Correspondence: Dr. Vincent Lévy, Centre d'Investigations Cliniques, Hôpital Saint Louis, 1 avenue Claude Vellefaux, 75010, Paris, France. Phone: international +33.1.42499494. Fax: international +33.1.42499397. E-mail: vincent.levy@sls.ap-hop-paris.fr

References

1. Dimopoulos MA, Panayiotidis P, Moulopoulos LA, Sfikakis P, Dalakas M. Waldenström's macroglobulinemia: clinical features, complications, and management. J Clin Oncol 2000; 18:214-26.
2. Foran JM, Rohatiner AZ, Coiffier B, Barbui T, Johnson SA, Hiddemann W, et al. Multicenter phase II study of fludarabine phosphate for patients with newly diagnosed lymphoplasmacytoid lymphoma, Waldenström's macroglobulinemia, and mantle-cell lymphoma. J Clin Oncol 1999;17:546-53.
3. Leblond V, Levy V, Maloisel F, Cazin B, Fermand JP, Remenieras JJ, et al. Results of a multicentric randomized study comparing the efficacy of fludarabine to that of cyclophosphamide, doxorubicine and prednisone in 92 patients with Waldenström's macroglobulinemia in first relapse or primary refractory disease. Blood 2001;98:2640-4.
4. Dhodapkar MV, Jacobson JL, Gertz MA, Rivkin SE, Roodman GD, Tuscano JM, et al. Prognostic factors and response to fludarabine therapy in patients with Waldenström's macroglobulinemia: results of United States intergroup trial (Southwest Oncology Group S9003). Blood 2001;98:41-8.
5. Morel P, Monconduit M, Jacomy D, Lenain P, Grosbois B, Bateli C, et al. Patients with the description of a new scoring system and its validation on 253 other patients. Blood 2000; 96:852-8.
6. Kaplan E, Meier P. Non-parametric estimation from incomplete observations. J Am Statist Assoc 1958;35:457-81
7. Cox D. Regression models and life tables. J R Statist Soc B 1972;34:187-201.
8. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residual. Biometrika 1994; 81: 515-26.
9. Hurvich CM, Tsai CL. Regression and time series model selection in small samples. Biometrika 1989;76:297-307.
10. Altman DG, Royston P. What do we mean by validating a prognostic model? Stat Med 2000;19:453-73.

Platelets

Effect of a single dose of rituximab in chronic immune thrombocytopenic purpura in childhood

Twenty-two children with immune thrombocytopenic purpura (ITP) with long-lasting thrombocytopenia, adversely affecting their quality of life, were treated with a reduced rituximab regimen in order to eliminate B cells producing anti-platelet antibody. A single dose of rituximab resulted in a response rate similar to that reported for cases in which 4 doses of rituximab were used.

haematologica 2005; 90:281-283
(http://www.haematologica.org/journal/2005/2/281.html)

Up to 30% of children suffering from immune thrombocytopenic purpura (ITP) fail to achieve remission within six months.[1] The quality of life in these children with chronic ITP is substantially reduced as they suffer from the permanent fear of bleeding, multiple physician visits or hospital admissions, side effects of treatment or recurrent bleeding events. In order to eliminate the B-cell clone producing the anti-platelet antibodies, the chimeric cytotoxic CD20-antibody rituximab was succesfully used in adults with chronic ITP[2-6] and in a small number of pediatric patients.[7,8] We report on the efficacy of a single dose of rituximab in childhood chronic ITP.

Table 1. Patients' characteristics itemized according to response to therapy, relapse rates, time to relapse and duration of continuous remission.

	Total n=22	*CR n=7*	*PR n=6*	*NR n=9*	*p**
Sex [n] (f-m)	14-8	6-1	4-2	4-5	0.231
Age at diagnosis [years] median (min-max)	5.8 (2.5-15.2)	11 (5-13.7)	4.3 (2.6-9.8)	5.0 (2.5-15.2)	0.044
ITP duration before rituximab [months] median (min-max)	44 (14-103)	53 (22-72)	47 (17-103)	38 (14-51)	0.283
Median PLT before rituximab [n×10⁹] median (min-max)	5 (2-27)	8 (2-27)	6 (2-11)	5 (2-9)	0.250
Duration of remission° [months] median (min-max)	12 (2-24)	12 (2-24)	10 (2-16)	–	0.885
Patients in continuous remission° [n]	8/13	3/7	5/6	–	0.266
Duration of continuous remission° [months] median (min-max)	13.5 (2-16)	12 (2-16)	15 (4-16)	–	0.647
Relapsed patients° [n]	5/13	4/7	1/6	–	0.266
Time to relapse° [months] median (min-max)	6 (2-24)	9 (4-24)	2	–	0.157

