Vincristine, dexamethasone and epratuzumab for older relapsed/refractory CD22+ B-acute lymphoblastic leukemia patients: a phase II study

The treatment of older patients with acute lymphoblastic leukemia (ALL) still represents an unmet medical need. Here we report the results of a chemoimmunotherapy approach combining vincristine/dexamethasone and epratuzumab, a humanized monoclonal therapeutic antibody against CD22, in patients over 55 years of age with relapsed/refractory CD22+ B-ALL. This approach shows low toxicity and some activity in this patient population.

The main goal in relapsed ALL patients is to obtain a new complete remission (CR) to subsequently allow allogeneic stem cell transplantation (allo-SCT), which is the best prospect for cure. Undoubtedly, this strategy only applies to younger fit patients who can receive aggressive salvage regimens.[1] Due to a higher incidence of poor prognostic factors, older ALL patients (generally considered as over 55-65 years old, depending on trials) have a significantly lower CR rate, much earlier mortality, a higher relapse rate, and a poorer survival compared with younger patients.[2] Progress in terms of treatment optimization is limited and there is a lack of clinical trials in this population. Since almost all B-ALL cells express the CD22 surface antigen, it represents a good target for immunotherapy.[3,4] As such, the anti-CD22 humanized antibody, epratuzumab (Immunomedics, Inc., Morris Plains, NJ, USA), which has been studied extensively in NHL,[5,6] is under active investigation in younger adults and children with B-ALL.[7,8]

Here, we have evaluated the addition of epratuzumab (hLL2, 360 mg/m²/d i.v. on days 1, 8, 15, and 22) to the combination of vincristine (2 mg i.v. on days 1, 8, 15 and 22) and dexamethasone (40 mg p.o. on days 1, 8, 15, and 22) in patients over 55 years of age with relapsed/refractory CD22+ B-ALL. Dexamethasone was administered before epratuzumab administration + paracetamol 1 g as prophylaxis in order to prevent epratuzumab infusion reactions. Also, intrathecal injections of methotrexate 15 mg, aracytine 40 mg, and depomedrol 40 mg were recommended on days 1 and 8. This prospective phase II study was conducted at six French centers and was approved by the Brest ethical committee and the clinical trial department of the "Agence Française de Sécurité Sanitaire des Produits de Santé" (AFSSAPS) (clinicaltrials.gov identifier 01219816). Epratuzumab was supplied by Immunomedics Inc. (Morris Plains, New Jersey, USA). Written informed consent was obtained from each patient. Eligibility criteria were: age over 55 years, B-ALL with 20% or more of blasts in the bone marrow (BM), CD22+ expression on 30% or more of the blast population, refractory B-ALL defined by treatment failure after two successive courses of induction therapy or relapse less than six months after first CR, first relapse or beyond, patients relapsed or refractory to at least one 2nd-generation tyrosine kinase inhibitor for Philadelphia positive (Ph+) B-ALL, performance status ECOG 0-2, creatinine clearance 50 mL/min or over, and serum bilirubin 30 µmol/L or under. The main objective of the study was to evaluate the CR rate defined by patients reaching CR + CR without platelet recovery (CRi). CR was defined as less than 5% marrow blasts, neutrophil count 1x10⁹/L or over, platelet count 100x10⁹/L or over, and no evidence of extramedullary disease. CRi was defined similarly but without recovery of platelet counts. Partial response (PR) was defined as a more than 50% decrease in blasts in the bone marrow. Responses were evaluated between four and six weeks post-salvage regimen. Responding patients (CR, CRi or PR) were allowed to receive a second cycle as consolidation. Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 4. Cytological, phenotypic, karyotype and BCR-ABL1 (for Ph+B-ALL) molecular analyses were performed on blood samples and/or BM aspirates according to standard methods.

Table 1. Characteristics of older CD22+ refractory/relapsed B-ALL patients who received the salvage chemoimmunotherapy.

