CD20 expression has no prognostic role in Philadelphia-negative B-precursor acute lymphoblastic leukemia: new insights from the molecular study of minimal residual disease

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ABSTRACT

The prognostic significance of CD20 expression in acute lymphoblastic leukemia has been investigated in children and adults but is still a subject of debate. The aim of our study was to correlate CD20 expression with clinical-biological characteristics and outcome in 172 Philadelphia-negative patients prospectively treated in a multicenter trial introducing the molecular evaluation of minimal residual disease for therapeutic purposes. We considered 20% as the threshold for CD20 positivity. Complete remission rate, minimal residual disease negativity rate at weeks 10, 16 and 22, and disease-free and overall survival were similar among CD20 positive and negative patients, even considering minimal residual disease results and related therapeutic choices. Our study failed to demonstrate any prognostic significance for CD20 expression in Philadelphia-negative acute lymphoblastic leukemia. This conclusion is supported for the first time by a comparable minimal residual disease response rate among CD20 negative and positive patients. *ClinicalTrials.gov ID, NCT00358072*

Key word: CD20, BCP-ALL, Philadelphia negative, minimal residual disease, molecular.

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Introduction

The prognostic significance of CD20 expression has been investigated in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) but is still a subject of debate. In childhood ALL, Borowitz *et al.*[1] found that high CD20 intensity correlated with poorer event-free survival, while Jeha *et al.*[2] did not recognize CD20 as an adverse prognostic factor. The first study in adults was carried out by Thomas *et al.*[3] CD20 positivity (20% or over positive ALL cells) was associated with worse disease-free survival (DFS) and overall survival (OS), an effect especially relevant in patients under 30 years of age. This adverse prognostic impact was confirmed by the French group[4] but only in patients with a white blood cell (WBC) count over 30×10^9/L. Another recent report failed to document such prognostic relevance in unselected patients and in discrete subsets identified by WBC count and genetics.[5] The aim of our study was to correlate CD20 expression with clinical-biological characteristics and outcome in Philadelphia-negative (Ph-) BCP-ALL patients prospectively treated within the multicenter NILG 09-2000 study,[6] designed to direct post-remission strategy from minimal residual disease (MRD) assessment. There is currently no information available on MRD response according to CD20 antigen expression which could shed light on the real clinical impact of this B-lineage differentiation molecule in adult ALL.

Design and Methods

Study design

The NILG 09-2000 prospective program was based on MRD results, assessed by real-time quantitative polymerase chain reaction (RQ-PCR). The study was approved by the institutional review board and patients were enrolled after giving their written informed consent in accordance with the Declaration of Helsinki. Quantitative evaluation of chimeric transcript was exploited for BCP-ALL cases harboring

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fusion genes. In all the other cases, leukemia-specific probes were generated by genomic amplification and sequencing of the VDJ/VJ regions of immunoglobulin heavy chain (IgH) or the kappa light chain (IgK). The treatment plan had 2 distinct phases. The first phase was applicable to all patients, allowing the MRD response to be defined. For MRD assessment, 3 serial bone marrow (BM) samples were prospectively withdrawn before cycles 4, 6, and 8, corresponding to the ends of treatment weeks 10 (MRD-1), 16 (MRD-2), and 22 (MRD-3). The critical points for assigning an MRD risk classification coincided with MRD-2 and MRD-3. Only patients bearing t(4;11) translocation could proceed straight to allogeneic stem cell transplantation (SCT). In all other patients, the second phase depended on MRD status, with maintenance therapy prescribed to MRD-negative (MRD-neg) patients, and sibling/unrelated allogeneic SCT or multiple "hypercycles" supported by autologous stem cells to MRD-positive (MRD-pos) patients. MRD negativity was defined by a RQ-PCR assay less than 10^{-4} at MRD-2 and completely negative at MRD-3. Patients without molecular probe were treated according to clinical risk. Patients with Ph^+ ALL received a modified treatment[7] and were excluded from this analysis.

Immunophenotyping

Immunophenotyping was performed according to the recommendations from the European Group for the Immunological characterization of Leukemias[8] in multiparameter flow cytometry with CD45 gating[9]. Phenotypic data were expressed as the percentage of CD20 positive cells on the whole leukemic population. The phenotypic analysis was performed on bone marrow and peripheral blood in 146 and 26 patients, respectively. We considered 20% as the threshold for positivity (CD20-pos).

