Multi-center randomized open label phase II trial on three rituximab dosing schemes in immune thrombocytopenia patien

Jaap J. Zwaginga,[1] Bronno van der Holt,[2] Peter A. te Boekhorst,[4] Bart J. Biemond,[3] Mark-David Levin,[5] René van der Griend,[8] Anneke Brand,[1] Sonja Zweegman,[7] Hans F.M. Pruijt,[9] Vera M.J. Novotny,[12] Art Vreugdenhil,[6] Marco R. de Groot,[13] Okke de Weerdt,[10] Elisabeth C.M. van Pampus,[11] Tanja M. van Maanen-Lamme,[14] Shulamiet Wittebol,[15] Martin R. Schipperus,[16] Matthijs H. Silbermann,[17] Peter C. Huijgens,[7] Marleen Luten,[2] Rene Hollestein,[2] Jan A.C. Brakenhoff,[18] Jolanda G. Schrama,[19] Fransje A.A. Valster,[20] Gerjo A. Velders,[21] Harry R. Koene;[10] on behalf of the Dutch HOVON 64 study group

[1]Dept. of Immunohematology and Blood Transfusion, Leiden University Medical Center and the Jon J van Rood Center for Clinical Transfusion Research, Sanquin-Leiden University Medical Center; [2]HOVON Data Center, Erasmus MC Cancer Institute – Clinical Trial Center, Rotterdam; [3]Dept. of Hematology, Academic Medical Center Amsterdam; [4]Dept. of Hematology, Erasmus MC, Rotterdam; [5]Dept. of Internal Medicine, Albert Schweitzer Hospital, Dordrecht; [6]Dept of Internal Medicine, Máxima Medical Center, Veldhoven; [7]Dept. of Hematology, VU University Medical Center, Amsterdam; [8]Department of Internal Medicine, Diakonessenhuis, Utrecht; [9]Dept. of Internal Medicine, Jeroen Bosch Hospital, Den Bosch; [10]Dept. of Internal Medicine, St. Antonius Hospital, Nieuwegein; [11]Dept. of Laboratory Medicine, Radboud University Medical Center, Nijmegen; [12]Dept. of Hematology, Radboud University Medical Center, Nijmegen; [13]Dept. of Internal Medicine, Medisch Spectrum Twente, Enschede; [14]Dept. of Internal Medicine, Westfriesgasthuis, Hoorn; [15]Dept. of Internal Medicine, Meander Hospital, Amersfoort; [16]Dept. of Internal Medicine, HagaZiekenhuis, Den Haag; [17]Dept. of Internal Medicine, Tergooiziekenhuizen, Blaricum; [18]Waterland hospital, Purmerend; [19]Dept. of Internal Medicine, Spaarne hospital, Hoofddorp; [10]Dept. of Internal Medicine, Lievensberg Hospital, Bergen op Zoom; and [21]Dept. of Internal Medicine, Gelderse Vallei Hospital, Ede, The Netherlands

Correspondence: j.j.zwaginga@lumc.nl
doi:10.3324/haematol.2014.110213

Supplemental data:

Patients and methods

Eligibility

Eligible were patients aged 18 years or older with immune thrombocytopenia (ITP) irrespective of their ITP being newly diagnosed (< 3 months from diagnosis), persistent (3 to 12 months from diagnosis) or chronic (> 1 year from diagnosis) [1]. However, patients needed to have an ITP relapsed on or refractory to high dose corticosteroids (> 1mg/kg longer than 3 weeks before start of rituximab) as indicated by at least 2 platelet counts less than 30×10^9/l. Further inclusion criteria were a WHO performance status 0-2, no previous rituximab treatment, no active bleeding (defined as grade 3 or 4 according to NCI CTCAE v3.0) [13], no abnormal liver or renal function, no active systemic infections (HBV, HCV, HIV, HTLV, CMV, and EBV by serologic screening), no active malignancies, no other autoimmune diseases (on clinical criteria and absence of for other AID indicative serology e.g. ANF, ANA), must be at least 3 weeks without IVIG, splenectomy or pulsed or high dose corticosteroids (>1 mg/kg prednisone or equivalents of dexamethason). Maintenance corticosteroids or tapering of the latter, tranexaminic acid (Cyklokapron) or stable non-immunosuppressive medication (e.g. danazol, dapsone) was allowed. Although advised nowadays, the protocol did not involve a pre-splenectomy like vaccination scheme or an active screening for Helicobacter pylori. Other treatments, which influence the immune system, platelet counts or platelet function, were prohibited. After written informed consent, patients were randomized, stratified by center and previous splenectomy using a minimization procedure and treated with rituximab. National approval for the study was obtained from the medical ethical committee of the Academic Medical Center Amsterdam and for local execution by the separate committees of all participating hospitals.

