



Rituximab in the treatment of immune thrombocytopenia: what is the role of this agent in 2019?

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ABSTRACT

The use of rituximab for the treatment of immune thrombocytopenia was greeted enthusiastically: it led to up to 60% response rates, making it, nearly 20 years ago, the main alternative to splenectomy, with far fewer side effects. However, long-term follow-up data showed that only 20-30% of patients maintained the remission. No significant changes have been registered using different dose schedules and timing of administration, while the combination with other drugs seemed promising. Higher response rates have been observed in young women before the chronic phase, but apart from that, other clinical factors or biomarkers predictive of response are still lacking. In this review we examine the historical and current role of rituximab in the management of immune thrombocytopenia, 20 years after its first use for the treatment of autoimmune diseases.

Introduction

Primary immune thrombocytopenia (ITP) is an autoimmune bleeding disorder due to a variable combination of increased platelet destruction and impaired platelet production, as a consequence of defects in central and/or peripheral immune tolerance which allow the escape of autoreactive lymphocytes.¹⁻³ B cells have a well-established role in the pathogenesis of the disease, as the source of antibodies directed against platelet-surface glycoproteins.⁴⁻⁶

Rituximab, a monoclonal antibody directed against CD20, a membrane glycoprotein expressed on the surface of B cells, was introduced for the treatment of B-cell lymphomas towards the end of the 1980s.⁷

Binding to an antigen that is only expressed on mature B cells, rituximab leads to a fast and deep, but reversible B-cell depletion.⁸ The transience of the B-cell depletion and the low toxicity profile represented the rationale for its use in the treatment of autoimmune conditions, especially those in which B-cell activity was considered the main pathogenic mechanism, such as ITP. Many studies have been carried out in this field: in monotherapy, with different dose schedules and in combination with other drugs, proving its efficacy, although some differences exist across certain studies.

Rituximab has also been explored in a number of other autoimmune auto-antibody-mediated diseases such as systemic lupus erythematosus,⁹ rheumatoid arthritis,¹⁰ autoimmune hemolytic anemia,¹¹ type II mixed cryoglobulinemia,¹² myasthenia gravis,¹³ multiple sclerosis,¹⁴ thrombotic thrombocytopenic purpura,¹⁵ Sjogren syndrome,¹⁶ pemphigus¹⁷ and others. Despite these extensive investigations, autoimmune conditions for which rituximab is licensed by the Food and Drug Administration and the European Medicines Agency are rheumatoid arthritis and ANCA-associated vasculitis.

In this review, we discuss the development and current role of rituximab in the management of ITP.

Pathophysiology of immune thrombocytopenia

The milestone role of autoantibodies in the pathogenesis of ITP was first reported in 1951 by Harrington *et al.*, who showed that the infusion of plasma from ITP

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patients into normal controls caused thrombocytopenia, thus imputing the cause of the disease to a plasma-derived factor.⁴ This “factor” was subsequently identified as an IgG anti-platelet antibody, directed against platelet glycoprotein (GP) IIb/IIIa and/or the GPIb-IX-V complex.⁵ Very rarely, antibodies against GPIa-IIa or GPIV can be found (5%).¹⁸

Antibody-opsonized platelets are then recognized through the Fc γ -receptors by macrophages in the spleen, liver and bone marrow, phagocytized and prematurely destroyed.¹⁹ Other mechanisms through which antibodies can mediate platelet destruction are complement deposition with intravascular lysis and induction of platelet apoptosis.^{20–23} Plasma from patients with ITP also inhibits megakaryocyte growth and function in the bone marrow.^{6,24}

The Ashwell-Morell receptors in hepatocytes have been invoked as a further pathogenic mechanism, because they physiologically remove desialylated, “old” platelets from the circulation. Anti-GPIb/IX autoantibodies are thought to enhance the desialylation of GPIb, increasing hepatic clearance of platelets.^{25,26}

Many abnormalities have been shown in T cells of patients with ITP: an altered Th1/Th2 balance, with an increased number of Th1 T-helper cells^{27,28} and a decrease in the number and function of regulatory T cells.^{29,30} The abnormal activation of cytotoxic CD8⁺ T cells may also have a role in the pathogenesis of ITP, contributing to both platelet destruction and impaired platelet production.³¹

Circulating thrombopoietin levels in ITP are not increased proportionally to the level of thrombocytopenia, and are usually normal or only slightly increased.³²

Early history of rituximab

In the late 1980s, the idea of using monoclonal antibodies that recognize tumor-associated antigens for the treatment of hematologic malignancies became reality, and rituximab became a well-tolerated and highly effective option initially used for patients with multi-refractory lymphoproliferative diseases.⁷ CD20, a transmembrane glycoprotein expressed on the surface of normal and malignant B cells, appeared ideal for targeted therapy, because it does not shed from the cell surface and is not internalized upon antibody binding.³³ CD20 is expressed from early pre-B to mature B lymphocytes, but is not expressed on hematopoietic stem cells, plasma cells or other cells of the body.³⁴ Rituximab is a type 1 IgG1- κ human-mouse chimeric monoclonal antibody directed against CD20, which acts through three mechanisms: complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity and induction of direct apoptosis of the target cell.³⁵

The first report of a case in which rituximab was used for the treatment of an autoimmune disease was published in 1998, when a patient with a cold agglutinin disease and a small IgM paraprotein was successfully treated with four weekly infusions of rituximab.³⁶ A few years later, in 2001, the first report of the successful use of rituximab for the treatment of ITP associated with a low-grade non-Hodgkin lymphoma was published.³⁷ Since then, rituximab has been widely used for the treatment of autoimmune manifestations associated with lymphoproliferative disorders, and, because it is a therapy borrowed from lymphomas, the dose schedule of 375 mg/m² weekly for 4

weeks also became the “standard dose” of rituximab in autoimmune diseases.

