# Rituximab therapy for childhood Evans syndrome

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*Funding: this work was supported by a grant from the Association Française pour le Syndrome d'Evans and by a grant from GIS Institut Maladies rares-INSERM.*

*Manuscript received March 22, 2007. Manuscript accepted September 21, 2007.*

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## ABSTRACT

The safety and efficacy of rituximab have been retrospectively assessed in 17 children with Evans syndrome. Patients received 4 or 3 weekly doses of rituximab (375 mg/m$^2$ per dose) associated with prednisone, alone (14 patients) or associated with other immunosuppressive drugs. Complete or partial remission of at least one cytopenia was achieved in 13 out of the 17 patients (76%), and lasted in 11 of them with a mean follow-up of 2.4 years (range 0.5-7 years). Steroid therapy was stopped or tapered at 50-100% of the baseline dosage in all long-term responders. Moderate side effects and infection occurred only in 4 and 1 children respectively.

Key words: Evans syndrome, children, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, rituximab.

Haematologica 2007; 92:1691-1694. DOI:10.3324/haematol.11540



Evans syndrome (ES) is a rare and severe disease in children, defined by the combination of autoimmune hemolytic anemia (AHAI) and autoimmune thrombocytopenia (ITP) in the absence of known underlying etiology.[1] Mortality is high in the 4 reported pediatric series, ranging from 7-36%.[2-5] Only a few non-controlled trials of treatment regimens containing a small number of patients with ES have been published.[6] The management of ES therefore remains a challenge and innovative approaches are needed. Although many aspects of ES pathogenesis remain unknown, a key role of B cells in producing auto-antibodies and co-stimulating T-cells may be considered. Rituximab, a chimeric anti-CD20 monoclonal antibody directed against the membrane-associated CD20 molecule, results in a marked B-cell depletion.[7] Therefore, it has been tested in the treatment of childhood AIHA[8, 9] and ITP,[10, 11] and recent reports have suggested its efficacy and safety in this field. This is a retrospective report on the widest series of anti-CD20 monoclonal therapy in childhood-onset Evans syndrome. It aims to describe its efficacy and safety in children.

## Design and Methods

### Patients

A French registry of pediatric Evans syndrome, diagnosed before 15 years of age, was compiled on behalf of the French Society of Pediatric Hematology and Immunology to assess the demographic presentation, clinical course and treatment response of the disease and to investigate its pathophysiology. Patients with ES, defined by the combination (either simultaneously or sequentially) of AHAI and ITP, were eligible for enrollment in the registry.

On study entry, patients and/or their parents (according to the age of the child) had signed an institutional review board-approved informed consent. All the 25 French hematology pediatric centers were contacted. The study was retrospective until 2004, and prospective thereafter. The cut-off date of follow-up was December 2006. To allow us to analyze a homogeneous population, among the 84 patients enrolled in the registry, we retrospectively reviewed all those meeting the following criteria: treatment with rituximab, absence of known associated underlying

**Table 1.** Characteristics of the seventeen patients at baseline.

| *Patient Ethnicity/ Sex* | *Familial history* | *Concomitant disease* | *Previous treatment (months elapsed from end of treatment to first infusion of RTX)* | *Transfusion dependence* | *Age at onset of AIHA/ age at onset of ITP (year)* | *DAT* |
|---|---|---|---|---|---|---|
| 1/Af/M | Consanguinity | AI enteropathy | PRED*, IVIg, CSA*, AZA | No | 3/4 | IgG |
| 2/Ca/M | ITP (father) | – | PRED*, IVIg | No | 5/7 | IgG, C |
| 3/Ca/M | – | – | PRED*, CYC, IVIg, splenectomy (104) | Yes | 10.5/0.7 | IgG, Ig A, C |
| 4/Ma/F | SLE (mother, aunt) | – | PRED*, IVIg | No | 0.8/0.8 | IgG |
| 5/Ca/M | – | – | PRED*, IVIg | No | 4/4 | IgG |
| 6/NA/M | – | Celiac disease | PRED*, IVIg, CSA (3), AZA (2), CYC (1), splenectomy (5) | Yes | 1.5/1.5 | IgG, C |
| 7/Ca/F | – | – | PRED*, IVIg | No | 13/12 | – |
| 8/Ca/F | – | – | PRED*, IVIg, VCR* | No | 3.5/4 | N |
| 9a/Ma/M | – | $\alpha$ thalassemia | PRED*, IVIg, CSA*, AZA (13) | No | 5/4 | IgG, C |
| 9b/Ma/M | – | | PRED*, IVIg, CSA*, AZA (20), RTX (7) | No | | |
| 10/Ma/F | – | – | PRED*, IVIg, VCR (2), CSA (1), CYC (1), plasmapheresis (2), splenectomy (1) | Yes | 4 /3.8 | IgG |
| 11a/Ca/M | – | – | PRED*, IVIg, VCR (84), Danazol (72), CSA (1), splenectomy (7) | Yes | 3/3.2 | IgG |
| 11b/Ca/M | – | | PRED*, IVIg, VCR (110), Danazol (98), splenectomy (33), RTX (26) | Yes | | |
| 12/Ca/F | – | Thyroiditis | PRED*, IVIg, splenectomy (36) | No | 14/12 | IgG, C |
| 13a/Ca/F | – | – | PRED*, CSA | – | 1/1 | IgG |
| 13b/Ca/f | – | | PRED*, IVIg, splenectomy (1), RTX (8) | – | | |
| 14/Ca/F | – | – | PRED*, IVIg, splenectomy (1) | Yes | 5/5 | IgG |
| 15/Ca/F | AIHI (maternal uncle) | – | PRED* | No | 12/7 | IgG, C |
| 16/Ca/F | – | – | PRED* | No | 2/2 | IgG |
| 17/Ca/F | – | – | PRED* CSA (0.3) | Yes | 12/12 | IgG |