Treatment

Dose: In arm A (375 mg/m^2 once a week for 4 weeks) patients received iv infusions of rituximab on day 1, day 8, day 15 and day 22 (4 doses in total). In arm C (750 mg/m^2) patients received high dose rituximab (750 mg/m2) on day 1 and day 8. In arm B (375 mg/m^2) patients initially received 2 doses of standard rituximab, one on day 1 and the other on day 8. Early responders (CR, GR or MR), as assessed on day 15, and who had a sustained or improved response at day 43, went off protocol. Patients in arm C who initially responded at day 15, but showed no sustained response until day 43, received dose 3 and 4 with a weekly interval; dose 3 was given immediately after the initial response is lost). The 3rd dose could be given on day 22 - 43 and dose 4 at day 29 -50. Patients of arm C who showed no response at day 15 will also receive dose 3 and 4 with a weekly interval (dose 3 given at day 22, dose 4 at day 29). **Administration**: The initial dose rate was 50 mg/hr for the first hour. If no AE's occurred with the previous infusion, the infusion rate at the start of following infusions was 100 mg/hour and, if no further AE's are observed, the infusion rate can be increased with 30 minutes intervals with increment steps of 50 mg/hour to a maximum of 400 mg/hr or lower according to local standards. Infusion was temporarily halted in patients experiencing adverse events like fever and rigors with infusion of rituximab. If AE's were transient during observation, infusion was continued, initially, at 1/2 the previous rate. If after one hour of infusion at 1⁄2 dose rate no AE's were observed, the dose rate was escalated in 30 minutes intervals with increment steps of 50 mg/hour to a maximum dose rate of 400 mg/hr. In case of an adverse event, patients were observed and severity of the AE's evaluated and if necessary treated according to best available local practices and procedures. Following the antibody infusion, the IV line was kept open for other medication. If there are no complications, the IV line may be removed after one hour of observation. If complications occured during infusion, the patient were observed for two hours after the completion of the infusion. **Cycle:** arm A once weekly 4 times, arm B once weekly 2 times in early and sustained responding patients (= within 15 days and still responding at 43 days) and another 2 weekly 375 mg/m^2 rituximab infusions to patients not fulfilling these criteria; arm C once weekly for 2 weeks. **Premedication**: allowed were acetaminophen (paracetamol) and chlorpheniramine.

Patient monitoring parameters: Vital signs (blood pressure, pulse, respiration, body temperature) were monitored every 15 minutes x 4 or until stable and then hourly until the IV line is discontinued and until stable. During rituximab infusion vital signs (blood pressure, respiration rate, pulse, and body temperature) were recorded.

Response assessment and definitions of endpoints

Responses were evaluated on weekly single platelet counts a, starting on the first day of rituximab treatment (day 1) up to 10 weeks (day 71). After this, only patients that responded before day 71 were followed monthly for at least 12 months. An additional questionnaire-based survey was conducted in order to define longer responses in these patients. For 85% of these patients longer follow up data were thus obtained. Complete response (CR) and Good/ Partial Response (PR) were respectively defined as platelet counts $\geq 150 \times 10^9$/l and $\geq 50 \times 10^9$/l, respectively, on 2 consecutive occasions. A moderate response (MR) was defined as a platelet count above 30×10^9/l with at least twice the baseline count. Response duration was measured as the time between first response and the last recorded response or relapse signifying the need of new intervention therapy e.g. because of bleeding and/or platelet decrease again below 30×10^9/l or platelet counts falling below 2-fold increase of baseline platelet count for all responders or death. Relapse free survival (RFS) in responding patients, was defined as time from response until relapse, emergency treatment, or death, whichever came first. Complete response duration was also determined for CR patients and expressed as time until platelet counts returned below 150×10^9/l or bleeding. Patients without a response at day 71 went off protocol and further data acquisition of this group was not obligatory. For patients with at least a MR, follow up data for at least 1 year were collected until relapse defined as platelets again below 30, new other treatments or death, whichever came first. Patients still alive at the date of last contact were censored. Thus expressed survivals are necessarily underestimates because responding patients can maintain this response after the last contact. Apart from this more detailed response score, we also retrospectively used the new International Working Group (IWG) ITP trial guidelines [1] defining CR as a platelet count above 100×10^9/l and combining both MR and PR in the single term Response both to be present in 2 of more measurements one week apart.