Since CD20 is also expressed in normal B cells, there was a strong rationale for using rituximab to deplete pathological antibodies in autoimmune diseases. One of the first cases, published in 2000, was that of a young man with refractory myasthenia gravis who, after four standard doses of rituximab, experienced a complete clinical response with disappearance of anti-acetylcholine receptor antibodies, and he did not relapse.¹⁵ This case was an impressive proof of principle that transient B-cell depletion secondary to rituximab treatment may positively modulate the immune system, inhibit the production of autoantibodies, and cause long-term clinical improvement.

Rituximab in immune thrombocytopenia

In 2001, Stasi *et al.* reported the results of the first prospective study in which 25 patients with chronic ITP were treated with four weekly infusions of rituximab at a dose of 375 mg/m². The overall response rate (ORR) was 52%, with 28% sustained responses. No clinical or laboratory parameters were found to predict treatment response, but they noticed that women and younger patients had a better chance of response, and that, in some patients who relapsed, retreatment was effective.³⁸

Subsequent studies showed that the overall initial response to rituximab used as second- or further-line of therapy ranges between 52% and 73%, with the complete response (CR) rate ranging between 20% and 54%^{39–48, 56, 58, 68} (Table 1). In a systematic review including 313 adult patients with chronic ITP, a response rate of 62.5% and a CR rate of 46.3% were found, with a median duration of response of 10.5 months.⁴²

Three different patterns of response can be distinguished: a first group of patients respond rapidly, within the first month; in the second group, the platelet count starts increasing after 3–4 weeks, and a CR is achieved within 8 weeks after treatment; in the third group of patients, the platelet count increases very slowly, only reaching normal values 3 months after therapy.⁴⁶

Only two randomized, placebo-controlled studies have been performed. One very small pilot randomized trial compared rituximab with placebo as second-line therapy. No difference in terms of treatment failure (65.6% vs. 80.8%)⁴⁷ was found between the two groups. Overall platelet count response (i.e. platelet count $\geq 30 \times 10^9/L$) was achieved by 62.5% of the patients in the rituximab group and 73.1% in the placebo group at 6 months.

In the much larger RITP trial,⁴⁸ the rate of treatment failure was not significantly different between patients given rituximab or placebo (58% vs. 69%), and the ORR was 81% in the rituximab group compared to 73% in the placebo group. Improvements in platelet counts were seen up to week 72 in the rituximab arm.

Rituximab doses

“Standard dose” rituximab results in a marked reduction of malignant and non-malignant B cells in peripheral blood and bone marrow.⁴⁹ Since the total mass of B cells is much smaller in patients with ITP than in patients with lymphoma, it was not clear whether a lower dose of rituximab or a different schedule could be equally effective.

Potential advantages of the lower dose include avoidance of severe side effects, steroid-sparing effects, the greater possibility of administering repeated courses, and decreased cost. Zaja *et al.* investigated the efficacy of rituximab given at a dose of 100 mg (“low dose”) weekly for 4 weeks in 48 ITP patients.⁵⁰ In an indirect, non-randomized comparison, both the initial response (ORR 60.5% and CR 39.5%) and the duration of response (12- and 24-month cumulative relapse-free survival rates of 61% and 45%, respectively) were moderately lower in this group than in patients treated with “standard dose”. The time to response was also longer than that observed with the “standard dose”. This may be due to the fact that the depth of B-cell depletion reached in peripheral blood might not correlate with the depletion in other organs, and the 100 mg dose is probably not enough.

A recent UK study retrospectively compared 113 patients who received “standard dose” rituximab to 169 who received the “low dose”. They found that the low dose was not significantly different from the standard dose with regards to ORR (at 2 months, 56% vs. 59%; at 6 months, 62% vs. 64%), time to maximum platelet count (77 vs. 74 days), time to next treatment (4.6 vs. 4.3 months) and duration of response.⁵¹

Some groups explored a fixed dose of rituximab, 1000 mg given twice on days 1 and 15, which is the dose schedule licensed for rheumatoid arthritis. Khellaf *et al.*, in a prospective registry including 248 patients, compared the “standard dose” to the “rheumatoid arthritis-like” regimen. They did not find any difference in terms of initial or long-term response between the two groups.⁴¹ In a multicenter, single-arm study (R-ITP1000 study), Tran *et al.*

found that the “rheumatoid arthritis-like” fixed dose led to an ORR of 44% at week 8 in patients with relapsed/refractory ITP.⁵²

A multicenter, randomized, phase II Dutch trial compared three rituximab dosing schemes in 156 patients with relapsed or refractory ITP: “standard dose” rituximab, two weekly 375 mg/m² doses and two weekly 750 mg/m² doses. Response rates were similar within the three arms (63%, 59% and 61%, respectively), with a relapse-free survival of 72% at 1 year and 58% at 2 years.⁵³

The results of the most relevant studies with different dosing schedules of rituximab are summarized in Table 2.

Factors predictive of response

Over the years, multiple factors have been investigated with the aim of predicting response to treatment. Several studies highlighted the correlation of age and gender with outcome. In the very first study, Stasi had already pointed out that women and young patients had better responses.³⁸ This finding was subsequently extended by Bussel *et al.*,⁵⁴ who showed that women of child-bearing age whose duration of ITP was less than 24 months had a long-term response comparable with that obtained after splenectomy (60% long-term treatment-free responses). Another study with the same treatment schedule pointed out that adolescent females with an ITP duration of less than 12 months had the longest duration of response.⁵⁵ Similar results were also reported with rituximab alone: young (<40 years) women had a significantly higher probability of achieving a response (73%), a complete response (56%), and as well as a better long-term response (47% after 72 months) compared with the other groups.⁵⁶ It must be noted, however,

Table 1. Most relevant studies with rituximab administered at a standard dose of 375 mg/m² weekly for 4 weeks in patients with immune thrombocytopenia.

