidney disorder	1 (2%)	(0–9%)	9 (1%)	(0·5–2%)

CI, confidence interval; ITP, immune thrombocytopenia; TPO, thrombopoietin; Q1, first quartile; Q3, third quartile.

*Includes *n* = 20 patients who had romiplostim antibodies both at baseline and post-baseline.

†Includes pain at bone marrow biopsy site, increased bone marrow reticulin, and bone marrow reticulin fibrosis.

‡Medical history groupings were based on clinical input.

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antibodies and six (25·0%) were positive only for anti-TMP antibodies.

One of 958 patients (0·1%) tested positive for neutralising antibodies to romiplostim at baseline; all post-baseline results in this patient were negative for neutralising antibodies, and this patient achieved a platelet response for 8 out of 10 weeks. The emergence of new anti-romiplostim-neutralising antibodies in four of 961 patients (0·4%) post-baseline did not appear to be related to romiplostim dose or platelet count (Table IV). Platelet profiles for the four patients who tested positive for neutralising antibodies to romiplostim are shown in Fig 4. Patients 15, 25 and 60 developed anti-romiplostim-neutralising antibodies and remained positive, whereas Patient 54 developed anti-romiplostim-neutralising antibodies at week 30, then turned negative after week 52. Two patients continued romiplostim treatment after the detection of neutralising antibodies and both continued to have platelet counts of $\geq 50 \times 10^9$/l. Patient 25 had platelet counts that dropped below 50×10^9/l when the neutralising antibodies were detected; increasing the romiplostim dose to 10 μg/kg/week and initiating rescue medication restored platelet counts to above 50×10^9/l through the last assessment. Patient 54 maintained platelet counts above 50×10^9/l for more than 2 years after the neutralising antibodies were detected, without rescue medication use. This patient decreased the dose of romiplostim, then discontinued romiplostim completely while maintaining platelet counts above 50×10^9/l. In all 5 patients, neutralising antibodies to romiplostim were directed against the peptide component (TMP) of romiplostim and did not neutralise native TPO.

At baseline, 31 of 956 patients (3·2%) tested positive for anti-TPO-binding antibodies. One patient (0·1%) tested positive for neutralising antibodies to TPO and had a low TPO level at baseline (31·25 pg/ml). This one patient did not have binding or neutralising antibodies to romiplostim at baseline and had 111 out of 121 (91·7%) weeks of platelet response to romiplostim. Post-baseline, 33 of 960 patients (3·4%) tested positive for anti-TPO-binding antibodies and no patients tested positive for neutralising antibodies to TPO. Of these 33 patients, 31 were negative at baseline but positive post-baseline, and all are considered *de novo*-positive based on validation assay criteria. Two of the 33 patients (6·1%) had tested positive for anti-TPO-binding antibodies at baseline. Six patients (0·6%) who had binding antibodies to TPO after baseline also had binding antibodies to romiplostim after baseline; none of these patients had anti-romiplostim-neutralising antibodies after baseline.

Antibodies to romiplostim or TPO in the postmarketing registry

Of 184 adult patients for whom spontaneous requests for antibody testing of blood samples were submitted between May 2009 and May 2016, nine patients (4·9%) tested positive for binding antibodies to either romiplostim, TMP or TPO: seven patients (3·8%) had binding antibodies to romiplostim or TMP, two patients (1·1%) had anti-TPO-binding antibodies, and two patients (1·1%) had both anti-romiplostim- and anti-TPO-binding antibodies. For these two patients with both anti-romiplostim- and anti-TPO-binding antibodies, therapeutic response decreased and, in one case, a platelet count decrease was reported.

One patient (0·5%) tested positive for anti-romiplostim-neutralising antibodies. This patient received romiplostim for 11 months at a dose of 2 μg/kg and then experienced an abrupt fall in platelet count, even as romiplostim dose was increased to 10 μg/kg. Romiplostim was discontinued and the patient was switched to alternative therapy. A follow-up sample, approximately 7 months later, tested negative for anti-romiplostim-neutralising antibodies. No patient in the registry tested positive for anti-TPO-neutralising antibodies.