*Ca: Caucasian ; Af: African ; Ma: Magrebian, PRED: prednisone per os; CYC: intravenous cyclophosphamide; AZA: azathioprine; IVIg: intravenous immunoglobulin; CSA: ciclosporin A ; VCR: vincristine; RTX: rituximab, DAT: direct antiglobulin test; AI: autoimmune; C: complement. *Indicates the treatment present at the time of rituximab initiation. a indicates the first course of rituximab and b the second course in the same patient.*

immunodeficiency, especially serum immunoglobulin class and subclass deficiency, or autoimmune disease. The study was approved by the Bordeaux hospital ethics committee. Patients were assessed for indication of rituximab, administrated rituximab regimen, clinical and biologic response, adverse events, B cell depletion. Complete remission of AHAI was defined as a sustained hemoglobin level >11 g/dL and reticulocyte count <120×$10^9$/L during 4 consecutive weeks. Partial remission of AHAI was defined as a sustained hemoglobin level of 7-11 g/dL or hemoglobin level > 11 g/dL and reticulocyte count >120×$10^9$/L during 4 consecutive weeks. Complete remission of ITP was defined as a sustained platelet count >150×$10^9$/L during 4 consecutive weeks. Partial remission of ITP was defined as a sustained platelet count of 50-150×$10^9$/L, associated with a twofold increase of platelet count during 4 consecutive weeks. Responses had to be independent of rescue and supportive care regimens between measurements. We used Fisher's exact test at an $\alpha$ level of 0.05 to compare characteristics of responders and non-responders.

## Results and Discussion

Seventeen patients, median age at first infusion of rituximab 7.7 years (range 0.7–15 years) were included. Three received a second course of rituximab after a relapse.

Demographic characteristics, duration and type of autoimmune cytopenias are shown in Table 1. Patients received rituximab because of the lack of efficiency of previous treatments. All of them had previously received prednisone, alone (8 patients) or associated with other immunosuppressive drugs, and 7 (41%) underwent previous splenectomy (Table 1). Rituximab treatment was indicated in 6 patients for AHAI alone (7 courses of rituximab), in 1 patient for ITP alone (2 courses of rituximab), and in 10 patients for both cytopenias. Four or 3 weekly doses of rituximab at 375 mg/$m^2$ per dose were administered after a premedication with diphenhydramine, paracetamol and/or steroids.

Prednisone dosage ranged from 0.2-3 mg/kg/d at the start of rituximab treatment, and was associated with vincristine or cyclosporine A in 3 patients (Table 2). All but 3 patients (patients 7, 16, 17) received substitutive intravenous immunoglobulin (IVIG) for 4-10 months to prevent therapy-induced hypogammaglobulinemia. Overall, a complete or partial remission of at least one cytopenia was achieved in 13 out of the 17 patients (76%) (Table 2). Complete or partial remission was achieved in 12 out of the 16 patients (75%) presenting with AIHA and in 7 of the 11 patients (63%) presenting with ITP. Among the 10 patients who presented with AIHA and ITP together at the start of the rituximab treatment, complete or partial remission of both hematologic involvements was achieved in 6 patients. The time to complete or partial remission of AIHA and ITP was short, with a mean of 1.5 months (range 0.2-4 months) (*Figure 1, online supplement*). In 11 out of 13 patients, duration of partial or complete remission was a mean of 2.4 years (range 0.5-7 years). Steroid therapy was stopped or tapered at 50-75% of the baseline dosage in 8 and 3 patients respectively. One of the