Author	Number of patients	Median age, years (range)	F:M	ITP phase	ORR	6 months	12 months	24 months	36 months	Last follow-up
Stasi 2001 ³⁸	25	46 (22-74)	64%:36%	Chronic	52% (20% CR) ^o	NA	NA	NA	NA	NA
Cooper 2004 ⁴⁶	57	46 (21-79)	68%:32%	Chronic	54% (32% CR) ^o	NA	NA	NA	NA	32% at a median FU of 72 weeks (18 months)
Zaja 2006 ^{5,58}	37	NA	NA	Median ITP duration: 34.5 (1-264) months	73% (54% CR) ^o	NA	NA	NA	NA	40.5% at a median FU of 25 months (3-55)
Medeot 2008 ⁶⁸	26	55 (18-76)	81%:19%	Median ITP duration 34.5 (4 - 264) months	69% (54% CR) ^o	NA	55%	45%	41%	35% at a median FU of 57 months
Godeau 2008 ^{5,39}	60	48 (18-84)	67%:33%	Chronic	NA	NA	40% (30% CR) ^o	33%	NA	NA
Cervinek 2012 ^{5,43}	114	55 (21-89)	53%:47%	16% ND; 84% chronic or persistent phase	NA	72% (48% CR)*	ORR 69% (45% CR)	NA	NA	NA
Patel 2012 ^{5,40}	72	39 (18-78)	65%:35%	Most chronic phase	57% ^o	NA	38%	31%	NA	21% at 5 years FU
Arnold 2012 ^{5,47}	33	40 (30-59)	58%:42%	50% ND	NA	62.5% (53% CR)*	NA	NA	NA	NA
Mahevas 2013 ^{5,45}	61	52 (34-70)	64%:36%	18% ND; 26% persistent phase; 56% chronic phase	54% (32% CR) at 3 months*	NA	36% (28% CR)	NA	31% (26% CR) median FU of 36 months	NA
Khellaf 2014 ^{5,41}	173	51 (21-71)	64%:36%	56% chronic	62%*	80%	62%	50%	38%	NA
Ghanima 2015 ^{5,48}	55	46 (27-61)	73%:27%	33% ND; 24% persistent phase; 44% chronic phase	73% (51% CR)*	60%	45%	NA	NA	24% at 78 weeks FU
Marangon 2017 ^{5,56}	103	46 (15-82)	59%:41%	Median ITP duration 20 (1 - 403) months	55% (36%CR)*	NA	NA	42%	40%	21% at 96 months

F:M: female to male ratio; ITP: immune thrombocytopenia; ORR: overall response rate; CR: complete response; NA: not available; FU: follow-up; ND: newly diagnosed. ^oConsidering as denominator all the patients. ^{*}Considering as denominator only patients who responded to rituximab. ^{*}response = platelet count $\geq 30 \times 10^9/L$. ^oresponse = platelet count $\geq 50 \times 10^9/L$

that in several other studies the predictive role of age and gender could not be confirmed.^{40,41,43,46,57} A disease duration of less than 12 months has also been frequently related to better outcomes,^{41,43,54,58} as well as the achievement of complete remission,^{39,40,46,54,56} while the influence of a previous splenectomy is not completely clear.^{40,46}

The role of antiplatelet autoantibodies (APA) as factors predictive of response is controversial. Differently from other autoimmune diseases, APA in ITP are neither very specific nor sensitive, and for this reason APA are not currently recommended as a diagnostic test for ITP.^{59,60} A reduction of APA levels has been associated with an increase in platelet count,⁶¹ and the presence of platelet-bound antibodies has been associated with a better response to rituximab.⁶² The persistence of autoantibodies in non-responders may suggest that rituximab had not removed the long-lived, antibody-producing plasma cells. There is also a small cohort of patients who respond despite undetectable antibodies: in those cases, either laboratory assays are not able to identify the antibody, or the response comes from the elimination of B-cell-mediated activation of T cells.⁶¹

A more recent study did not show any correlation between the presence of APA and the response to ritux-

imab, but found that rituximab resulted in a significant reduction of anti-GPIIb/IIIa but not anti-GPIb/IX levels.⁶³ In an unconfirmed study, patients with anti-GPIb/IX were shown to have a lesser response to intravenous immunoglobulins and steroids.^{64,65} In patients treated with rituximab, the presence of antibodies against GPIIb/IIIa led to a higher response rate than that in patients without anti-GPIIb/IIIa, while the presence of anti-GPIb/IX did not significantly influence the outcome.⁶⁶ This is potentially due to the different modes of action of the autoantibodies: anti-GPIIb/IIIa antibodies induce platelet destruction by Fc-dependent phagocytosis, while the action of anti-GPIb/IX may be FcR-independent⁶⁷ and instead increase the hepatic clearance of desialylated platelets.

In summary, the role of APA in response to rituximab remains unclear and may depend on the laboratory doing the testing, the phase of the disease, and which tests are performed.

Long-term outcome of rituximab treatment of immune thrombocytopenia

Only a small proportion of patients maintain a long-term remission after rituximab: in a prospective French

Table 2. Most relevant studies with different dose schedules of rituximab.