Discussion

The analysis of adult patients with ITP who participated in 13 clinical trials of romiplostim showed that 60 of 961 patients (6·2%) developed new anti-romiplostim-binding antibodies post-baseline. Another 35 patients had anti-romiplostim-binding antibodies at baseline, including 20 patients who had anti-romiplostim-binding antibodies at least once post-baseline and 15 patients with evidence of transient anti-romiplostim-binding antibodies only at baseline. Of the 60 patients who developed new anti-romiplostim-binding antibodies post-baseline, 48 had repeat testing a median of 12 weeks later. Of these, only 30% had another positive antibody test subsequently, providing further evidence of the transient nature of anti-romiplostim-binding antibodies. Platelet counts and adverse events within 30 days after the first antibody detection provided no evidence that binding antibodies against romiplostim were associated with reduced platelet response; there was no obvious pattern of romiplostim dose increase when immunogenicity to romiplostim was detected. 16 out of 60 patients (26·7%) experienced bleeding or hypersensitivity events (subject incidence: 13/60 bleeding; 5/60 hypersensitivity; 2/60 both).

Our examination of patient characteristics before and during romiplostim treatment suggested that patients with indicators of more severe disease (longer duration of ITP, prior splenectomy and a higher number of previous ITP treatments) may have higher incidences of developing anti-romiplostim-binding antibodies. However, these and other baseline characteristics did not differ statistically between the patients with or without post-baseline binding antibodies against romiplostim. In the absence of evidence showing that anti-romiplostim-binding antibodies influence clinical outcomes in adults, the ability to predict which patient will be more likely to develop antibodies has limited clinical utility.

Antibody testing followed a sequential analysis approach, wherein samples that were confirmed to bind to romiplostim were tested further for their neutralising activity against

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Table III. Listing of adults who developed romiplostim-binding antibodies post-baseline in clinical trials.

Patient	At first binding antibody				After first binding antibody
	Week	Dose	Platelets (10^9/l)	Adverse events*	% Platelet responses†
1	116	1·0	12		265/296 (89·5%)
2	123	1·0	12		
3	109	1·0	34		270/274 (98·5%)
4	90	1·0	34		276/276 (100·0%)
5	99	1·0	10	Rash	261/265 (98·5%)
6	45	1·0	10	Injection site rash	20/40 (50·0%)
7	30	1·0	24		266/272 (97·8%)
8	99	1·0	24	Epistaxis, injection site bruising	
9	40	1·0	26		57/270 (21·1%)
10	31	1·0	26	Contusion	269/271 (99·3%)
11	22	1·0	22	Thrombocytopenia	106/129 (82·2%)
12	18	1·0	22		7/8 (87·5%)
13	111	1·0	18	Blood blister	75/81 (92·6%)
14	69	1·0	18	Allergic sinusitis	
15	104	2·0	6	Epistaxis	76/79 (96·2%)
16	8	9·0	7		187/234 (79·9%)
17	8	2·0	24	Skin hemorrhage, epistaxis	208/208 (100·0%)
18	8	11·0	14	Injection site bruising	11/233 (4·7%)
19	9	2·0	14		208/208 (100·0%)
20	8	11·0	26	Hypersensitivity, postmenopausal hemorrhage	1/173 (0·6%)
21	16	1·0	26	Hemoptysis (haemorrhages)	
22	9	2·0	2		67/81 (82·7%)
23	9	1·0	2		224/224 (100·0%)
24	9	4·0	2	Epistaxis, mouth hemorrhage ecchymosis, petechiae, rash	1/21 (4·8%)
25	116	10·0	13		0/11 (0%)
26	8	1·0	28		188/188 (100·0%)
27	8	3·0	13		182/182 (100·0%)
28	83	6·0	31		
29	11	4·0	33		58/62 (93·5%)
30	66	10·0	260		
31	12	3·0	15		62/66 (93·9%)
32	17	3·0	11		121/121 (100·0%)
33	12	6·0	46		140/140 (100·0%)
34	10	7·0	90		200/206 (97·1%)
35	30	10·0	15		
36	58	3·0	112		
37	34	2·0	88		59/61 (96·7%)
38	6	5·0	77		228/231 (98·7%)
39	23	2·0	270		48/48 (100·0%)
40	11	3·0	53		56/56 (100·0%)
41	12	3·0	483		131/131 (100·0%)
42	11	3·0	166		101/101 (100·0%)
43	11	2·0	3		128/128 (100·0%)
44	12	5·0	167		104/111 (93·7%)
45	11	6·0	216		
46	12	3·0	60		94/98 (95·9%)
47	11	3·0	74	Epistaxis	108/108 (100·0%)
48	11	10·0	230		
49	11	5·0	151	Hemolysis	113/113 (100·0%)
50	12	3·0	136		168/171 (98·2%)
51	12	8·0	137		129/131 (98·5%)
52	12	10·0	310		
53	23	1·0	71	Epistaxis, gingival bleeding	57/57 (100·0%)