Patients	N=25
Male	13 (52%)
Median age: years (range)	65 (32-84)
>55 years	23 (92%)
Status	
First relapse	18
Second relapse	4
Third relapse	1
Fourth relapse	1
Refractory	1
Median white blood count at time of relapse	4.250 (0.170-39.230)x10⁹/L
Karyotype	
Normal	8
t(9;22)	6
Hyperdiploidy	2
Hypodiploidy	2
MLL rearrangement	1
t(1;19)	1
t(13;14)	1
9p deletion	1
17p duplication	1
14 abnormality	1
Complex	1
Median % of blasts in bone marrow	72%
Median CD22-RFB4 expression	100% (75-100)
Median CD22-SHCL-1 expression	100% (75-100)
Median interval between diagnosis and salvage chemoimmunotherapy: months (range)	16 (2-48)
Previous allotransplant	3 (12%)

Between November 2010 and December 2013, 26 patients were enrolled. One case was excluded because of progression before receiving the treatment, while 2 younger patients were inappropriately included (a 49-year old female in fourth relapse and a 32-year old female with refractory second relapse). All the 25 treated patients were considered for analysis. Patients' characteristics are shown in Table 1. The overall response rate was 40%, including 4 CR + one CRi (20%) and 5 PR (20%). Patients obtaining CR/CRi included 2 cases with normal karyotype, 2 with Ph+ B-ALL (1 in first relapse and 1 in third relapse), and one case with hypodiploidy. Regarding the 6 Ph+ B-ALL patients, 2 obtained a CR and one a PR. The patient with MLL-rearranged B-ALL obtained a PR. There was no difference between the median percentage of BM blasts at inclusion between responders (CR/PR: 70.5%) *versus* non-responders (median 72%). The 2 younger patients inappropriately included showed no response. All patients in CR/CRi and one patient in PR received a second cycle as consolidation. None of the responders was consolidated by

allo-SCT. Minimal residual disease (MRD) evaluated by cytometry in CR/CRi patients was undetectable in 2, and estimated at $5x10^{-4}$, $7x10^{-3}$ and 1.2% and 1.2% for the 3 other responding patients, respectively (Figure 1). Two patients died during treatment due to progression of the disease. The median overall survival (OS) was four months (range: 0.5-43), with a 1-year OS estimated as 13+7% (Figure 2). Median leukemia-free survival for patients achieving CR/CRi was 3.8 months (range: 1-8). One of the non-responding patients had an unexpected CR after receiving rituximab/6-mercaptopurine/methotrexate therapy,[9] and is currently alive at three years after allo-SCT. At time of analysis, all patients have died of progression, except 2 non-responding cases.

Salvage regimen was generally tolerated well, since the large majority of grade 3/4 toxicities were expected pancytopenia. One grade 3 allergic toxicity was related to the first epratuzumab infusion, but the patient received the three