**Table 2.** Rituximab regimen, hematologic outcome and B cell depletion in seventeen Evans patients.

| *Patient/age at onset of RTX* | *Rituximab regimen* | *Cible/ Duration of cytopenia (years)* | *Response (months from onset of RTX)* | *Relapse (months from onset of RTX)* | *Treatment at cut off in responding patients* | *Duration of the follow-up in long-term responders (years)* | *B cell depletion (% of CD19 B cells)* |
|---|---|---|---|---|---|---|---|
| 1/6 yr. | 4×375 mg/m²; PRED 2mg/kg/d, CSA, AZA | AHAI (3) | CR (0.5) | No | PRED (0.4 mg/kg/d) | 4 | Yes (0) |
| 2/7 yr. | 3×375 mg/m²; PRED 0.6 mg/kg/d | AHAI (2)<br>ITP (0.1) | NR<br>NR | | –<br>– | – | Yes (0) |
| 3/10 yr. | 4×375 mg/m²; PRED 0.2mg/kg/d, | AHAI (0.05) | NR | | – | – | Yes (0) |
| 4/0.7 yr. | 4×375 mg/m²; PRED 3.3 mg/kg/d | AHAI (0.5)<br>ITP (0.5) | CR (1)<br>CR (1) | No<br>No | No | 7 | Yes (0) |
| 5/4 yr. | 4×375 mg/m²; PRED 2 mg/kg/d | AHAI (0.1)<br>ITP (0.1) | CR (0.5)<br>PR (2) | No<br>No | No | 1.3 | NA |
| 6/7 yr. | 4×375 mg/m²; PRED 2 mg/d | AIHA (0.7)<br>ITP (0.7) | NR<br>NR | | – | – | NA |
| 7/2 yr. | 4×375 mg/m²; PRED 1mg/kg/d | AHAI (0.3)<br>ITP (0.3) | CR (0.5)<br>CR (0.2) | No<br>No | No | 1.2 | NA |
| 8/6 yr. | 4×375 mg/m²; PRED 2 mg/kg/d, VCR | AHAI (2) | CR (1.2) | Yes (12) | | – | Yes (1) |
| 9a/14 yr. | 4×375 mg/m²; PRED 0.6 mg/kg/d, CSA | AHAI (11.5) | CR (2) | Yes (3) | CSA, AZA | – | NA |
| 9b/14.3 yr. | 4×375 mg/m²; PRED 0.14 mg/kg/d, CSA | AHAI (11.8) | CR (4) | No | CSA, AZA | 2 | NA |
| 10/4 yr. | 4 x 375 mg/m²; PRED 1 mg/kg/d | AHAI (0.3) | CR (2) | No | No | 4 | Yes (0) |
| 11a/11 yr. | 4×375 mg/m²; PRED 1 mg/kg/d | ITP (8.5) | CR (2 ) | No | MMF, AZA | 1.7 | Yes (0) |
| 11b/12.5 yr. | 4 ×375 mg/m²; | AIHA (10.3) | NR | | | – | NA |
| 12/15 yr. | 3×375 mg/m²; PRED 0.3 mg/kg/d | AIHA (1.2)<br>ITP (1.2) | CR (1)<br>NR (1) | No<br>No | PRED (0.08 mg/kg /d) | 1.5 | NA |
| 13a/2 yr. | 4×375 mg/m²; PRED 0.8 mg/kg/d | ITP (1) | NR | – | | – | Yes (0%) |
| 13b/2.7 yr. | 3×375 mg/m²; PRED 1.2 mg/kg/d | ITP (1.7) | NR | – | | – | |
| 14/9 yr. | 4×375 mg/m²; CS 2 mg/kg/d | AIHA (3.8)<br>ITP (3.8) | CR (2)<br>CR (0.5) | No<br>No | PRED (0.2 mg/kg/d) | 4 | Yes (0) |
| 15/11 yr. | 4×375 mg/m²; PRED 1 mg/kg/d | AIHA (4) | PR (1) | No | No | 0.5 | Yes (0) |
| 16/4 yr. | 4×375 mg/m²; PRED 2 mg/kg/d | AIHA (1.5)<br>ITP (2) | CR (2)<br>CR (2) | No<br>No | No | 0.7 | Yes (1) |
| 17/12 yr. | 4×75 mg/m²; PRED 1.3 mg/kg/d, CSA | AIHA (0.3)<br>ITP (5) | CR (2)<br>PR (1) | No<br>No | No | 1 | Yes (1) |

*PR: partial remission; CR: complete remission; Pred: prednisone per os; CYC: intravenous cyclophosphamide; AZA: azathioprin; IVIG: intravenous immunoglobulin; CSA: ciclosporin A; MMF: mycophenolate mofetyl; VCR: vincristine; RTX: rituximab; NA: not available; NR: not response.*

2 patients (patients 8 and 9) who relapsed 3 and 12 months after treatment respectively was successfully retreated with a second course of rituximab.