Author	Rituximab dose	Number of patients	Median age, years (range)	F:M	ITP phase	Early response	6 months	12 months	24 months	36 months	Last follow-up
Mahevas 2013 ⁴⁵	375 mg/m ² weekly x 4	61	52 (34-70)	64%:36%	ND 18%; P 26%; C 56%	54% (32% CR) at 3 months*	NA	36% (28% CR)	NA	NA	31% (26% CR) Median FU 36 months
	1000 mg on days 1 and 15	46	55 (34-76)	67%:33%	ND 17%; P 26%; C 57%	54% (32% CR) at 3 months*	NA	50% (41% CR)	NA	NA	48% (41% CR) Median FU 20.5 months
Khellaf 2014 ⁴¹	375 mg/m ² weekly x 4	173	51 (21-71)	64%:36%	56% chronic	62%*	80%	62%	50%	38%	NA
	1000 mg 2 weeks apart	72	53 (33-73)	65%:35%	66% chronic	61%*	90%	70%	43%	/	NA
Tran 2014 ⁵²	1000 mg on days 1 and 15	108	49 (19-85)	57%:43%	Median ITP duration 24.8 months (1.2 - 470)	43.5% (CR 9.3%) at week 8°	NA	29% [†] 66% [‡]	NA	NA	NA
Zaja 2010 ⁵⁰	100 mg weekly x 4	48	41 (16-74)	62%:38%	Median ITP duration 16 months (2 - 451)	60.5% (CR 39.5%)°	NA	61%	45%	40%	NA
Zaja 2012 ⁵⁴	100 mg weekly x 4	25	43 (14-74)	NA	Median ITP duration 24 months (2-324)	52% (28% CR)*	NA	32%	28%	23%	23% at 48 months
	375 mg/m ² weekly x 4	32	51 (16-80)	NA	Median ITP duration 31 months (3-264)	66% (50% CR)*	NA	50%	45%	40%	35% at 48 months
Gracie 2018 ⁵¹	100 mg weekly x 4	169	57 (30-70)	57%:43%	Median ITP duration 12.9 months (3.1-62)	NA	61.7% (35% CR)*	60%	53%	50%	NA
	375 mg/m ² weekly x 4	113	59 (35-72)	42%:58%	median ITP duration 12 months (2.9-43.5)	NA	64.1% (42% CR)*	60%	51%	40%	NA

F:M: female to male ratio; ITP: immune thrombocytopenia; ND: newly diagnosed; P: persistent phase ITP; C: chronic phase ITP; NA: not available; CR: complete response; FU: follow-up. [†]Response rates calculated considering as denominator only patients who responded to rituximab. [‡]Response rates calculated considering as denominator all treated patients. *response = platelet count $\geq 30 \times 10^9/L$. ° response = platelet count $\geq 50 \times 10^9/L$.

study in 248 adult patients, Khellaf *et al.* found that after a median follow-up of 24 months, 39% of patients were still responding.⁴¹ In other studies a long-term response, at approximately 2 years after initial treatment, was observed in about 40% of patients.^{39,56,68} However, the only study with a 3-5 year follow up showed that the response was maintained after 5 years in only 21% of adults and 26% of children treated with rituximab.⁴⁰

Given that a long-term response greater than 20-30% would be desirable, several strategies have been implemented in order to augment response rates and sustained remissions.

Anticipated use of rituximab in patients with immune thrombocytopenia

Although not supported by randomized, controlled trials, some studies pointed out that better outcomes can be achieved if rituximab is administered in an early stage of the disease.^{46,58} Following this observation, two studies^{69,70} compared the combination of “standard dose” rituximab and dexamethasone (40 mg/day for 4 days) to dexamethasone alone as first-line therapy in adult patients with ITP. In both studies the combination led to higher sustained response rates (63%⁶⁹ vs. 36% and 58%⁷⁰ vs. 37%), compared to those achieved with dexamethasone alone.

Combination treatment to improve long-term outcome with rituximab

Since rituximab affects almost exclusively B cells, without directly affecting the activity of other cells of the immune system (in particular T cells and plasma cells), the combination with other drugs with different modes of action looked appealing. The addition of 28 mg/m² dexamethasone (as an anti-plasma cell treatment), given for three 4-day cycles at 2-week intervals, to “standard-dose” rituximab was explored in a cohort of 67 patients (41 adults and 26 children) with ITP, of whom only five were treatment-naïve. This combination led to a 75% initial response rate and an almost 50% estimated long-term cure rate at 5 years.⁵⁴ However, the good long-term responses were seen almost exclusively in women of child-bearing age within 1 year of diagnosis.

The combination of high-dose dexamethasone, low-dose rituximab and cyclosporine (TT4) administered over 1 month was tested in 20 ITP patients (including 7 with newly diagnosed ITP and 5 with secondary ITP), with the aim of targeting, in addition to B cells, also plasma cells and T cells. The response rate at 6 months was 60% and among responders, the relapse-free survival rate was 92% at 12 months and 76% at 24 months. The treatment was well tolerated.⁷¹

Two Chinese studies explored the combination of low-dose rituximab with recombinant human thrombopoietin. The first study enrolled 14 patients with refractory ITP, who had an ORR of 93%.⁷² The second was a randomized, open-label study in which the combination was compared with rituximab 100 mg weekly for 4 weeks in patients with relapsed or refractory ITP. The group treated with the combination had a substantially shorter time to response (7 vs. 28 days).⁷³ The long-term response rate (79.2% vs. 71.1%) was not significantly different between the two groups.