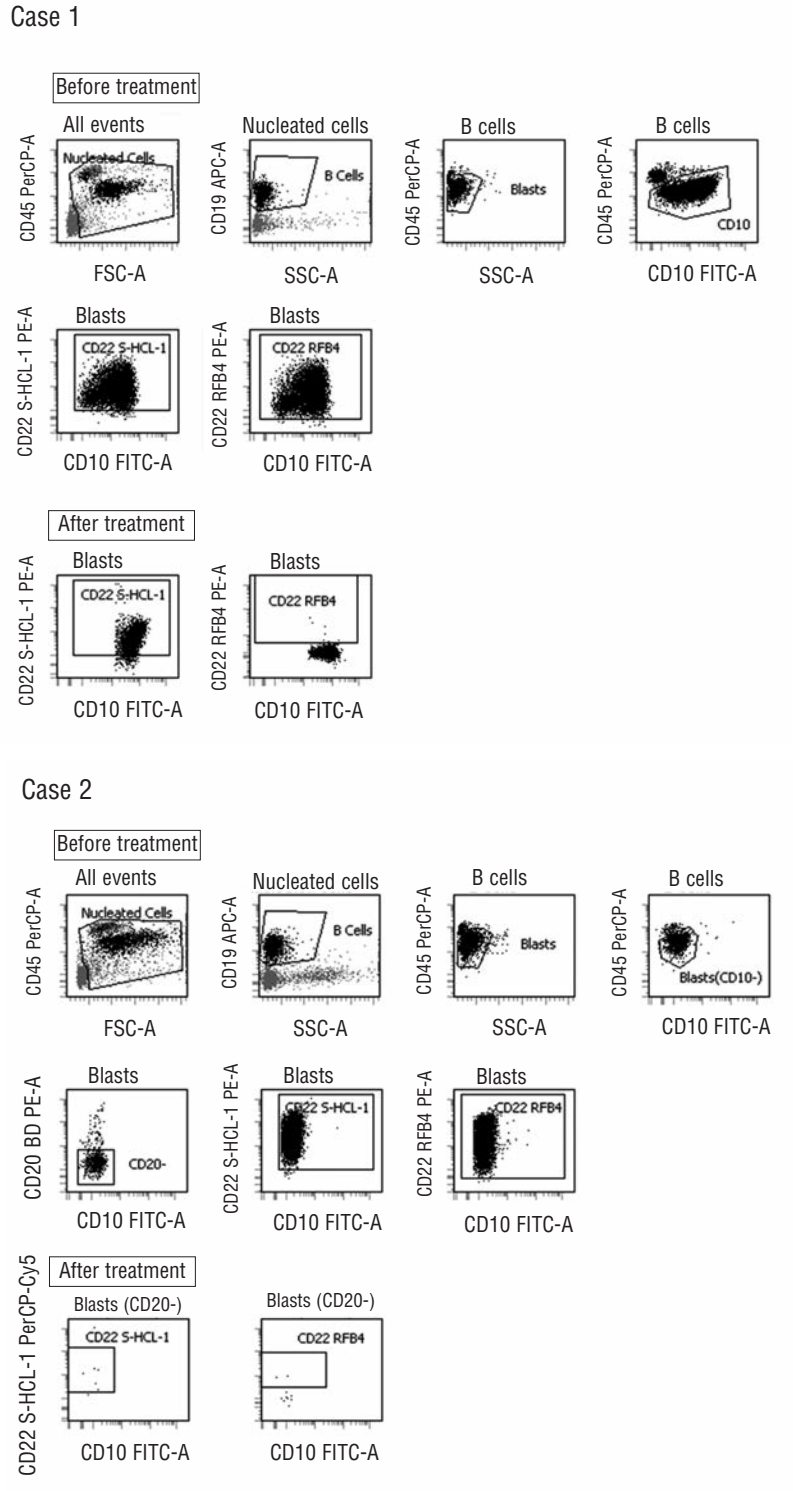

Figure 1. Minimal residual disease (MRD) evaluated by flow cytometry. All patients were monitored at the CHU of Nantes for CD22 expression before treatment and then for phenotypic MRD (Dr Nelly Robillard). Leukemic blasts were evaluated using an 8-color panel including antibodies to CD45, CD19, CD10, CD34, CD38, CD58, CD20 and CD22. MRD was assessed, concomitantly to responses, between four and six weeks after beginning the salvage regimen using flow cytometry (FACS CANTO II, BD, Biosciences, San Jose, CA, USA) performed on bone marrow samples. Two different anti-CD22 antibodies were used. The RFB4 antibody (PE conjugated, Invitrogen, Camarillo, CA, USA) and epratuzumab binds competitively with RFB4, meaning that in the presence of epratuzumab, RFB4 binding is blocked. By contrast, the SHCL-1 antibody (PE or PerCp-Cy5.5 conjugated, BD Biosciences, San Jose, CA, USA) binds to a non-cross-blocking epitope and can be used to assess the modulation of the CD22 antigen on the blast surface. None of the non-responders or the 3 patients with detectable MRD showed CD22 expression as assessed with RFB4 antibody. By contrast, all showed full CD22 expression with the SHCL-1 antibody. This demonstrates a persistent targeting of epratuzumab on blasts without loss of the CD22/epratuzumab complex from the cell surface. Case 1: An example of positive MRD. Leukemic blasts were both CD22-RFB4 and CD22-SHCL-1 positive before treatment. After treatment, the percentage of blasts is estimated at 0.05%. They are CD22-RFB4 negative and CD22-SHCL-1 positive, suggesting that epratuzumab is bound to these leukemic cells, and that the CD22-epratuzumab complex is not internalized and remains on the cell surface. Case 2: An example of "negative" MRD. Leukemic blasts (CD10/CD20 negative) are both CD22-RFB4 and CD22-SHCL-1 positive before treatment. After treatment, MRD was less than 10^{-5}.

other infusions without events. One grade 3 renal toxicity and one grade 4 hypertriglyceridemia of unclear etiology were also documented.