Statistics

The probabilities of favorable outcome (CR, MRD negativity) were compared using the χ^2 test with Yates correction. DFS and survival curves were plotted using the Kaplan-Meier method and compared by the log rank test. Multivariate analyses were carried out by Cox's linear regression model, including all variables expressing significant *P* values in univariate analysis. $P<0.05$ was considered significant.

Results and Discussion

Between March 2000 and September 2008, 403 consecutive ALL patients were enrolled in the study, including 172 patients with Ph-negative BCP-ALL (Table 1). Fifty-two (30.2%) patients resulted CD20-positive. Median value of CD20 expression within CD20-positive patients was 67% (range 25-100%). As expected, the distribution of EGIL classification was correlated to CD20 status: the B-I phenotype (pro-B CD10-) was more frequent in the CD20-negative group (38.4%) than in the CD20-positive group (7.6%; $P<0.0001$). On the contrary, B-II phenotype was more frequently associated with CD20 positivity (77.0%) than negativity (45.8%; $P<0.0001$). A prevalence of hepatosplenomegaly was also noted in CD20-positive patients. As regards outcome, overall CR rate was 84.3% (n=145), with no difference seen between the two groups (43 CD20-negative 82.7%; 102 CD20-positive 85%). Since treatment was MRD-oriented, it was possible to look for correlations between CD20 expression and MRD course. One or more sensitive ($\geq 10^{-4}$) patient-specific molecular probes were available for 124 of 172 (72.1%) study patients (Figure 1). A non-significant trend for more markers being available was noted in the CD20-negative subset, despite the fact that 29 patients with highly specific single fusion gene as molecular marker belonged to this group (25 with t[4;11] and 4 with t[1;19]). The association between t(4;11) and CD20 negativity accounted for fewer MRD data available in this group, due to early treatment failure or shift to allogeneic SCT. The rate of MRD negativity achieved at the three time points was similar for CD20-positive and CD20-negative patients (Figure 1) and CD20 expression had no impact on DFS and OS in the entire patient cohort (Figure 2A and B), nor did it affect outcome of specific subgroups defined by patient age, WBC count, EGIL immunophenotype and cytogenetics/genetics (*Online Supplementary Appendix*). The exclusion of t(4;11)+ ALL from analysis did not alter this result, and no difference was documented varying CD20 positivity threshold from 20% to 10 and 30%, respectively. Focusing on the decisional time points for final treatment allocation (MRD-2 and MRD-3), we investigated the significance of CD20 within MRD-defined groups receiving either maintenance because MRD-negative or transplantation-based therapy because MRD-positive. Eighty-nine of 145 CR patients (61.4%) could be classified according to MRD study results. This group included 86 patients assessable at MRD-2 and MRD-3 time points as per protocol design (Figure 1), plus 3 further patients without MRD-3

Table 1. Patients' characteristics.

	Overall (172)	CD20+ (52)	CD20- (120)	*P* value
Age, median (range)	37 (16-68)	41 (16-68)	37 (16-65)	ns
WBC $\times 10^9$/L, median (range)	10.0 (0.5-730)	7.9 (0.5-330)	11.5 (1-730)	ns
LDH U/L, median	851	815	922	ns
PB blasts %, median (range)	56 (0-100)	53 (0-100)	60 (0-100)	ns
Splenomegaly, n (%)	70 (40.7%)	28 (53.8%)	42 (35.0%)	0.067
Hepatomegaly, n (%)	55 (32.0%)	24 (46.2%)	31 (25.8%)	0.014
EGIL B-I, n (%)	50 (29.1%)	4 (7.6%)	46 (38.4%)	<0.0001
EGIL B-II, n (%)	96 (55.8%)	40 (77.0%)	55 (45.8%)	<0.0001
EGIL B-III, n (%)	26 (15.1%)	8 (15.4%)	19 (15.8%)	ns
Cytogenetics, unfavorable	51 (29.7%)	11 (21.2%)	40 (33.3%)	ns
t(4;11)/MLL	25 (14.6%)	0	25 (20.8%)	N/A
other*	26 (15.1%)	11 (21.2%)	15 (12.5%)	ns
Cytogenetics, standard	121 (70.3%)	41 (78.8%)	80 (66.6%)	ns