Gómez-Almaguer *et al.* recently published the results of a single-center, pilot study conducted to assess the safety and efficacy of the combination of eltrombopag, low-dose

rituximab and dexamethasone in 13 newly diagnosed ITP patients. The ORR was 100%, with 92% CR rate and a relapse-free survival rate of almost 80% at 12 months.⁷⁴

The results of the most relevant studies of the use of rituximab in combination with dexamethasone and other drugs in ITP are summarized in Table 3a and 3b, respectively.

Efficacy of rituximab retreatment

Limited data are available concerning retreatment with rituximab. In a retrospective study, Hasan *et al.* explored the response to retreatment with standard-dose rituximab in patients with chronic ITP: 80% of the retreated patients responded again to standard-dose rituximab.⁷⁵

Zaja *et al.* showed that even low-dose rituximab can be effective in patients who previously responded to the standard dose of the drug, although only three patients received a second course of rituximab.⁵⁰ Khellaf *et al.* reported that most of 11 patients retreated with rituximab responded again.⁴¹ In other studies, very small numbers of patients were retreated with rituximab, in most of cases with good responses.^{38,56,68,76-78}

Rai *et al.*, in 17 patients with autoimmune cytopenias (including 11 cases of ITP) who previously responded, but then relapsed after a standard course of rituximab, explored the use of rituximab maintenance: a single 375 mg/m² infusion every 4 months for a total of 2 years: 88% of patients achieved a CR, with a mean duration of response of 48 months.⁷⁹

B-cell recovery after rituximab

The depletion of circulating B cells after rituximab administration is rapid (within 1 week) and deep, with B-cell counts remaining low in the peripheral blood for at least 6-12 months.⁸ The repopulating pool is dominated by immature B cells, while memory B cells recover after 2 years.⁸⁰ A similar depth of peripheral B-cell depletion has also been observed with low-dose rituximab.⁵⁰ In the vast majority of patients who achieve a complete remission, the recovery of B cells is not associated with disease relapse,⁴⁶ while in non-responders or in patients who relapse, B cells tend to reappear sooner in the peripheral blood and increase to higher levels.^{40,46} Rituximab also induces nearly complete depletion of splenic B cells, and in patients who do not respond the recovery of B cells in the spleen is faster.⁸¹

Effects of rituximab on T cells

In rituximab responders, all the immune-system abnormalities seen in patients with active disease tend to revert towards normal: in the peripheral blood, there is restoration of the Th1/Th2 and Tc1/Tc2 ratios, a decreased expression of Fas ligand and Bcl-2 mRNA, an increased expression of Bax mRNA and an increased number of regulatory T cells^{82,83}. The numbers of interleukin-10-producing B cells (regulatory B cells) and interleukin-6-producing B cells are also normalized after treatment.⁸⁴

In contrast, these abnormalities are still detectable in the spleens of non-responders: reduced regulatory T cells, an increased Th1/T-regulatory cell ratio,⁸¹ and persistence of the Tc1 polarization with CD8⁺ cytotoxic T cells displaying the phenotype of effector memory T cells with a restricted T-cell receptor repertoire.⁸⁵ These findings suggest that the action of rituximab is not limited to B cells and humoral immunity, but that rituximab also affects cel-

lular immunity. In a mouse model of T-cell-mediated ITP, B-cell depletion resulted in a significantly decreased proliferation of splenic CD8⁺ T cells *in vitro*, which correlated with an *in vivo* normalization of platelet counts.⁸⁶

However, T-cell remodeling in responders seems not to be a specific effect of rituximab since similar changes have also been observed in patients who respond to dexamethasone or thrombopoietin receptor agonists.⁸⁷⁻⁸⁹ It is possible that other biological factors concur to this effect. In particular, it has been proposed that the platelet count increase itself, either directly or via release of transforming growth factor- β , may cause a positive immune-modulating effect.⁹⁰

Why a proportion of patients do not respond to rituximab is unknown. This could be caused by either the persistence of long-lived, antibody-secreting plasma cells in the spleen^{81,91} and/or in the bone marrow or by the abnormal

activation of T cells, whose activity is not switched off by B-cell depletion. Finally, if the patient does not actually have ITP this would likely explain non-response as well.

Adverse events

Rituximab is generally a well-tolerated therapy, and adverse events are usually mild and easily manageable. The major concerns derive from the possible induction of hypogammaglobulinemia, and the increased risk of certain infections.

Infusion reactions after the first administration of rituximab are experienced by a variable proportion of patients, ranging from nearly 60% in the first trials⁴⁶ to 15% in the more recent reports,⁴¹ and are related to immediate cytokine release. They are usually easily manageable, and

Table 3a. Studies with rituximab plus dexamethasone.

Author	Treatment	Number of patients	Median age, years (range)	F:M	ITP phase	Early response	6 months	12 months	24 months	36 months	Last follow-up
Zaja 2010 ⁸⁹	375 mg/m ² x 4 weekly + 1 cycle dexamethasone (40 mg/day for 4 days)	49	49 (33-65)	55%:45%	Previously untreated	37% ^o at week 4	63%	50%	47%	43%	43% 30-months estimated probability of duration of response
Gudbrandsdottir 2013 ⁷⁰	375 mg/m ² x 4 weekly + up to 6 cycles dexamethasone (40 mg/day for 4 days every 1 to 4 weeks)	62	51 (36-63)	58%:42%	ND	NA	57% ^o	53%	NA	NA	NA
Bussel 2014 ⁵⁴	375 mg/m ² x 4 weekly + 3 cycles dexamethasone (28 mg/m ² /day for 4 days)	41	36 (18-64)	53%:46%	Median ITP duration: 16 (1-286) months	88% at week 8 ^o	82%	65%	58%	50%	47% estimated sustained response at 64 months FU
Chapin 2016 ⁵⁵	375 mg/m ² x 4 weekly + 3 cycles dexamethasone (28 mg/m ² /day for 4 days)	49	37	55%:45%	Duration of ITP: range 0 – 258 months	NA	NA	NA	NA	NA	33.3% sustained response at 72 months FU ^o

F:M: female to male ratio; ITP: immune thrombocytopenia; dexamethasone; ND: newly diagnosed; NA: not available; FU: follow-up; yrs: years. Response rates are calculated using as denominator all the patients treated with rituximab. ^oresponse = platelet count $\geq 50 \times 10^9/L$.