**Pearson's χ^2 test for categorical variables, Kruskal Wallis test for continuous variables. °NR excluded.*

The study protocol was approved by the local ethics committee and the patients were enrolled in the study after written consent from their guardians. Twenty-two children (8 boys, 14 girls) suffering from chronic ITP with documented platelet counts <30×10⁹/L for longer than 12 months were treated. The median age of the patients at diagnosis of ITP was 5.8 years (range 2.5-15.2), the median duration of documented thrombocytopenia was 44 months (range: 14-103) and the median platelet count before treatment was 5×10⁹/L (range: 2-27). In 18/22 patients the platelet count before rituximab was <10×10⁹/L. Only one patient had a platelet count >20×10⁹/L because of steroid treatment for a second intracranial hemorrhage. Bleeding symptoms were mild (grade 2)[9] in 13/22 patients, 7/22 patients suffered from moderate bleeding (grade 3)[9] requiring intervention and 2/22 patients had documented intracranial hemorrhage (grade 5)[9] in their ITP history. Prior to rituximab patients had been treated with intravenous immunoglobulins (IVIG) and/or steroids (21/22 IVIG, 19/22 steroids). A transient response was documented in 12/21 IVIG-treated patients, and 12/19. Anti-D treatment was given to 4/22 patients without response. Splenectomy prior to rituximab treatment was performed in 2/22 patients and partial embolization of the spleen in one patient; none of these 3 patients had a response. Bone marrow aspiration was performed in all patients before treatment to exclude thrombocytopenia due to megakaryocytopenia. All patients received a single intravenous dose of rituximab (375 mg/m²). The patients received no other treatment in addition to rituximab. Criteria for response to therapy were defined as follows: complete remission (CR), PLT > 100×10⁹/L; partial remission (PR) PLT >30×10⁹/L; no

©Ferrata Storti Foundation

Table 2. Comparison of the data on the effect of four doses and one dose of rituximab treatment in chronic ITP. Different authors used different response criteria; in order to allow comparison, the previously published results were fitted as best possible to the response criteria.

Author	*Age*	*Rituximab dose*	*n*	*CR*	*PR*	*Relapse**	*Patients in continuous remission*
Zaja *et al.*[5]	Adult	4×375 mg/m²	15	6/15 (40%)	2/15 (13%)	3/8 (38%)	5/15 (33%)
Shanafelt *et al.*[3]	Adult	4×375 mg/m²	12	5/12 (42%)	1/12 (8%)	2/6 (33%)	4/12 (33%)
Giagounidis *et al.*[4]	Adult	4×375 mg/m²	12	5/12 (42%)	4/12 (33%)	2/9 (22%)	7/12 (58%)
Cooper *et al.*[2]	Adult	4×375 mg/m²	57	18/57 (32%)	13/57 (23%)	13/31 (42%)	18/57 (32%)
Braendstrup *et al.*[6]	Adult	4×375 mg/m²	39	7/39 (18%)	10/39 (26%)	–	–
Wiley *et al.*[8]	Children	4×375 mg/m²	19	11/19 (58%)	4/19 (21%)	9/15 (60%)	6/19 (32%)
Summary of published data			154	**52/154 (34%)**	**34/154 (22%)**	**29/69 (42%)**	**40/115 (35%)**
Taube *et al.* (this study)	Children	**1×375 mg/m²**	22	**7/22 (32%)**	**6/22 (27%)**	**5/13 (38%)**	**8/22 (36%)**

**NR excluded.*

response (NR), PLT <30×10⁹/L. The patients' characteristics, itemized according to response, duration of remission, relapse rates as well as time to relapse, are given in Table 1. Older age at diagnosis of ITP was associated with a higher rate of complete response. No other significant differences were seen between complete, partial and non-responders (Table 1).

