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Chronic Lymphoproliferative Disorders

A pilot study of low-dose subcutaneous alemtuzumab therapy for patients with hemotherapy-refractory chronic lymphocytic leukemia

Subcutaneous low-dose alemtuzumab (10 mg t.i.w. for 18 weeks) induced a 50% response rate, including 25% complete response, in 16 patients with refractory chronic lymphocytic leukemia (CLL) patients. The responses were substantial even in patients with unfavorable cytogenetics, fludarabine/rituximab refractoriness, Rai stage IV, previous infections, and age over 65 years. Subcutaneous low-dose alemtuzumab is effective in poor prognosis B-CLL, and has a particularly favourable toxicity profile.

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Patients with chronic lymphocytic leukemia (CLL) who are resistant/refractory to alkylating agents and/or fludarabine¹⁻³ have a poor prognosis, with a median survival duration of only 10 months.⁴

In this subset of high-risk patients, alemtuzumab has been shown to induce a significant overall response rate of 33%.² However, treatment with alemtuzumab according to the conventional administration schedule (30 mg three times a week intravenously) is associated with a consistent number of reactions and significant infectious morbidity.^{5,6} It has recently been reported that both the percentage and severity of *first dose* reactions can be dramatically reduced by the *subcutaneous* administration of alemtuzumab 30 mg three times weekly for 18 weeks.⁷

On the basis of these data we decided to assess the efficacy and safety of prolonged treatment with subcutaneous low-dose alemtuzumab (10 mg three times a week for 18 weeks) in a cohort of 16 heavily pre-treated B-CLL patients.

Sixteen patients were enrolled. The patients had received a median of three prior lines of therapy, all were refractory to alkylating agents, fourteen were refractory to fludarabine, and two were not allowed a purine analog-containing therapy due to previous Coombs'-positive anemia. Half of the patients were also refractory to rituximab-containing regimens. Half of the patients had had infections during the six months preceding the start of alemtuzumab therapy. Two patients with hepatitis B virus (HBV) reactivation were on lamivudine therapy, which was continued during treatment with alemtuzumab (Table 1).

All patients received at least four weeks of alemtuzumab therapy; twelve patients completed all 18 weeks of treatment. The reasons for treatment withdrawal during weeks 4-14 were the achievement of a complete response (3 patients), and infection (1 patient).

The overall response, according to NCIWG criteria,⁸ of the patients enrolled in this pilot study was 50%, including 25% complete responses. No progressive disease was observed during treatment. An objective response was documented in 50% of the patients refractory to both alkylators and fludarabine. Three of the eight patients resistant to rituximab responded to alemtuzumab. Overall response was 43.3% in the patients with an abnormal karyotype and 37.5% in patients with unfavorable cytogenetic alterations. The two patients with p53 gene deletions [del(17)(p13.1)] both showed a partial response after alemtuzumab therapy. The therapy was exceptionally well tolerated by the older patients, with remarkable responses in terms of percentage and quality (Table 2). A higher proportion of patients achieved responses in blood (93.7%) and bone marrow (62.5%), as compared with lymph nodes and spleen (50% and 42.9%, respectively). The time to achieve a 1-log depletion of peripheral blood lymphocytes (PBL) was more rapid in responders than in non-responders (3 weeks versus 8 weeks), and was particularly quick in complete responders (2.5 weeks). This observation, simply made by assessing PBL counts using a hematologic analyzer, is in line with the results of the elegant study by Rawstron *et al.*⁹

Four patients died of progressive disease or infectious complications after a median follow-up of nine months, all of whom were non-responders. The median survival of our non-responding patients is therefore comparable with that observed in CLL patients failing purine analog therapy. In contrast, the patients who achieve a response after low-dose alemtuzumab are all alive after a median

Table 1. Patients' characteristics (n=16).

Characteristic	Patients
Age, y (median, range)	67 (48-83)
Binet stage, %	
A	0
B	56.2
C	43.8
Cytogenetic %	
Abnormal	56.2
>2 abn.	18.8
13q- (single)	6.2
+12	12.5
11q-	18.8
17p-	12.5
Previous infections %	50.0
Pseudomonas pneumonia	6.2
Pseudomonas otitis	6.2
HBV reactivation	12.5
CMV reactivation	6.2
Mycobacterium chel. disseminated cutaneous infection	6.2
VZV	12.5
Previous chemotherapy %	100.0
Failed alkylating agents	100.0
Failed fludarabine	87.5*
Failed rituximab	50.0
AIHA	25.0°

*Fludarabine was not allowed in two patients with AIHA; °previous therapy with fludarabine in two out of four AIHA patients.

Table 2. Response (%) to low-dose subcutaneous alemtuzumab in 16 refractory B-CLL patients

Group	OR	CR	PR	SD	PD
All patients (n=16)	50.0	25.0	25.0	50.0	0
Failed fludarabine (n=14*)	50.0	21.4	28.6	42.9	0
Failed rituximab (n=8)	37.5	25.0	12.5	63.5	0
Stage IV C (n=7)	42.9	14.3	28.6	57.1	0
Previous infection (n=8)	50.0	25.0	25.0	50.0	0
Unfavorable cytogenetic (n=8)	37.5	12.5	25.0	62.5	0
Age < 70 years (n=11)	54.6	18.2	36.4	45.4	0
≥70 (n=5)	60	40	20	40	0

*Fludarabine was not allowed in two patients with active AIHA; OR, overall response; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

follow-up of 12.5 months, with non-progressive disease in seven of the eight patients.