**Other : del(6q) (n=2), -7 (n=2), +8 (n=7), low hypodiploidy (30-39 chromosomes) (n=5), near triploidy (60-78 chromosomes) (n=7), complex with ≥ 5 abnormalities (n=3). ns: non-significant P value; N/A: not applicable.*

analysis but heavily positive at MRD-2 (MRD-positive, n=2) or totally negative at MRD-1 and MRD-2 (MRD-negative, n=1). The remaining CR patients were excluded from MRD analysis because of early removal (relapse, toxicity, transfer to SCT) or because they lacked a molecular probe. Five-year survival rates were similar among CD20-positive

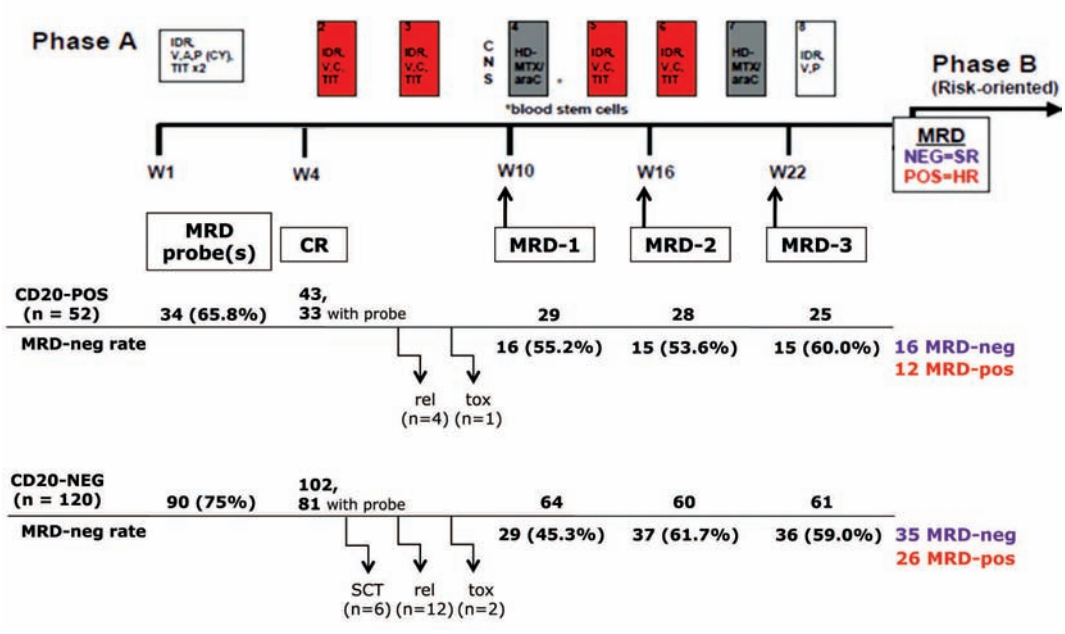

Figure 1. Outline of protocol NILG-ALL 09/00, MRD study, and treatment flow in the whole cohort and according to CD20 expression. MRD-neg rate: minimal residual disease negativity rate; SCT: stem cell transplantation; rel: relapse; MRD probe(s) refers to the availability of a clonality marker.