Table 3b. Studies with rituximab in combination with drugs other than dexamethasone.

Author	Treatment	Number of patients	Median age, years (range)	F:M	ITP phase	ORR	6 months	12 months	24 months
Zhou 2015 ^{5,73}	Rituximab 100 mg weekly for 4 weeks + rhTPO 300 $\mu g/kg/day$ for 14 days	77	42 (13-82)	65%:35%	Median ITP duration 12.5 months (3-72)	93% (50% CR)*	67.2%	24.6%	NA
	Rituximab 100 mg weekly for 4 weeks	38	42.5 (12-68)	66%:34%	Median ITP duration 11 months (3-65)	93% (50% CR)*	55.6%	18.5%	NA
Choi 2015 ⁵⁷¹	Rituximab 100 mg weekly for 4 weeks + dexamethasone 40 mg days 1-4 + cyclosporine 2.5-3 mg/kg/day (days 1-28)	20	NA	55%:45%	ND or Persistent ITP: 7; Chronic ITP: 13	NA	60%*	92%	76%
Li 2015 ⁵⁷²	Rituximab 100 mg weekly for 4 weeks + rhTPO 300 $\mu g/kg/day$ for 14 days	14	52 (18-76)	93%:7%	Unknown	93% (50% CR)*	71% (40% CR)	NA	NA
Gomez-Almaguer 2017 ⁵⁷⁴	Eltrombopag 50 mg/day, days 1-28 + dexamethasone 40 mg days 1-4 + rituximab 100 mg weekly for 4 weeks	13	40 (16-61)	62%:38%	ND	100% (92% CR)*	NA	80%	70%

F:M: female to male ratio; ITP: immune thrombocytopenia; ORR: overall response rate; rhTPO: recombinant human thrombopoietin; CR: complete response; NA: not available; dexamethasone; ND: newly diagnosed. *Response rates calculated considering as denominator only patients who responded to rituximab. ^{*}Response rates calculated considering as denominator all treated patients. ^{*}response = platelet count $\geq 30 \times 10^9/L$; ^oresponse = platelet count $\geq 50 \times 10^9/L$.

serious adverse reactions are exceptional, especially if prednisone is included in premedication.

Serum sickness is a much rarer adverse reaction, characterized by fever, rash, polyarthralgia or arthritis, proteinuria, hematuria, elevated inflammatory markers and decreased complement, which usually arises 10-14 days after treatment.⁹² It is the result of immune activation against the chimeric mouse-human drug, with the formation and deposition of immune complexes, and consequent activation of the complement cascade.⁹³ This type III delayed hypersensitivity reaction to rituximab has been reported more commonly in autoimmune disorders than in hematologic malignancies, but overall it remains a very rare occurrence in adults, with less than 50 cases having been reported.⁹²

Hypogammaglobulinemia

Since rituximab does not affect pre-existing long-lived plasma cells, there are not significant changes in the IgG or IgM levels in patients treated with one "standard dose" course.^{38,46-48} However, patients treated with multiple courses are more likely to develop hypogammaglobulinemia, which usually recovers spontaneously in a few months.⁹⁴ Since dexamethasone also affects plasma cells, 10-20% of patients treated with the combination may experience a marked hypogammaglobulinemia, which usually recovers within 1 year.^{50,54,70}

Some reports suggest that a pre-existing hypogammaglobulinemia can be aggravated by rituximab, which can in some cases trigger or accelerate the development of a real common variable immunodeficiency (CVID).^{95,96} It is worthwhile remembering that ITP may be the initial manifestation of CVID and can precede its diagnosis by years.⁹⁷ It is therefore important, in ITP patients treated with rituximab, to monitor immunoglobulin levels before and after therapy. In addition to monitoring and possibly even temporarily giving intravenous immunoglobulin replacement, prior assessment of genetic markers and vaccine responses^{98,99} may be useful.

The efficacy and safety of standard-dose rituximab in patients with CVID-associated ITP or autoimmune hemolytic anemia was assessed in a multicenter, retrospective, French study. The ORR was 85%, with a 74% CR rate and a sustained response rate of 60% at a median follow up of 39 months; severe infections occurred in 24% of patients, four of whom were not on immunoglobulin replacement therapy. The authors concluded that rituximab is highly effective and relatively safe in the management of CVID-associated immune cytopenias, and that immunoglobulin replacement is strongly recommended in this cohort of patients.¹⁰⁰

Infections

An increased risk of infections after rituximab therapy is generally uncommon and more frequently observed in severely immunocompromised patients.¹⁰¹ Chugh *et al.*, in a meta-analysis including five trials and 463 ITP patients treated with rituximab, did not find an increased risk of infection.¹⁰² Khellaf *et al.* concluded that the risk of infections was acceptable (cumulative incidence of 2.3 infections per 100 patient-years at a median follow up of 24 months), with the most severe infections occurring in adults older than 70 years of age, who suffered from severe comorbidities.⁴¹