Statistical analysis

The primary objective of this study was to evaluate whether each of the treatment arms was sufficiently promising for further study, but not to compare the treatment arms. The sample size calculation was based on the percentage of patients reaching a response within 10 weeks after starting rituximab treatment in each arm. The design of each treatment arm followed an optimal Simon two-stage design[14]. A true response rate of 30% or less was considered insufficient, while a true response rate of at least 50% would warrant further research of that specific treatment arm in clinical trials. For $\alpha = 0.10$ and $\beta = 0.10$, a total of 46 patients per treatment arm were required. Taking into account a drop-out rate of 8%, a total of 150 patients were included in the trial. All analyses were performed according to the intention-to-treat principle, irrespective of patients' compliance with the protocol. Ineligible patients, however, were excluded from all analyses.

The primary analysis was to estimate the response rate with a 90% confidence interval (CI) in each arm separately. RFS was analysed using the actuarial Kaplan-Meier method, and Kaplan-Meier curves were generated to illustrate RFS. Exploratory analyses regarding the association of baseline characteristics with response and RFS were performed using respectively logistic regression and Cox regression.

Ineligibility, inclusions, demography, off protocol events and follow up

Between November 2005 and May 2010, 156 patients from 26 centres, consented after being informed, to be included, and were randomized in the study. Of these 156, 15 patients (3 patients of arm A and C each, and 9 of arm B) were considered ineligible. One patient was wrongfully diagnosed and appeared to have AML, 6 patients showed high platelet counts between randomisation and start of treatment, 6 patients still had high dose (> 1 mg/kg) corticosteroids 3 weeks before starting the study medication;

lower stable or decreasing dosing prior to study medication was allowed. One patient's thrombocytopenia was secondary to Lupus Erythematodus and one splenectomized patient was not followed due to the finding of an accessory spleen and the decision to perform re-surgery. Additionally, 3 patients were randomized but excluded because they did not start with rituximab treatment (one patient in arm C died of an intra-cerebral bleeding 2 days after registration, another patient in arm C was non-compliant and one patient in arm A already had a normal platelet count-before start of rituximab (Consort flow diagram: figure s1). The number of eligible and informed patients that refrained from participation in the study period was not recorded.

The demography of the included patients per study arm is shown in table 1. The total group of 138 patients consisted of 44% males and 56% females with a median age of 53 years (range 17-82 years; one patient aged 17 years and 9 months was allowed to enter randomisation). Splenectomy (Sx) prior to the study had been performed in 12 patients with a range of 2 months to 29 years before start of rituximab, with 8 patients showing an initial CR or PR after Sx before relapsing again. Response on previous corticosteroid treatment was 64% (21% CR, 33% PR, 10% MR), 25% had no response on steroids and in 12% the response was unknown/not reported. Median time after high dose prednisone and inclusion for R treatment was 4.1 months and a maximum of almost 19 years. Forty one % of patients were still on stable or decreasing < 1 mg/ kg prednisone at the start of rituximab treatment.
Marrow aspirates were performed in 81% of patients: 3 marrow evaluations indicated dysmegakaryocytopoiesis, hematopoiesis with some maturation lack, and dysplastic megakaryopoiesis each in 1 patient. The latter aspirate was reviewed and found not sufficient for MDS; moreover, follow up of all these patients did not change the ITP diagnosis.

The median platelet count at inclusion was 17×10^9/L (range, 1- 38). 4 patients with platelet counts between 31 and 38×10^9/L were not excluded because of stable or lower platelet counts at least during the first 2 weeks of rituximab treatment.
Except for the mentioned patient in arm C, bleeding events and symptoms at study entry were limited and not of clinical significance. In this respect only 3% of the patients experienced epistaxis at study entry. For oral bleeding this was 4%. Even petechiae as the most common bleeding symptom were present in only 11% of patients at study entry, while 35% of all patients had never experienced petechiae. Underestimation is this respect, might be possible because these data were not derived from validated bleeding questionnaires.

