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Haematologica 2014 [Epub ahead of print]

doi:10.3324/haematol.2014.120220

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Vincristine, dexamethasone and epratuzumab for older relapsed/refractory CD22+ B-acute lymphoblastic leukemia patients: a Phase 2 Study

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Letter to the Editor

The treatment of older patients with acute lymphoblastic leukemia (ALL) still represents an unmet medical need. Here we report the results of a chemoimmunotherapy combining vincristine/dexamethasone and epratuzumab, a humanized monoclonal therapeutic antibody against CD22, in patients > 55 years old, with relapsed/refractory CD22+ B-ALL, showing low toxicity and some activity for this 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 and 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), 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 iv on days 1, 8, 15, and 22) to the combination of vincristine (2 mg iv, days 1, 8, 15 and 22) and dexamethasone (40 mg/d po, days 1, 8, 15, and 22) in patients > 55 years old, 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 2 study was conducted at 6 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). This trial is registered at [http://clinicaltrials.gov/ct no.NCT01219816](http://clinicaltrials.gov/ct no.NCT01219816). Epratuzumab was supplied by Immunomedics, Inc. (Morris Plains, New Jersey, USA). Written informed consent was obtained from each patient. Eligibility criteria were: Age > 55 years, B-ALL with ≥ 20% of blasts in the bone marrow (BM), CD22+ expression on ≥ 30% of the blast population, refractory B-ALL defined by treatment failure after 2 successive courses of induction therapy or relapse < 6 months after first CR, first relapse or beyond, patients relapsed or refractory to at least one second generation tyrosine kinase inhibitor for Philadelphia positive (Ph+) B-ALL, performance status ECOG 0-2, creatinine clearance ≥ 50 mL/min, and serum bilirubin ≤ 30 µmol/L. The main objective of the study was the CR rate defined by patients reaching CR + CR without platelets recovery (CRi). CR was defined as <5% marrow blasts, neutrophils ≥ 1x10⁹/L, platelets ≥ 100x10⁹/L and no evidence of extramedullary disease. CRi was defined similarly but without recovery of platelets counts. Partial response (PR) was defined as a decrease of >50% of blasts in the bone marrow. Responses were evaluated between 4 and 6 weeks post-salvage regimen. Patients in response (CR, CRi or PR) were allowed to receive a second cycle as consolidation. Toxicity was evaluated according to the NCI-CTC criteria version 4. Cytologic, phenotypic, karyotype and BCR-ABL1 (for Ph+B-ALL) molecular analyses were performed on blood samples and/or BM aspirates by standard methods.

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 (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. The patient demographics are given in Table 1. The overall response rate was 40%, including 4 CR + 1 CRi (20%) and 5 PR (20%). Patients obtaining CR/CRi included 2 cases with normal
karyotype, 2 with Ph+ B-ALL (one in first relapse and one in third relapse), and 1 case with hypodiploidy. Regarding the six Ph+ B-ALL patients, 2 obtained a CR and 1 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%) vs no – responders (median 72%). The two younger patients inappropriately included showed no response. All patients in CR/CRi and 1 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 three other responder patients (Figure 1).

Two patients died during treatment due to progression of the disease. The median overall survival (OS) was 4 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-responder patients had an unexpected CR after receiving rituximab/6-mercaptopurine/methotrexate therapy,9 and is currently alive at 3 years from allo-SCT. At time of analysis, all patients have died of progression, except two non-responder 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 other infusions without events. One grade-3 renal toxicity and one grade-4 hypertriglyceridemia of unclear etiology also were documented.

This original study reports the results of a chemoimmunotherapy 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 population. Also, these results suggest that older patients may be retreated 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, while reduced-intensity conditioning regimens now allow to perform this cellular therapy up to 70-75 years of age. Unfortunately, none of the responders here could be consolidated by an allo-SCT, 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 achieving CR2 might be a better approach to achieve 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. Also, the recent use of liposomal vincristine for relapsed/refractory ALL showed results comparable to those we now describe (35% overall response and 20% CR/CRi). 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. In a recent study (submitted for publication) by Raetz et al, the addition of epratuzumab did also not translate in a higher CR rate in pediatric ALL patients in relapse, but of 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 of Advani et al, which tested a combination of clofarabine/cytarabine/epratuzumab 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 which the median OS was 5 months.8

The question remains 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), second generation purine analogues (nelarabine, clofarabine) or chimeric antigen receptor T-cell targeting CD19 may have their place in older patients and also should be tested in this setting. A radiation approach, using epratuzumab conjugated with a therapeutic radionuclide (90Y-epratuzumab tetraxetan radioimmunotherapy) could be also a promising therapeutic option for some CD22+ B-ALL patients.15

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

This study was supported by a grant from the French National Cancer Institute (PHRC 2010).