Rituximab response was not associated with prior splenectomy ($p$=0.25), age at onset of Evans syndrome under or over 6 years ($p$=1), or duration of the cytopenia for more or less than 1 year ($p$=1).[6] This retrospective study was limited by the heterogeneity of the rituximab regimens which does not allow any conclusions about the optimal regimen. Three responding patients received immunosuppressive drugs at the start of rituximab treatment. However, these drugs were discontinued for 2 of them within five months of the first infusion of rituximab, without relapse. Therefore, rituximab probably induced the remission by itself. The small number of patients, does not allow a comparison of response according to the number of courses of rituximab.

The treatment was well tolerated. Only 3 children experienced moderate side effects consisting of vomiting, facial edema and urticarial rash during the infusions. These were promptly resolved with appropriate therapy. A fifth child (patient 16) developed transient neutropenia ranging from 0.22-0.4×10⁹/L within 2 weeks of rituximab administration. Patient 14 experienced a non-severe pneumonia seven days after the fourth infusion of rituximab while receiving substitutive IVIG. Depletion of peripheral B cells ($CD19^+$) occurred in all 12 patients tested from a baseline mean of 28% to less than 1%, including three non-responding patients. This lasted 2-7 months in the 5 patients tested.

This first pediatric series of rituximab therapy for childhood-onset Evans syndrome suggests that rituximab in combination with steroids may be effective in most of the children with severe Evans syndrome refractory to standard agents. Similarly, a pooled analysis of ES pediatric cases[8, 9, 11, 12] showed a high response rate of 77% among 13 patients, although publication bias may have overestimated the response rate. Additionally, ES was weakly associated with achievement of the primary outcome in children treated for chronic immune thrombocytopenic purpura.[11] Repeated courses of rituximab were, as in our series, safe and efficient in previous studies[8, 13] and may be an alterna-

tive therapy to splenectomy in relapsing patients. However, the long-term safety of B cell therapy must still be clarified.[7] The present study confirms the good tolerability of rituximab in the treatment of autoimmune disorders.[14] However, the recent report of severe hematologic side-effects and of a higher rate of serum sickness than in adults emphasizes the need for careful monitoring of this treatment in children.[10, 11, 15] A low infection rate and an absence of significant hypogammaglobulinemia were shown in adults and children over 10 years old receiving rituximab for autoimmune diseases.[11, 16, 17] Conversely, younger children were kept on prophylactic IVIG replacement therapy,[9] but data on serum immunoglobulin levels were not provided. The only severe infection reported in children was an enteroviral meningoencephalitis which occurred after severe immunosuppression.[18]

These features suggest that IVIG may be unnecessary in children over 10 years old without underlying immunodeficiency. However, serum immunoglobulins levels must be carefully monitored in all patients, and further evaluation is needed to provide pediatric guidelines.

CD19 B cell depletion occurred in all 12 patients tested, and did not parallel the improvement of hematologic disease. This agrees with the report by Zecca *et al.*[8] of children treated with rituximab for AIHA. Conversely, in older children with systemic lupus erythematosus, B cell depletion induced by rituximab is variable and often paralleled with clinical remission.[16,17] This suggests that the mechanisms of action of rituximab may differ according to the nature of the auto-immune disease and/or with the age at onset of treatment. The polymorphism of Fcγ receptors, serum rituximab level, inherited or acquired deficiencies in complement and the occurrence of human antichimeric antibodies could interfere with the efficiency of rituximab,[13, 19, 20] but these features were not investigated in our patients.

Finally, regarding the rapid response to rituximab in most of the patients who were refractory to standard agents, and to the safety of this drug in our small retrospective study,[8] we suggest that it should be promptly proposed as second line treatment early in the course of pediatric Evans syndrome refractory to steroids. Nevertheless, further prospective trials in larger series of children are needed to confirm the efficiency of this treatment, to assess its long-term safety, and to identify predictive factors of response to rituximab.

### Authors' Contributions

*GL, FM, AR, BN, MM, CA-A, KY, CP, FB, BB-M, NA, YP, YB, FL, AC and AP have all substantially contributed to conception and design, or acquisition of data, or analysis and interpretation of data of this multicentric study, contributed to drafting the article, revising it critically and approving the final version to be published; AP performed statistical analysis. Each author has participated sufficiently in the work to take public responsibility for appropriate portions of the content.*

### Conflicts of Interest

*The authors reported no potential conflicts of interest.*

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