Ninety-one % of patients completed the treatment according to protocol. Twelve patients went off protocol treatment for various reasons other than normal completion (Consort flow diagram: figure s1). In three patients (2 in arm A and 1 in arm C) of these twelve, insufficient data follow up led to a major protocol violation. In 2 patients, these protocol violations were possibly related to the occurrence of an adverse event: i.e. fever after the second dose and a serum sickness (CTC 3) after the second dose of rituximab. One patient of the 12 off protocol patients in arm B experienced a life threatening but rituximab-unrelated infection with hospitalisation. Two (arm C) patients out of 12 refused further monitoring (non compliance) 6 weeks after study initiation. Two of 12 patients went off protocol due to treatment-related toxicity: 1 patient in arm A (serum sickness) and 1 in arm C (allergic reaction) because of R-related toxicity. The last 4 of the 12 off protocol patients involved emergency treatments: in arm B one patient received this for a planned conization and one for progressive disease; in arm C one patient needed higher platelet counts for a fibula fracture and one was thus treated during hospitalization for a respiratory tract infection. For CTC and SAEs look below and table s1.

Response modulating factors

Early vs late responders: Patients throughout the 3 arms who responded within 14 days after start of R, clearly showed more CRs (18/28 vs 8/40, $P < 0.001$) within 70 days and also more CRs (19/28 and 14/40

p = 0.01) in follow up as compared to later responding patients. Comparing these early or later responding patients as well as early or late responding patients with an additional CR within 70 days, both groups showed a similar response duration and RFS.

Quality of response vs. response duration: Response duration was also determined from day 71, and compared between patients who were then in CR (n=26), PR (n=25) and MR (n=14). RFS at 2 years was 68%, 48% and 63%, respectively (p=0.92)

Disease duration: Disease duration was studied as a response-modifying factor in 2 ways. First we compared if disease duration was different between responders and non-responders. The duration of ITP, defined as time between initial diagnosis and start rituximab, was indeed significantly shorter both in patients reaching response (median 322 days; range 7-8964; p=0.008) and CR (237 days; range, 29-7726; p=0.042) versus patients without response (median 783 days; range 23-11487). Alternatively, we compared the responses between chronic ITP patients (disease duration > 1 year) and patients with less than one year ITP. After comparing 65 patients with ITP less than one year, with 73 patients with ITP for more than a year, respectively 37 (57%) and 31 (42%; p=0.09) had a response. 17 of these patients (27%) and 16 (22%; ns) had a CR. Less than 1 year disease duration also tended to have better RFS at 2 years after first response: 66% vs. 46%; p=0.06).

Additional corticosteroids/ patient age/ gender/ prior splenectomy: To detect possible synergistic effects between rituximab and corticosteroids, we studied if having corticosteroids at the start of rituximab influenced response rate and quality. This appeared not to be the case, as 39 of 81 patients who were not on maintenance corticosteroids 4 weeks prior to inclusion did respond to treatment (48%, including 15% CR), versus 29 of 57 patients (51%, including 25% CR) on maintenance corticosteroids, p= 0.75. Moreover, patient age was not found associated with the probability to obtain a response (p=0.25) and RFS (p=0.57). Response according to patient sex, however, tended to be more and better in the 77 participating females, with a total response of 56% with 18 CR, 15 PR and 10 MR, while the total response in the 61 participating males was only 41% (p=0.08) with 8 CR, 12 PR and 5MR.
The small numbers of splenectomized patients and their unequal distribution among the arms did not allow separate analysis per study arm, but together these 12 patients showed 5 responses (42%): 1 reached CR, 1 PR and 3 MR.

Secondary endpoints:
Emergency treatments. As mentioned above under off protocol events, only 4 patients received emergency treatment on study; these treatments however were not caused by bleeding grade 3 or 4. In case of non-response or relapse, patients went off protocol and often received other treatments. The varying rescue strategies that were used in non-responding or relapsing patients who went off protocol were not further analysed except for splenectomy. Splenectomy was observed in only 4 of 68 (6%) initial responding patients within one year after study initiation. In the 70 non-responding patients, not all follow up data was complete, but splenectomy was already reported in 15 patients (21%) within one year after initiation of the study and therefore significantly more than in responders (p=0.01).