This innovative study reports the results of a chemoimmunotherapy approach with epratuzumab, an anti-CD22 humanized antibody, in the setting of older patients with B-ALL. The results, although modest, are encouraging because the patients studied here represented a very high-risk refractory/relapsed older patient population. These results also suggest that older patients may be re-treated even after relapse or with refractory disease. This is of particular interest in case of donor availability. Indeed, allo-SCT remains the best consolidation for patients achieving CR2,[1] while reduced-intensity conditioning regimens now allow this cellular therapy to be performed in patients up to 70-75 years of age.[9] Unfortunately, none of the responders here could be consolidated by an allo-SCT; this was not because of their age (4 out of 5 were under 70 years old), but because they relapsed shortly after the second chemoimmunotherapy cycle. Thus, proceeding straight to transplant as soon as CR2 is achieved might be a better approach to obtain long-term remission in these patients.

Considering epratuzumab, it remains questionable whether this antibody improves the results of the chemotherapy. To our knowledge, there is no series reporting the results of a combination of vincristine/corticosteroids in an older relapsed/refractory B-ALL population, although this remains probably one of the most routinely mild re-induction chemotherapies used in this setting. In first-line therapy, the vincristine/corticosteroid combination provides an up to 53% CR rate that is not inferior to a vincristine/corticosteroid/anthracycline-based regimen.[11] Also, the recent use of liposomal vincristine for relapsed/refractory ALL showed results comparable to those we describe here (35% overall response and 20% CR/CRi).[12] Thus, it is difficult to conclude whether epratuzumab will increase the therapeutic results of a non-intensive salvage chemotherapy in the particular setting studied here. Nevertheless, more interestingly, epratuzumab may provide an important addition in terms of induction of MRD. Indeed, 2 of our 5 responders were documented with negative MRD, which is currently considered, at least in younger patients, as a marker of long-term disease-free survival.[13]

In a recent study (submitted for publication) by Raetz *et al.*,[14] the addition of epratuzumab also did not translate into a higher CR rate in pediatric ALL patients in relapse. But among the children in CR, those treated with epratuzumab were significantly more likely to become MRD negative as compared to those treated without epratuzumab. Moreover, in the series reported by Advani *et al.*, in whom a combination of clofarabine/cytarabine/epratuzumab was investigated in younger patients with refractory/relapsed B-ALL, one patient achieved negative MRD and survived 11 months, much longer than all of the other patients for whom the median OS was five months.[8]

The question remains as to how to improve the results in older B-ALL patients. Clearly, there is a need for new treatment protocols designed for the elderly ALL patient, as well as a better understanding of the unique biological characteristics of the disease in this age group. Of course, other therapeutic monoclonal antibodies (inotuzumab, blinatumomab, rituximab), 2nd-generation purine analogs (nelarabine, clofarabine) or chimeric antigen receptor T-cell targeting CD19 may have their place in treatment approaches for older patients and should also be tested in this setting. A radiation approach, using epratuzumab conjugated with a therapeutic radionuclide (^{90}Y-epratuzumab tetraxetan radioimmunotherapy) could also be a promising therapeutic option for some CD22$^+$ B-ALL patients.[15]

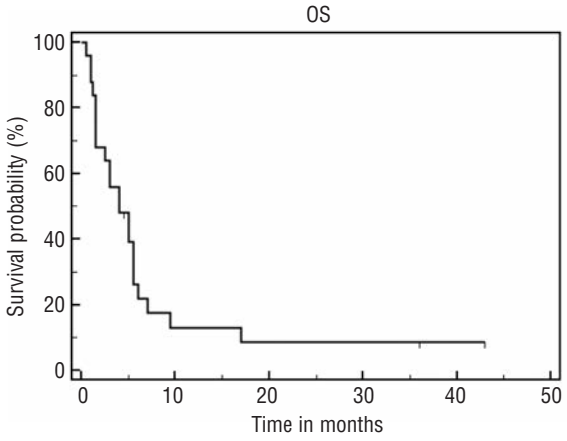

Figure 2. Overall survival for the whole cohort (n=25).

In conclusion, our results show some activity of epratuzumab combined with vincristine and dexamethasone in the very high-risk, refractory/relapsed, older CD22$^+$ B-ALL population studied here. These results pave the way for integrating epratuzumab within first-line chemotherapy approaches in older CD22$^+$ B-ALL patients, in order to possibly improve the rate of negative MRD in this setting.

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