Rituximab therapy reduces the genesis of new long-

lived plasma cells, thus impairing the immune response to vaccines. Vaccines against encapsulated bacteria (*Streptococcus pneumoniae*, meningococci and *Haemophilus influenzae*) should be administered before rituximab, considering that patients, especially non-responding ones, may require a subsequent splenectomy.¹⁰³

Reactivation of hepatitis B virus (HBV) is a well-recognized complication of immunosuppressive therapy. Patients who are HBcAb positive (likely occult carriers) should receive antiviral prophylaxis with lamivudine, while patients with HBsAg or HBV-DNA positivity (active carriers and inactive carriers) should be referred to a hepatologist and treated with entecavir or tenofovir.¹⁰⁴

A rare but life-threatening infection that has been linked to rituximab is progressive multifocal leukoencephalopathy (PML), caused by the activation of Jakob-Creutzfeldt virus and its spread to the central nervous system.¹⁰⁵ This complication, although rare, has been almost only reported in patients with lymphoproliferative disorders, and only a very few cases have been observed in patients with autoimmune diseases, especially in systemic lupus erythematosus.^{106,107} Furthermore, patients who develop PML are usually profoundly immunocompromised from combination chemotherapy, and rituximab is often just one of the many drugs received.^{108,109} There is only one well-studied case of PML in ITP and the course was unusual with the PML occurring more than 3 years after exposure to rituximab.

The occurrence of late-onset (>4 weeks after treatment) neutropenia has been described in patients treated with rituximab for both malignant¹¹⁰ and non-malignant conditions,^{111,112} including a few ITP patients.⁴⁰ Most cases appear to be self-limiting and resolve without issue,¹¹³ and according to some authors retreatment with rituximab is safe.⁸⁶

Malignancies

Immunosuppression secondary to rituximab could increase the risk of second primary malignancies. In large follow-up studies, rituximab has not been shown to increase the risk of cancer in patients with rheumatoid arthritis,¹¹⁴ among non-Hodgkin lymphoma patients¹¹⁵ and in patients with ANCA-associated vasculitis.¹¹⁶

No malignancies were reported in ITP by Arnold *et al.* in a meta-analysis including more than 300 patients,⁴² or in other studies,^{40,41,58} including combination studies with dexamethasone.^{54,69,70} In other reports nearly 3-4% of patients developed a second primary malignancy after having received rituximab for ITP, but the low percentage and the heterogeneity of the neoplasia led the authors to conclude that a causative relationship with rituximab could not be assessed.^{39,44,56}

Use of rituximab in children

Following the development of rituximab for adults with ITP, studies soon migrated to pediatric patients (Table 4).

The first large series was reported in 2005 and included 24 chronic ITP patients who were refractory to or relapsed after previous treatments. The ORR to "standard-dose" rituximab was 78%, with a 63% CR rate and an overall sustained response rate of 37%.¹¹⁷

The first prospective phase I/II study of rituximab in children and adolescents with chronic ITP included 36

Table 4. Rituximab in children.

Author	Rituximab dose (mg/m ²)	Number of patients	Median age years (range)	F:M	ITP phase	ORR (CR)	12 months	24 months	36 months	Last follow-up
Wang 2005 ¹¹⁷	375 mg/m ² x 4 weekly	24	12 (2-19)	58%:42%	Chronic	78%* (63%)	NA	NA	NA	NA
Taube 2005 ¹²¹	375 mg/m ²	22	5.8 (2.5-15.2)	64%:36%	Chronic	59%* (32%)	NA	NA	NA	NA
Bennett 2006 ¹¹⁸ & Mueller 2009 ¹¹⁹	375 mg/m ² x 4 weekly	36	11.2 (2.6-18.3)	42%:58%	Chronic	31% ^o	31%	NA	NA	NA
Parodi 2009 ¹²³	375 mg/m ² x 4 weekly	49	7.4 (0.7-17.6)	67%:33%	77% chronic	69% ^o (53%)	NA	NA	60%	NA
Grace 2012 ¹²⁴	NA	80	7.5 (4.9-12)	56%:44%	Chronic	64% ^o	NA	NA	NA	NA
Patel 2012 ⁴⁰	375 mg/m ² x 4 weekly	66	12 (2-17)	61%:39%	Median duration of ITP 20 months (1 month – 9 years)	57% ^o	33%	NA	NA	26% at 5 years
Matsubara 2014 ¹²⁵	375 mg/m ² x 4 weekly	22	4.2 (0.25-11.6)	50%:50%	Median duration of ITP 18.5 months (2 – 120 months)	50%* (41%)	27%	18%	NA	14% at 5 years
Oved 2017 ¹²²	Rituximab 375 mg/m ² x 4 weekly + dexamethasone 28 mg/m ² days 1-4; 15-18; 29-32	33	11 (1-17)	58%:42%	Persistent/ chronic ITP	48% ^o	NA	NA	NA	27% at a median FU of 35 months (range 10-58)

F:M: female to male ratio; ITP: immune thrombocytopenia; ORR: overall response rate; CR: complete response; NA: not available; FU: follow-up; Response rates are calculated using as denominator all the patients treated with rituximab. *response = platelet count $\geq 30 \times 10^9/L$; ^oresponse = platelet count $\geq 50 \times 10^9/L$; ^ssustained platelet count over $50 \times 10^9/L$ in 4 consecutive weeks.

patients with severe refractory ITP or Evans syndrome treated with “standard-dose” rituximab. After a follow up of 1 year, 31% of them maintained a platelet count $>50 \times 10^9/L$.^{118,119}

A systematic review including 14 studies with a total of 323 pediatric ITP patients reported a pooled response rate of 68%, and a pooled CR rate of 39%, with a median duration of response of 12.8 months.¹²⁰