Secondary endpoints, toxicity and side effects :

Emergency treatments. As mentioned above, only 4 patients received emergency treatment on study and went off protocol treatment, though this was not due to bleeding grade 3 or 4. In case of non-response or relapse, patients went off protocol and often received other treatments. The varying rescue strategies that were used in non-responding or relapsing patients who went off protocol were not further analysed except for splenectomy. Splenectomy was observed in only 4 of 68 (6%) initial responding patients within one year after study initiation. In the 70 non-responding patients, not all follow up data was

complete, but splenectomy was already reported in 15 patients (21%) within one year after initiation of the study and therefore significantly more than in responders (p=0.01).

Toxicity and side effects: Toxicities scored according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 3.0 were comparable in all study arms as were side effects (table 1). In arm A: 5, 9 and 1 CTC grade 2,3 and 4 non-infectious events were observed in respectively 3, 8 and 1 patients while 2 CTC 2 infectious events were observed in one patient. In arm B: 6, 2 and 1 CTC grade 2,3 and 4 non- infectious events were observed in 4,2, and 1 patient, while 2 patients had a CTC 3 and a CTC 4 infectious event each. One further non-responding patient in arm B died 3 years and 5 months after study initiation at 83 years of age; the cause of death was not reported. In arm C 11, 6 and 1 grade 2,3 and 4 events were observed in 7,6 and 1 patients respectively while 3 patients had 2 grade 2 and 1 grade 3 infectious event.

In conclusion, although 22 patients (16%) experienced CTC 3 or 4 events, only 8 cases (6 %) with CTC 3 and 4 events were recorded as SAEs, which in respect of imputability were reported with a possible (3 events), probable (4 events) or definite (1 events) relation to R-administration. A definite R- related CTC 4 seizure should have been reported as SAE.

In arm A, the CTC grade 4 non-infectious event involved a seizure definitely associated with the 3rd rituximab (R) infusion. In arm A, 2 SAE's were reported: one CTC 3 haemolytic anaemia (with an aspecific positive direct agglutination test, and for haemolysis typical laboratory values which were noticed at the moment of the first R dose graded as possible related but on review not likely caused by R; the hemolysis after some increase started resolving without the need for additional treatment around the 4th R dosing), and one CTC 3 serum sickness probably related to the second R dose.

In arm B 5 SAE's were reported. A non-infectious CTC grade 4 event involved bleeding after a trauma in a non-responding patient. This event was considered unrelated to R. A CTC grade 4 infectious event involved a possible therapy related beta haemolytic streptococcus bacteriaemia. This event led to ICU admittance and long hospitalisation; the patient recovered completely. Besides, the described CTC grade 4 bleeding and the CTC grade 4 beta haemolytic streptococcus bacteriaemia, also one probably R related grade 3 serum sickness was reported. Two SAE reports on recurrence of ITP 6 months after protocol initiation needing dexamethason treatment and the unplanned pregnancy of a patient's partner were questionable and should be considered unrelated to the treatment protocol. An additional CTC grade 3 "infectious" event in arm B involved fever after the 2nd dose of R, was not reported as possible related SAE; cultures remained negative.

In arm C, the non-infectious grade 4 CTC event involved a severe allergic reaction including seizures and mild hypotension. This event was considered definitely related to the first R infusion. The grade 3 infectious event involved a possibly R-related Staphylococcus bacteriaemia for which antibiotics were needed; the patient recovered completely. In arm C a total of 7 SAEs were reported. Besides the grade 4 and grade 3 events, a probably second R infusion-related CTC grade 2 fever needing acetaminophen, and a R-unrelated grade 2 viral upper airway infection with fever were reported as SAE, the 3 other SAE events were a CTC grade 3 R unrelated fibula fracture after trauma, a probable second dose R related combined skin reaction with myalgia (grade 3) , and an R-unrelated hypertensive period with non-specific chest pain (grade 3).

Table s1: SAE and AE's

Patients with SAE's and additional CTC 4 events: **n**	**Arm A:** **2**+1	**Arm B:** 5	**Arm C:** 7
Patients with non infectious adverse events: maximum CTC 2/3/4 per patient Any CTC 2/3/4 events: #	3/8/1 5/9/1	4/2/1 6/2/1	7/6/1 11/6/1
Patients with infectious adverse events*:			

maximum CTC 2/3/4 per patient	1/0/0	0/1/1	3/1/0
Any CTC 2/3/4 events: #	2/0/0	0/1/1	3/1/0

Figure s1: Consort flow diagram.