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Table III. (*Continued*)

Patient	At first binding antibody				After first binding antibody
	Week	Dose	Platelets (10^9/l)	Adverse events*	% Platelet responses†
54	30	6·0	199		43/43 (100·0%)
55	23	2·0	73		131/132 (99·2%)
56	23		0		9/9 (100·0%)
57	23	2·0	49		132/136 (97·1%)
58	23	5·0	89		129/133 (97·0%)
59	59	8·0	168		
60	53	3·0	30		
Median	14	2	29·5	–	98·5%
Range	6–123	1–11	0–483	–	0–100%

*Bleeding or hypersensitivity adverse events that started or worsened within 30 days from the sample collection date of the positive antibody result.

†Platelet response was defined as a weekly platelet count ≥50 × 10^9/l during the treatment period without a rescue medication in the past 8 weeks.

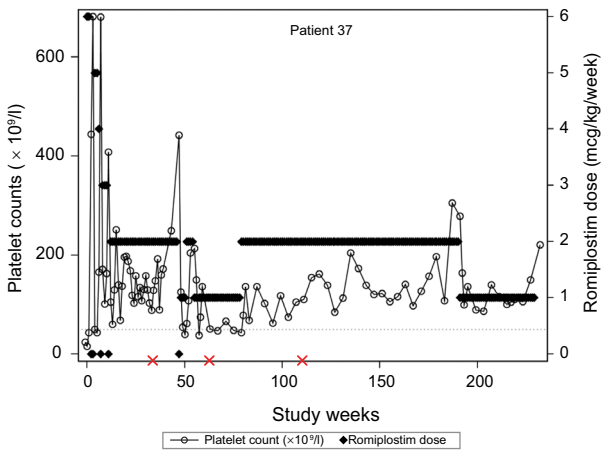

Fig 3. Sample platelet profile from Patient 37, who developed romiplostim-binding (but not romiplostim-neutralizing) antibodies post-baseline in a clinical trial. Red "x" represents time of antibody detection. [Colour figure can be viewed at wileyonlinelibrary.com]

romiplostim. Development of anti-romiplostim-neutralising antibodies was uncommon in the clinical trials, occurring in only four of 961 patients (0·4%) with prospective, scheduled testing. When neutralising antibodies to romiplostim were identified in these patients, they did not cross-react with native TPO, and no patient in the clinical trials had anti-TPO-neutralising antibodies post-baseline. Two patients continued romiplostim after the neutralising antibodies to romiplostim were detected, and both patients who continued had subsequent platelet counts of ≥50 × 10^9/l. One patient reported Grade 1 and 2 bleeding events both prior to and after neutralising antibody detection, and also reported rescue medication use; the other patient did not report either bleeding events or rescue medication use after neutralising antibody detection. The observation of a transient neutralising anti-drug antibody response against romiplostim is related to its generally weak neutralising capacity. As with many other protein therapeutics, it is not unexpected and is a known phenomenon. The exact mechanism for romiplostim is not well understood, but may be due to a lack of a robust T-dependent B cell response, limiting somatic mutation and isotype switching.[21]

Our examination of the antibody specificity in patients who developed a persistent anti-romiplostim-binding antibody response post-baseline in the clinical trials showed that most patients developed binding antibodies to both romiplostim and TMP, which indicated peptide-specific antibodies. A few patients developed binding antibodies that scored positive for romiplostim binding but negative for TMP binding, suggesting that some anti-romiplostim-binding antibodies are low-level and may be cross-reactive to the Fc domain; or they scored negative to romiplostim, owing to a slightly more sensitive TMP assay surface. Despite the handful of discordant results, it suggests that these are all low-level binding antibodies, none of which demonstrated neutralising activity to romiplostim.

Data from 184 patients in a postmarketing registry also support a low risk of clinically significant immunogenicity, with respect to neutralising antibodies to romiplostim. The postmarketing registry comprises a selected subset of patients who received romiplostim in clinical practice that either demonstrated a suboptimal platelet response initially or lost their platelet response with continued treatment. In either case, the healthcare provider requested antibody testing. As the registry was established based on spontaneous postmarketing requests for antibody testing, this data source may not represent the general population of romiplostim patients. However, the referral of patient samples for which antibody positivity was suspected implies that the proportion we

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Table IV. Overview of adults who developed neutralizing antibodies to romiplostim post-baseline in clinical trials.

