patients with partial responses to FC.

The data we present suggest that EBV-reactivation in CLL may not be rare, in fact the condition could be significantly under-diagnosed. All 11 cases had signs of severe CLL associated secondary immunodeficiency. The clinical presentation of EBV reactivation was as varied as in BLPD associated with primary immunodeficiencies, and can mimic the symptoms of active CLL. Therefore, EBV-reactivation must be considered in febrile CLL patients with high-risk biological risk features and/or fludarabine-refractory disease. In the absence of clinical trials for the management of EBV reactivation in CLL, the treatment strategy should include rituximab as in the non-CLL setting, possibly in combination with chemotherapy as recommended for Richter's syndrome.[7] Further research is needed to determine a threshold for differentiation between a significant and non-significant increase in EBV copies, and to determine when rituximab therapy should be initiated.

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Rituximab in patients with hairy cell leukemia relapsing after treatment with 2-chlorodeoxyadenosine (SAKK 31/98)

We assessed the efficacy of rituximab, 375 mg/m^2 weekly x 4, in 26 patients with hairy cell leukemia (HCL) relapsed or progressed after prior 2-chlorodeoxyadenosine (CDA). Overall response rate (RR) was 80%, with 32% complete remission (CR). Median relapse-free-survival (RFS) was 27 months and median remission duration (RD) 33.6 months.

HCL is an indolent B-cell neoplasm. Hairy cells typically coexpress CD11c, CD25 and CD103 antigens in addition to the pan B-cell antigen CD20.[1] Patients require treatment for pancytopenia, infections or symptomatic splenomegaly and CDA has emerged as the treatment of choice.[2] A single course of CDA produces CR in up to 85% of patients and partial response (PR) in 5-25%.[3,4] With longer follow-up, relapses of HCL are common. Goodman *et al*. reported a 37% relapse rate in 209 patients treated with CDA and had at least seven years of follow-up.[5] The RR (CR and PR) after re-treatment with CDA ranges from 60 to 90% but with shorter RD. Bone marrow aplasia, prolonged cytopenias and infections increase with repetitive CDA courses.[2] Hairy cells exhibit an especially high CD20 antigen density,[6] and the anti-CD20 monoclonal antibody rituximab is considered an attractive treatment option.

We conducted a multicenter phase II trial to investigate rituximab in pre-treated HCL patients of any age who had received at least one previous course of CDA. Diagnosis and assessment of remission was established by morphology including peripheral blood smear and bone marrow examination, supported by a positive tartrate-resistant acid phosphatase (TRAP) stain and characteristic immunophenotyping. Relapse was defined as the reappearance of hairy cells in the bone marrow or as any other new disease manifestations after documented CR. Progressive disease (PD) was defined as >50% increase in the percentage of residual tumor cells, or as >50% increase of residual disease-related organomegaly after documented PR.

Between February 1998 and July 2002, 26 patients were accrued. One patient was not evaluable. Of 25 patients, 24 had classical HCL and one a prolymphocytic sub-type. Pre-treatment consisted of CDA (n=25), splenectomy (n=4), interferon-α (n=9) and alkylating agents (n=2). Nine patients had relapsed and 16 had PD. Patients' characteristics are summarized in Table 1.

Patients received rituximab 375 mg/m^2 weekly x 4 doses using standard infusion guidelines. Re-staging was carried out two months after treatment and then every three months during the first three years and every six months thereafter until relapse. Twenty-four of the 25 patients received all of the four planned infusions. One patient stopped treatment after the first infusion because of a dermal vasculitis. Across all follow-up visits the RR was 80% (20/25 patients; 95% CI: 64.32, 95.68) and the rate of CR was 32% (8/25 patients; 95% CI: 13.71,

Table 1. Patients' characteristics at study entry (n=25).

Factor	*N. patients (%)*
Gender: Male	20 (80)
ECOG performance status:	
0	17 (68)
1	7 (28)
2	1 (4)
Hairy cell leukemia sub-type	
Classic HCL	24 (96)
prolymphocytic subtype	1 (4)
Status at study entry	
Relapse	9 (36)
Progressive disease	16 (64)
Organ involvement:	
Splenomegaly	9 (36)
Lymphadenopathy	2 (8)
Previous therapy types administered	
2-chlorodeoxyadenosine	25 (100)
Interferon-α	9 (36)
Splenectomy	4 (16)
Chemotherapy	2 (8)
Total number of previous therapy types administered	
1	15 (60)
2	5 (20)
3	5 (20)
Median (quartile range)	
Bone marrow infiltration (%)	55.00 (41, 80)
Hemoglobin (g/dL)	13.20 (11.9, 13.9)
Leucocytes ($\times 10^9$/L)	2.80 (2.4, 3.2)
Thrombocytes ($\times 10^9$/L)	95.00 (83, 177)
Creatinine (μmol/L)	96.00 (83, 101)
Lactate dehydrogenase (IU/L)	281.00 (199, 367)
Uric acid (μmol/L)	386.00 (299, 431)

Table 2. Rates of remission.