In terms of efficacy, our results are therefore comparable to those obtained using i.v. alemtuzumab, but with less toxicity. In fact, no infusion-related effect was observed except for a mild local reaction at the site of injection in about half of the patients, and transient low-

grade fever in a quarter of them. Overall, five patients had suspected or proven infective complications and one died because of polymicrobial infection. No CMV infection was observed, but only transient and asymptomatic reactivation in two patients, which subsided without any specific treatment. Our patients were all treated with cotrimoxazole, and no PCP was observed. The association of alemtuzumab with reverse transcriptase inhibitors was well tolerated and effectively inhibited HBV proliferation in two patients with active HBV infection. We therefore confirm the observation by Heider *et al.* on lamivudine efficacy in a patient on alemtuzumab therapy.¹⁰ Furthermore, we show that the addition of adefovir is able to completely abrogate HBV viral flare-up in a patient resistant to lamivudine. Most of our patients experienced transient cytopenias, which were generally reversible. Only three of the patients received granulocyte colony-stimulating factor (CSF) (a median of sixteen doses). Alemtuzumab was safely administered to patients with a history of autoimmune hemolytic anemia (AIHA). In fact, only one patient showed reactivation of hemolysis six weeks after the end of antibody therapy, whereas no signs of active hemolysis could be detected in the other three who had had AIHA.

This regimen seems to be as effective as standard intravenous infusion and has a particularly favorable toxicity profile. It may therefore represent a more convenient approach for patients and physicians by allowing home administration and reducing health care costs. Further studies and a longer follow-up are necessary to validate our results.

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Key words: alemtuzumab, B-CLL, overall response, complete response, safety, HBV, autoimmune hemolytic anemia.

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Acute Myeloid Leukemia

Low frequency of exon 3 *PTPN11* mutations in adult *de novo* acute myeloid leukemia. Analysis of a consecutive series of 173 patients

A total of 173 samples obtained from adult patients with *de novo* acute myeloid leukemia (AML) were assayed for exon 3 *PTPN11* mutations by single strand conformation polymorphism (SSCP) analysis and direct sequencing. Only three monocytic leukemias had point mutations (1.73%).

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The *PTPN11* gene encodes the non-receptor-type protein tyrosine phosphatase SHP-2 (src homology region 2-domain phosphatase-2) which plays an important role in the response to extracellular stimuli and is required for the activation of the RAS/MEK/ERK kinase pathway. *PTPN11* has been identified as the gene causing Noonan syndrome and it has also been found to be mutated in 34% of cases of juvenile myelomonocytic leukemia.^{1,2} Reported acquired mutations in myeloid malignancies cause a gain-of-function of the SHP-2 protein.^{3,4}

To assess the relevance of acquired *PTPN11* mutations in *de novo* adult AML we performed a mutational analysis in a consecutive series of 173 patients enrolled in the Spanish CETLAM protocol. DNA obtained from the diagnostic bone marrow was purified by digestion with proteinase K, extraction by the *salting out* method, and precipitation with ethanol. The configuration of the *MLL* locus was analyzed by hybridizing *Bam*HI- and *Hind*III-digested DNA to the B859 probe. AML1-ETO and CBF-MYH11 transcripts were assayed by reverse transcription polymerase chain reaction (RT-PCR) methods. The *FLT3* tandem duplication of the *JM* domain was investigated by genomic PCR. The *D835* mutation was ruled out by means of DNA-PCR followed by *Eco*RV enzyme digestion. Detection of exon 3 *PTPN11* gene mutations was studied by means of radioactive SSCP. The primers and PCR protocols have been published elsewhere.^{1,2} Direct sequencing was performed using forward and reverse primers in these cases with abnormal conformers on

Table 1. *PTPN11* mutated AML cases.

Pt.	Sex	Age	Dx	WBC	Cytogenetics	FLT3, MLL	PTPN11	Outcome
1	M	59	M5	73×10 ⁹ /L	45,XY,-7	GL	G60V	Dead 5m
2	F	48	M5	145×10 ⁹ /L	46,XX	GL	A72V	Dead 1m
3	M	35	M5	19×10 ⁹ /L	47,XY,+8	GL	H53Q	Dead 3m

M: male F: female; Dx: diagnosis according to FAB classification; GL: germ line.

SSCP analysis using the ABI Prism dRhodamine Terminator Cycle Sequence Ready Reaction kit (PE Biosystems, Warrington, UK) and the ABI PRISM 310 Genetic Analyzer (Foster City, CA, USA).

We detected three M5 AML cases harboring point mutations (Table 1), representing a prevalence of 1.73%. These findings are in line with those reported by Johan *et al.*⁵ in 64 AML cases enrolled in the MRC trials and suggest that this molecular lesion is rare in *de novo* adult AML, and clearly less common than in AML in children. Interestingly, most pediatric cases corresponded to monocytic leukemias. Mutations at codon 60 and 72, located at interaction sites between the N-SH2 and PTP domains, have been previously reported whereas the mutation at codon 53 has not been described to date. One of the cases reported here showed a +8, another a -7, and the remaining case had a normal karyotype. We were not able to detect the associated *FLT3* or *MLL* rearrangements commonly encountered in monocytic leukemias. All three patients died within a relatively short period: patient #1 from infectious complications arising in the context of graft-versus-host disease, patient #2 from severe bleeding in the induction phase and patient #3 relapsed. It can be concluded that *PTPN11* mutations affect a low percentage of patients with *de novo* AML and appear to be restricted to cases with a monocytic differentiation.

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