In all published reports on rituximab treatment of chronic ITP the antibody was administered in four weekly doses of 375 mg/m², in analogy to the treatment schedule used in oncology patients.[2-4,6-8] However, it was shown that the first dose of rituximab leads to a depletion of CD20⁺ cells in peripheral blood, measured one week after administration of the antibody.[10] In the light of these data and the fact that the numbers of CD20⁺ cells to be depleted are small in non-malignant B-cell disorders, such as chronic ITP, compared to in the oncology setting, we tested a single dose of rituximab instead of four weekly doses for the treatment of chronic ITP. Different response criteria were used in the various previous reports on rituximab treatment in chronic ITP. Some defined CR as a platelet count >150×10⁹/L,[2,4,8] others as a platelet count >100×10⁹/L.[3,5,6] Because, from a clinical point of view, a platelet count >100×10⁹/L is rarely associated with a considerable bleeding risk, we used this to define CR. To compare the data from previously published reports the results were fitted as best possible to the response criteria. In this comparison our data suggest that, in chronic ITP, the response rate after a single dose of rituximab is very similar to that after four doses and, likewise, that the relapse rate is not different (Table 2). Unfortunately, no parameter was found to predict the response to treatment. Therefore, further investigations are required to define parameters associated with the response to rituximab therapy in childhood chronic ITP.

In conclusion our results suggest that a single dose of rituximab in childhood chronic ITP may be efficacious, resulting in a response rate comparable to that achieved by the standard regimen. Therefore, a single dose of rituximab should be considered useful for the treatment of chronic ITP in order to reduce costs and patients' strain.

Tillmann Taube, Hansjörg Schmid,° Harald Reinhard,# Arend von Stackelberg,* Günter Henze,* Ursula Schulte Overberg**

**Charité - Universitätsmedizin Berlin, Otto-Heubner-Center for Pediatric and Adolescent Medicine, Clinic of Pediatrics Specializing in Oncology and Hematology, Berlin; °Medizinische Hochschule Hannover, Department of Pediatric Hematology/Oncology, Hannover; #University Hospital Homburg, Department of Pediatric Oncology, Homburg/Saar, Germany*

Key words: rituximab treatment, ITP, childhood.

Correspondence: Tillmann Taube, M.D., Charité-Universitätsmedizin Berlin, Campus Virchow-Klinikum, Otto-Heubner Centrum für Kinder- und Jugendmedizin, Klinik für Pädiatrie mit Schwerpunkt Onkologie/Hämatologie, Augustenburger Platz 1, 13353 Berlin, Germany. Phone: international +49.30.450666215. Fax: international +49.30.450566906. E-mail: tillmann.taube@charite.de

References

1. Blanchette VS, Price V. Childhood chronic immune thrombocytopenic purpura: unresolved issues. J Pediatr Hematol Oncol 2003;Suppl 1:S28-33.
2. Cooper N, Stasi R, Cunningham-Rundles S, Feuerstein MA, Leonard JP, Amadori S, et al. The efficacy and safety of B-cell depletion with anti-CD20 monoclonal antibody in adults with chronic immune thrombocytopenic purpura. Br J Haematol 2004;125:232-9.
3. Shanafelt TD, Madueme HL, Wolf RC, Tefferi A. Rituximab for immune cytopenia in adults: idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, and Evans syndrome. Mayo Clin Proc 2003;78:1340-6.
4. Giagounidis AA, Anhuf J, Schneider P, Germing U, Sohngen

©Ferrata Storti Foundation

D, Quabeck K, et al. Treatment of relapsed idiopathic thrombocytopenic purpura with the anti-CD20 monoclonal antibody rituximab: a pilot study. Eur J Haematol 2002;69:95-100.
5. Zaja F, Vianelli N, Sperotto A, De Vita S, Iacona I, Zaccaria A, et al. B-cell compartment as the selective target for the treatment of immune thrombocytopenias. Haematologica 2003; 88:538-46.
6. Braendstrup P, Pedersen M, Bjerrum OW, Nielsen OJ, Jensen BA, Nielsaage TC, et al. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with refractory idiopathic thrombocytopenic purpura. Blood 2003;11:[Abstract #1066].
7. Shenoy S, Kelly M, Grossman W, Ramadas J, Musselman M. Rituximab therapy in children with chronic refractory immune cytopenia: long term efficacy and immune function analysis. 2003 2003;11:[Abstract #1020].
8. Wiley JM, Wang J, Luddy RE, Bussel JB. Efficacy and safety of rituxan treatment in children with chronic immune thrombocytopenic purpura (ITP). Blood 2003;11:[Abstract #1062].
9. Buchanan GR, Adix L. Grading of hemorrhage in children with idiopathic thrombocytopenic purpura. J Pediatr 2002; 141:683-8.
10. Stasi R, Pagano A, Stipa E, Amadori S. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. Blood 2001; 98:952-7.