Disease status at trial registration (n=25)
Number of events (%)

Follow-up time	*Outcome*	*Relapsed*	*Progressive disease*	*Total (plus 95% confidence interval)*
At month three	CR	1 (4)	4 (16)	5 (20) (4.32, 35.68)
	CR+PR (RR)	4 (16)	9 (36)	13 (52) (32.42, 71.58)
Total follow-up (best response)	CR	2 (8)	6 (24)	8 (32) (13.71, 50.29)
	CR+PR (RR)	6 (24)	14 (56)	20 (80) (64.32, 95.68)

Number of treatment lines received previously (n=25)
Number of events (%)

Follow-up time	Outcome	1	2	3
At month three	CR	3 (12)	2 (8)	0 (0)
	CR+PR (RR)	7 (28)	5 (20)	1 (4)
Total follow-up (best response)	CR	5 (20)	2 (8)	1 (4)
	CR+PR (RR)	11 (44)	5 (20)	4 (16)

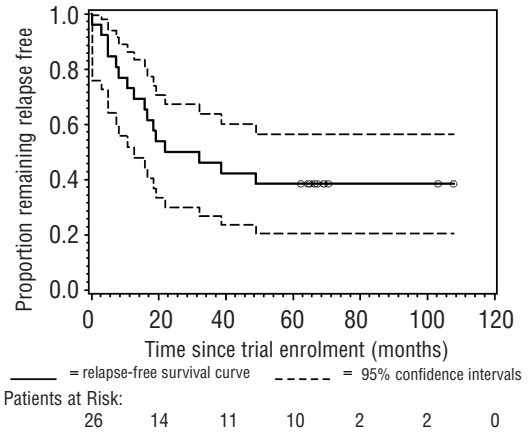

Figure 1. Kaplan-Meier curve for relapse-free survival with 95% confidence interval.

50.29). Patients with PD at trial registration had higher RR and CR rates compared to relapsed patients (56% *vs.* 24% and 24% *vs.* 8% respectively). The RR depends on the number of previous treatments (Table 2). The median RFS was 27 months (95% CI: 15.90, N/A) (Figure 1). Median follow-up time was 27 months. Eight of the 16 patients who achieved CR or PR had a PD. The median RD was 33.6 months. Seven serious adverse events have been recorded: one patient died of cardiac failure one day after the first infusion of rituximab, one patient developed a grade 3 dermal vasculitis and another patient a grade 4 thrombocytopenia. Two patients experienced a basalioma and one patient a prostate cancer during follow-up. One patient died as a result of gastrointestinal bleeding 12 weeks after treatment termination. Most adverse events were grade 1 and 2 expected infusional events that occurred during the first course of rituximab administration.

Rituximab at 375 mg/m^2/week × 4 in previously treated HCL has been studied by others. Hagberg and Lundholm[7] reported a RR of 64% and Lauria *et al.*[8] 50% in a series of 11 and 10 patients respectively. However, Nieva *et al*. demonstrated a RR of only 25% in 24 CDA-failed patients using the same dose and schedule of rituximab.[9] This difference may be due to patient selection. The majority of our patients were less pre-treated, having received only one course of CDA. Recently, Thomas *et al*. published the results of a phase II trial involving 15 patients with relapsed or refractory HCL, using a regimen of eight weekly doses of rituximab at 375 mg/m^2. The overall RR was 80%, with 53% CR.[10] The higher CR rate suggests that four weekly doses of rituximab may be inadequate to achieve the optimal response.

In conclusion, rituximab has good activity and is well tolerated in patients with HCL. However, the optimal dose and schedule has to be clarified. The efficacy of rituximab as first line treatment for HCL, as treatment of minimal residual disease or in combination with CDA, should be tested in future trials.