We want also to thank local investigators and data managers and the DRC of Nantes (especially Mrs Evelyne Cerato).

Authorships and Disclosures

PC designed, performed, and coordinated the research, collected, analyzed, interpreted the data, and wrote the manuscript.

NR performed flow cytometry analyses and commented on the manuscript.

MCB performed statistical analyses, produced the figures, and commented on the manuscript.

W.A. Wegener and D. M. Goldenberg provided epratuzumab, and revised the manuscript.

FH, ER, AE, TL, FL, TG, JD, AC, AP, PP, HD included patients, contributed data and commented on the manuscript.

Drs. Wegener and Goldenberg are senior employees with stock or stock options in Immunomedics; Dr. Goldenberg also is a patent inventor of epratuzumab. All other authors declare no potential financial conflicts.
References:


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

<table>
<thead>
<tr>
<th>Patients</th>
<th>N=25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender: male</td>
<td>13 (52%)</td>
</tr>
<tr>
<td>Median age: years (range)</td>
<td>65 (32-84)</td>
</tr>
<tr>
<td>&gt;55 years</td>
<td>23 (92%)</td>
</tr>
<tr>
<td>Status</td>
<td></td>
</tr>
<tr>
<td>First relapse</td>
<td>18</td>
</tr>
<tr>
<td>Second relapse</td>
<td>4</td>
</tr>
<tr>
<td>Third relapse</td>
<td>1</td>
</tr>
<tr>
<td>Fourth relapse</td>
<td>1</td>
</tr>
<tr>
<td>refractory</td>
<td>1</td>
</tr>
<tr>
<td>Median White blood count at time of relapse</td>
<td>4.250 (0.170-39.230) x10^9/L</td>
</tr>
<tr>
<td>Karyotype</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>8</td>
</tr>
<tr>
<td>t(9;22)</td>
<td>6</td>
</tr>
<tr>
<td>Hyperdiploidy</td>
<td>2</td>
</tr>
<tr>
<td>Hypodiploidy</td>
<td>2</td>
</tr>
<tr>
<td>MLL rearrangement</td>
<td>1</td>
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<tr>
<td>t(1;19)</td>
<td>1</td>
</tr>
<tr>
<td>t(13;14)</td>
<td>1</td>
</tr>
<tr>
<td>9p deletion</td>
<td>1</td>
</tr>
<tr>
<td>17p duplication</td>
<td>1</td>
</tr>
<tr>
<td>14 abnormality</td>
<td>1</td>
</tr>
<tr>
<td>Complex</td>
<td>1</td>
</tr>
<tr>
<td>Median % of blasts in bone marrow</td>
<td>72%</td>
</tr>
<tr>
<td>Median CD22-RFB4 expression</td>
<td>100% (75-100)</td>
</tr>
<tr>
<td>Median CD22-SHCL-1 expression</td>
<td>100% (75-100)</td>
</tr>
<tr>
<td>Median interval between diagnosis and salvage chemoimmunotherapy: months (range)</td>
<td>16 (2-48)</td>
</tr>
<tr>
<td>Previous allotransplant</td>
<td>3 (12%)</td>
</tr>
</tbody>
</table>
**Figure Legends**

**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 a 8 colors panel including antibodies to CD45, CD19, CD10, CD34, CD38, CD58, CD20 and CD22. MRD was assessed, concomitantly to responses, between 4 and 6 weeks after beginning the salvage regimen using flow cytometry (FACS CANTO II, BD, Biosciences, San Jose, CA) performed on bone marrow samples. Two different anti-CD22 antibodies were used. The RFB4 antibody (PE conjugated, Invitrogen, Camarillo, CA) and epratuzumab binds competitively to 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) binds to a non-crossblocking epitope and can be used to assess the modulation of the CD22 antigen on the blasts surface. None of the non-responders nor the three 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 <10^{-5}.

**Figure 2:** Overall survival for the whole cohort (N=25).
Figure 1:

Case 1

Before treatment

After treatment