Disorders of Hemostasis

Identification of 32 novel mutations in the factor VIII gene in Indian patients with hemophilia A

Seventy-five unrelated hemophilia A patients from India were analyzed for factor VIII gene defects. Intron 22 inversion was identified in 22 patients and intron 1 inversion in 2 patients. In the remaining 51 patients without inversions screening the FVIII gene by denaturing high performance liquid chromatography (DHPLC) revealed 42 different mutations in 44 unrelated subjects. These included 14 missense, 7 nonsense, 9 splice site, 8 deletional, 3 insertional mutations and one indel mutation. Of these, 32 were novel gene alterations. The hotspots included intron 22 inversion, CpG and adenine runs.

haematologica 2005; 90:283-284
(http://www.haematologica.org/journal/2005/2/283.html)

Hemophilia A (HA) is an X chromosome-linked bleeding disorder, caused by defects in the factor VIII gene (*F8*). Apart from intron 22 and 1 inversions, all other mutations are distributed heterogeneously throughout the gene (*http://europium.csc.mrc.ac.uk*). There is a paucity of data on HA mutations in India. We report here, for the first time, the distribution of causal mutations in the *F8* gene of Indian HA subjects. Seventy-five patients with HA were studied. DNA was extracted from blood and polymerase chain reaction (PCR) was performed to detect inversions of intron 1 and 22.[1] In patients negative for these inversions mutation screening was done by denaturating high performance liquid chromatography (DHPLC).[2] Splice site mutations were predicted by a program from *http://www.fruitfly.org* which predicts potential splice sites in a given sequence. It assigns them a score from 0.4 to 1.0, with values less then 0.4 predicting absence of a splice site. The fragments with abnormal chromatograms were sequenced. We used the nomenclature for nucleotides and amino acids proposed by Dunnen *et al*.[3]

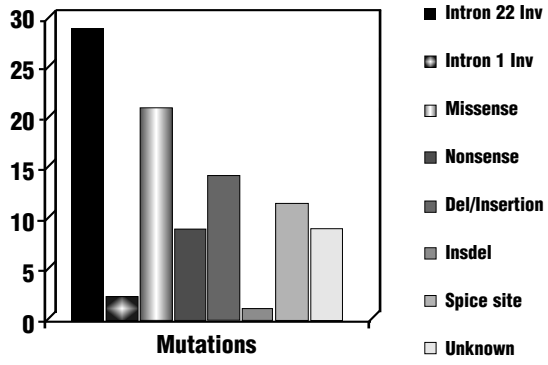

Figure 1. Mutation profile in hemophilia A patients.

Of the 75 patients with HA studied, 50 had severe HA (66.6%), 13 had moderate disease (17.3%) and 12 had mild HA (16%) were studied. Twenty-two had intron 22 inversion, 2 had intron 1 inversion, 16 had missense, 7 had nonsense and 9 had splice site mutations. Eight patients had deletions, 4 had insertions. Thirty-two of these were novel mutations, previously unreported in the Hemophilia A Mutation Database (HAMSTeRS). These novel mutations included 8 missense, 5 nonsense and 9 splice junction mutations and 10 deletions and insertions (Figure 1). The hotspots included intron 22 inversion, adenine runs and CpG.

Novel missense mutations were seen throughout the *F8* gene, but not in the B domain (Table 1). This further reinforces the view that single nucleotide substitutions within the B domain are largely unimportant.[4] Novel missense mutations identified may be causative. Moreover, polymorphisms in the coding regions of *F8* gene are rare and these changes were not found in normal individuals or as second site changes in hemophilia A patients.

These mutations replace amino acids that are conserved in humans, pigs, murine and canine species. The nonsense novel mutations were seen in A2, B, A3, C1, and C2 domains. These resulted in truncated proteins and were considered pathogenic. Nine novel splice junction mutations were seen in domains A1, A2 and C1. Six out of these had a score < 0.4, predicting absence of a splice site (Table 1). In 3 patients the program did not predict a splicing error. As no other gene alteration was found in these patients, further studies on the RNA are being undertaken to confirm the causal nature of these defects.

Ten of the 12 novel deletions and insertions caused a frame shift, leading to the introduction of a premature termination codon and thus a truncated protein (Table 2). One small deletion c.5293_5295delCCC was an in-frame mutation leading to deletion of proline at position 1746, which leads to a milder phenotype with 9% FVIII:C. In patient HA50, screening for all fragments except exon 26 showed normal amplification but amplification of exon 26 was unsuccessful despite a number of repetitions and multiplexing, suggesting a deletion of the whole exon in this patient. Large deletions are reported to be the causal defects in 3.9 to 7% of patients with hemophilia A,[5] which is mirrored in our study. Three severe hemophiliacs

©Ferrata Storti Foundation