	At time of neutralizing antibody detection				After first antibody detection	
Patient	Week of treatment*	Platelet count, $\times 10^9$/l*	Romiplostim dose, μg/kg*	Cumulative romiplostim dose, μg/kg*	Received romiplostim	Weekly platelet responses†
25	116	13	10·0	973	Yes	0/11 (0%)‡
15	182	37	1·0	190	No	–
60	53	30	3	152	No	–
54	30, 37, 52	199	6·0	104	Yes	43/43 (100%)

*Week of treatment, platelet count, dose and cumulative dose at the time antibodies were detected.
†Platelet response was defined as a weekly platelet count ≥50 × 10^9/l during the treatment period without a rescue medication in the past 8 weeks.
‡After antibody detection, all platelet counts were ≥50 × 10^9/l for this patient, but rescue medication was also given during this time.

observed in this sample is unlikely to be lower than what would be observed in the general patient population. Although baseline antibody testing was not performed in any of these samples, a previous report has shown that romiplostim and TPO-binding antibodies may be observed in patients before romiplostim exposure (7% and 5%, respectively).[20] That previous report postulated that pre-existing binding antibodies in patients with ITP could be attributed to a sensitive screening assay capable of detecting low-affinity binding antibodies, as well as the autoimmune disease state associated with ITP.[22]

Carpenedo *et al.* reported antibody testing results for four adult patients with ITP who were treated with romiplostim and had lost response to treatment.[23] This information would therefore have been captured in the postmarketing registry. The authors of that case series stated that three of four samples tested positive for anti-romiplostim antibodies, two of three patients tested negative for anti-romiplostim antibodies on subsequent retesting and none of these antibodies cross-reacted with endogenous TPO. However, data from the postmarketing registry identified only one adult subject who tested positive for anti-romiplostim-neutralising antibodies. To address this discrepancy, we conducted a review of the Carpenedo *et al.* case series data and found that one of the patients developed anti-romiplostim-neutralising antibodies but was a subject from clinical trial 20080435 (NCT01143038) and was not part of the postmarketing registry. The remaining two patients were both confirmed to be part of the postmarketing registry; one of the patients developed binding antibodies but did not develop neutralising antibodies to romiplostim, and the other patient developed neutralising antibodies to romiplostim.

Clinicians may question whether their ITP patients treated with romiplostim should be tested for neutralising antibodies. In cases where there is a loss of response or failure to maintain a platelet response with romiplostim within the recommended dosing range, clinicians should search for causative factors, including immunogenicity and increased bone marrow reticulin.

A potential limitation of our analysis was the fluctuation of platelet levels in adults with ITP due to disease state and varying rates of turnover in the bone marrow and spleen, which could complicate the interpretation of platelet response rates after the development of binding or neutralising antibodies to romiplostim. Prior use of rituximab or corticosteroids at baseline was not different between the patients with or without development of post-baseline antibodies to romiplostim, but the number of patients with prior use of these medications was small and the studies that were included in this retrospective analysis were not designed to examine this relationship.

In the analysis of prospective clinical trial data, the incidence of binding antibodies to romiplostim developed infrequently, the majority of which were transient in nature. A few patients positive for binding antibodies developed