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Increased cytotoxic T-lymphocyte-mediated cytotoxicity predominant in patients with idiopathic thrombocytopenic purpura without platelet autoantibodies

Idiopathic thrombocytopenic purpura (ITP) is a common hematologic disorder manifested by autoantibody-mediated platelet destruction. In the majority of ITP patients, autoantibodies were found to be specific to GPIIb/IIIa or GPIb/IX.[1] It may not only damage platelets via the reticuloendothelial system,[2] but may also inhibit platelet production.[3,4] However, platelet autoantibodies can be detected in only 50-70% of ITP patients,[5] indicating other mechanisms could be involved. Recently, *in vitro* studies suggested that $CD8^+$ cytotoxic T-lymphocyte (CTL) mediated lysis of autologous platelets may contribute to platelet destruction in ITP.[6,7] In the present study, we prospectively measured CTL-mediated cytotoxicity toward autologous platelets between ITP patients with and without autoantibodies, and evaluated the effect of high dose dexamethasone (HD-DXM) on this action.

Forty-eight previously-untreated ITP patients were enrolled by diagnostic criteria for ITP.[8] Blood sampling was performed before and two weeks after treatment with HD-DXM.[9] The control group consisted of 17 healthy adult volunteers with no history of blood transfusion or pregnancies (Table 1). The study was approved by the Medical Ethical Committee of Qilu Hospital. Informed consent was obtained from all subjects.

All plasma samples for modified monoclonal antibody specific immobilization of platelet antigen (MAIPA) assay were obtained from ethylenediaminetetraacetic acid (EDTA) anticoagulated blood by centrifugation and stored at -80°C prior to use. Platelets were prepared by differential centrifugation from EDTA-anticoagulated blood and used as target cells. $CD8^+$ T lymphocytes were isolated by magnetic microbeads (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) from peripheral blood mononuclear cells by density gradient centrifugation and used as effector cells. The purity of $CD8^+$ T cells was > 92%. The concentration of effector and target cells was adjusted to 10^5/mL and 10^6/mL respectively, and then diluted to achieve a final effector/target (E/T) ratio of 1:10. All cells were washed free from plasma.

Detection of specific anti-platelet GPIIb/IIIa and/or GPIb autoantibodies was performed as described by Hou *et al.*[10] Measurement of CTL-mediated cytotoxicity was carried out according to the protocol developed by Goldberg *et al.*[11] based on two-color flow cytometry to identify different populations of live targets ($CD41a^+$ Annexin V-) and apoptotic targets ($CD41a^+$ Annexin V^+) using antibodies from BD Biosciences (San Jose, CA, USA). Anti-CD3 antibody (BD Biosciences, San Jose, CA, USA) was added at a final concentration of 0.32 μg/mL to stimulate cytolytic effector T cells. Spontaneous lysis was determined in control tubes holding only culture media and platelets. A minimum of 10,000 events were acquired and data were analyzed using CellQuest Software on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA, USA). Percent lysis and specific lysis were calculated as follows: percent lysis=total ($CD41a^+$, Annexin V^+)=Total ($CD41a^+$); specific lysis=percent induced lysis=percent spontaneous lysis, and expressed as a percentage.

In the plasma of 48 patients with ITP, antibodies against GPIIb/IIIa and/or GPIb/IX were detected in 22 samples (group I). Negative reactions to both glycoproteins were displayed in the remainders (group II). Before HD-DXM treatment, positive platelet lysis was seen in 11 of group I and 21 of group II (group I vs. group II, $p<0.05$) and in 4 of group I and 7 of group II after treatment (group I *vs.* group II, not significant (NS); pre-treatment *vs.* post-treatment in group I, $p<0.05$, and in group II, $p<0.01$) (Figure 1A-F). On the other hand, before treatment, both group I and group II had increased platelet lysis compared with controls (group I *vs.* controls, $p<0.05$; group II *vs.* controls, $p<0.01$; group I *vs.* group II, $p<0.01$), whereas platelet lysis was substantially decreased in both groups after treatment (pre-treatment *vs.* post-treatment in group I, $p<0.01$, and in group II, $p<0.01$) (Figure 1G). Interestingly, positive

Table 1. Subjects' characteristics.

Characteristics	*ITP patients*	*Controls*
Age (median, range)	29 (11-73)	27 (18-40)
Sex (number, %)		
female	30 (62.5)	10 (58.8)
male	18 (37.5)	7 (41.2)
Platelet count ($\times10^9$/L) (median, range)		
pre-treatment	10.5 (1-55)	239 (156-350)
post-treatment	78.5 (10-305)–	