“Low-dose” rituximab has also been tested in children with ITP: Taube *et al.* explored the efficacy of a single dose of rituximab (375 mg/m²) in 22 patients with chronic ITP; the ORR was 59%, with a 27% CR rate, and 36% of patients maintained a long-term remission (median duration of remission 13.5 months; range 2-16 months).¹²¹

Oved *et al.* explored the addition of three 4-day cycles of dexamethasone (28 mg/m²) to “standard-dose” rituximab in 33 children with persistent/chronic ITP. The ORR was close to 50%, and 62% of the responders maintained the remission for a median of 35.5 months.¹²²

Factors predictive of response were also sought in children. Bennett *et al.*¹¹⁸ found a weak association between response and Evans syndrome, female sex and black race. Parodi and colleagues performed a retrospective study including 49 children (77% with chronic ITP) treated with “standard-dose” rituximab (ORR 69%, 60% relapse-free survival at 36 months), and found a significantly higher probability of relapse-free survival in males aged >14 years and females aged >12 years (88.9% vs. 56.7%), in patients who achieved a CR (70.2% vs. 25%) and in patients who achieved the response within 20 days of treatment (73.7% vs. 22%).¹²³

Among 80 pediatric patients with chronic ITP treated with rituximab (ORR 64%), Grace *et al.* found a higher

response rate in patients who had previously responded to steroids (87.5% vs. 47.9%) and in those with secondary ITP (89.5% vs. 55.7%).¹²⁴

In a study by Oved *et al.*,¹²² female adolescents with ITP lasting less than 24 months had a higher sustained remission rate (47%) than that of either the entire group (27%) or the male patients (7%).

The drug is usually well tolerated also in children, with a toxicity profile superimposable to that of adult patients in terms of immediate and long-term toxicity.^{117,118,125,40,123} Significant hypogammaglobulinemia was observed in 15% of patients treated with the combination of rituximab and dexamethasone,¹²² although this adverse event was transient.

The reported rate of serum sickness was higher in pediatric patients than in adults, particularly in the first series, occurring in up to 10% of the former.^{117,118} Clinical manifestations ranged from severe to mild. Laboratory confirmation can be sought by checking levels of C3 and C4; of note, certain pediatric patients may have a congenitally low C4 level (which may predispose to ITP) and C4 levels should, therefore, be checked prior to rituximab treatment.

Discussion

Twenty years after the first use of rituximab in ITP, published studies show an ORR of nearly 60% and a CR rate of 50%. Long-term remissions occur in 20-30% of patients, with slightly different outcomes possibly related to different selection of populations of patients.

In the interpretation of the results it is worth noting that

heterogeneous criteria for response were adopted across different studies (according to older or updated criteria); moreover, older studies included mostly chronic, pluri-refractory patients, while in the more recent studies patients with newly diagnosed/persistent ITP were also included. Finally, the ability to compare studies is often only partial because of different follow-up periods.

The data from placebo-controlled studies seem discouraging, but some considerations should be made: in the experimental treatment and placebo arms, patients were allowed to continue corticosteroid therapy, which could have biased the results: in the placebo arms the ORR were 67% (39% CR)⁴⁵ and 73% (46% CR).⁴⁷ Probably a more meaningful observation is that the median time to relapse in patients who achieved an overall response was 36 weeks in the rituximab group and 7 weeks in the placebo group.⁴⁸

The main criticism that only 20% to 30% of patients achieve long-term remission for more than 3-5 years deserves further consideration. The main “error” was probably the mistaken belief that rituximab could represent the medical substitute of splenectomy: a single treatment administered once in a lifetime that could definitively cure many or even most patients with ITP. In some groups of patients, it is worth considering that even a sustained response of 12-18 months can have a significant, positive effect on a patient’s quality of life: during this period of time, they do not have to take any ITP medication and can avoid frequent hospital checks.

Furthermore, several clinical studies found that in young women a course of rituximab administered before the chronic phase (ITP duration <12 months) can lead to response rates and at least mid-term remission rates comparable to those obtained with splenectomy. This finding suggests that at least in some selected patients the treatment outcome may be much better.

Retreatment with rituximab is effective in most cases, especially in patients who maintained the preceding remission for more than 12 months.

Biomarkers predictive of response, including the controversial role of anti-platelet antibody testing, are still lacking and further studies are needed for their establishment and subsequent application in clinical practice.

The efficacy and safety of combinations of treatment with rituximab and other agents, such as thromboietin-receptor agonists, immunosuppressive agents, agents targeting plasma cells or yet others, need to be better evaluated and proven in comparison studies with rituximab monotherapy.

As far as concerns different rituximab doses, comparisons between the “standard dose” and “rheumatoid arthritis dose” did not reveal different results in terms of efficacy and toxicity. Low-dose rituximab could be equally effective, although prospective studies comparing the standard dose and low doses are lacking.

Rituximab is a well-tolerated drug; serious side effects are extremely rare and life-threatening infectious complications are usually only seen in patients with other concomitant causes of immunodeficiency.

Conclusions

In conclusion, based on what has been published in the last 20 years, it is still difficult to give clear indications on when, to whom and how rituximab should be administered. In 2019, the choice of rituximab over other treatment options has to be weighed considering the individual patient’s features and expectations, the disease’s characteristics, the availability of the drug and the single center’s experience.

The authors of this review think that rituximab still represents a valuable therapeutic option for patients with ITP and, based on current knowledge, believe that it should be considered especially (although not exclusively) at an early stage of the disease, as second- or third-line therapy, in young patients (particularly young women) and in patients treated with curative purposes.

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