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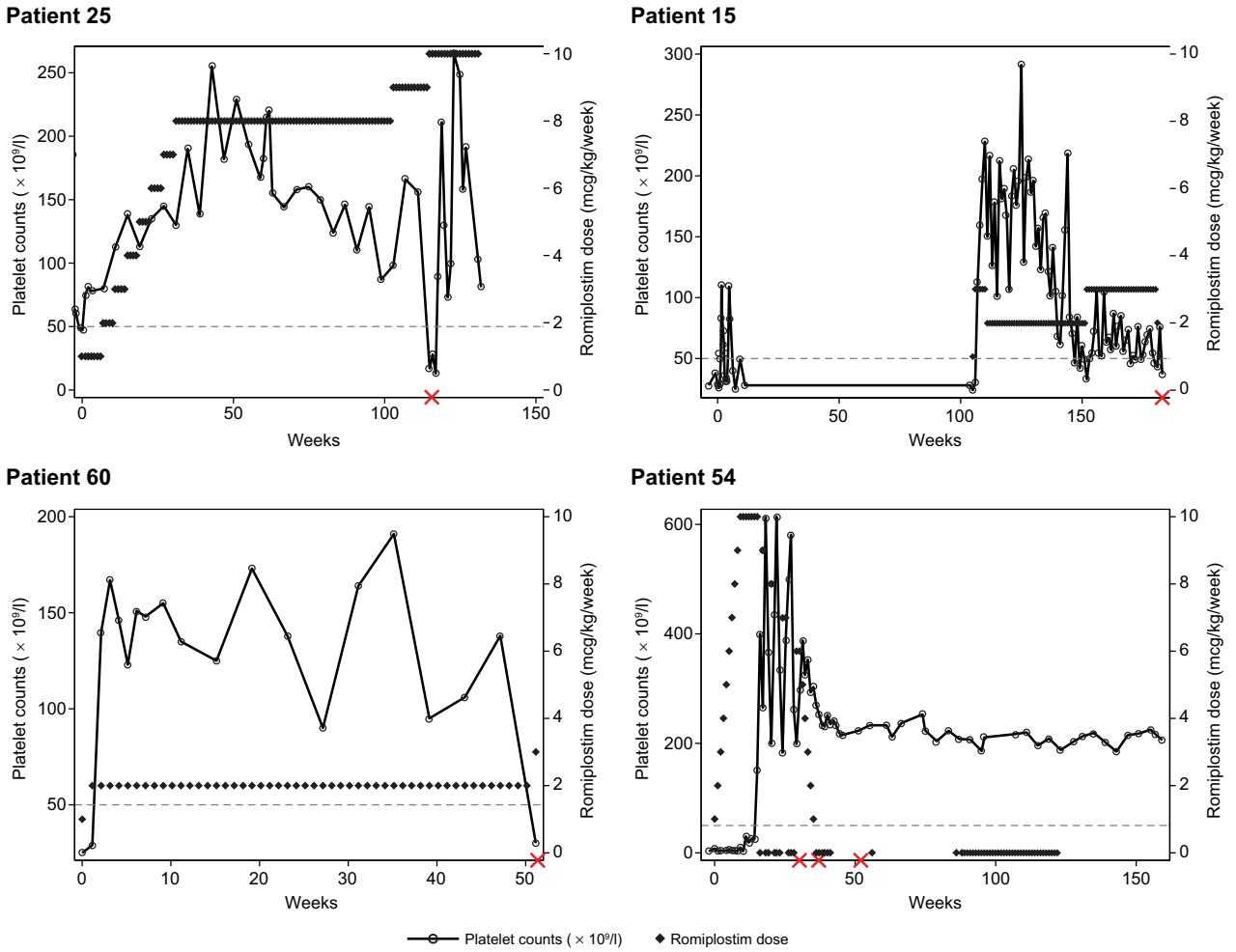

Fig 4. Platelet profiles for patients who developed romiplostim-neutralizing antibodies post-baseline in clinical trials. Red "x" represents the time-point at which the sample was taken in which romiplostim-neutralizing antibodies were detected. [Colour figure can be viewed at wileyonlinelibrary.com]

neutralising antibodies (0·4%). Most importantly, neutralising antibodies to romiplostim did not cross-react with TPO, and they were not associated with loss of response. Conversely, 95% of the patients in the postmarketing setting for whom a sample was submitted did not have evidence of antibodies to romiplostim when they responded suboptimally to romiplostim. In either setting, cross-reactive antibodies to native TPO, which halted clinical development of one first-generation recombinant TPO molecule, were not observed for the second-generation TPO receptor agonist, romiplostim. Collectively, these results suggest that immunogenicity to romiplostim occurs infrequently, and is generally not associated with loss of platelet response or other negative clinical implications in adults with ITP.

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Data sharing

Qualified researchers may request data from Amgen clinical trials. Complete details are available at http://www.amgen.com/datasharing.

Author contribution

All authors contributed to the analysis of data, interpretation of the results, drafting and editing of the manuscript, and have approved submission of the manuscript.

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Conflict of interest

D.T.M., J.K.P., J.K., T.E.B. and A.B. are employees and stockholders of Amgen Inc. V.J. was an employee and stockholder of Amgen Inc. when the analysis was initiated. D.J.K. has received research funding from Syntimmune, Bristol-Myers Squibb, Rigel, Principia and Protalex, and is a consultant for Amgen Inc., Novartis, Pfizer, Genzyme, Zafgen, Syntimmune, Fujifilm, Argenx, Alnylam, Shionogi, Dova, ONO, 3SBio, CRICO, Shire, Protalex, Principia and Rigel.

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