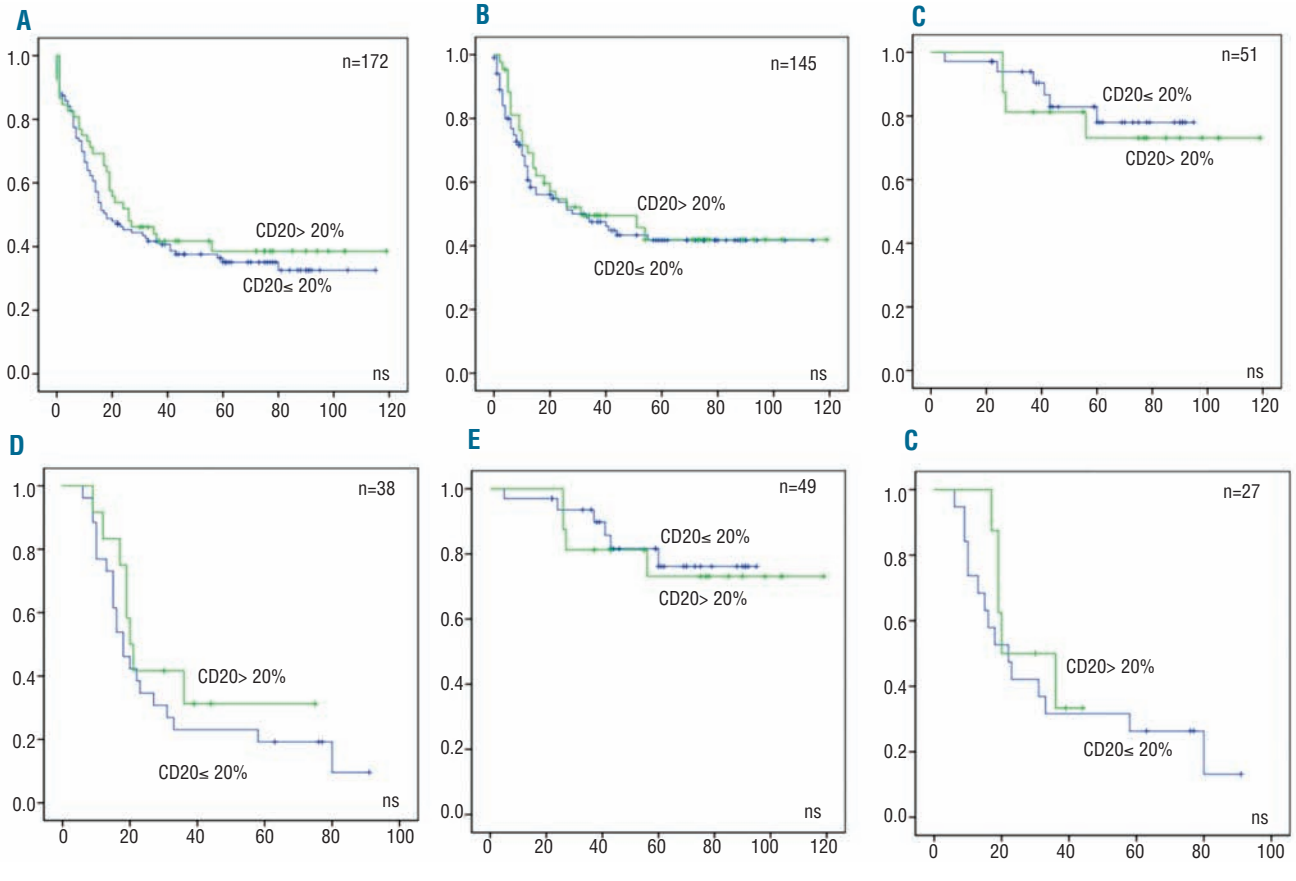

Figure 2. (A) Overall survival and (B) disease-free survival according to CD20 status in the whole cohort. Overall survival according to CD20 status in patients resulting MRD-neg (C) and MRD-pos (D), by treatment intention. Overall survival according to CD20 status in patients resulting MRD-neg and treated with maintenance (C) or MRD-pos and receiving allogeneic SCT or high-dose therapy with autotransplantation (D). Follow-up intervals in months.

and CD20-negative subsets in patient populations classified as MRD-negative or -positive, respectively (Figure 2C and D), according to intention to treat. With regards to actual treatment, 49 of 51 MRD-neg patients received maintenance, with a superimposable outcome between CD20-positive (n=16) and CD20-negative (n=33) cases (Figure 2E); and 27 of 38 MRD-positive patients received allogeneic SCT (n=14, 10 CD20-negative and 4 CD20-positive) or high-dose treatment with autologous rescue (n=13, 9 CD20-negative and 4 CD20-positive), once again without any prognostic influence related to CD20 expression (Figure 2F).

In conclusion, our study failed to demonstrate a prognostic significance for CD20 expression in BCP-ALL. While the discrepancy between our data and that of others might be related to differences in study design and therapeutic strategy, the study confirmed the leading prognostic relevance of MRD response in Ph- BCP-ALL, the rate of molecular CR being the same among CD20-positive and CD20-negative patients (60%), with an excellent survival plateau of over 70% at 5-8 years in both CD20-negative/MRD-negative and CD20-positive/MRD-negative patients treated with chemotherapy only. These findings are consistent with the results of another large trial investigating the significance of phenotype in BCP-ALL, in which no prognostic role was documented for the EGIL B-I subgroup (pro-B), that is most often CD20-negative[10]. An exception to that is represented in our study by t(4;11)+ ALL, that is consistently CD20-negative and is a well known very high-risk subset, with high propensity for relapse and clear indications for allogeneic SCT. Therefore, it could still be possible that a high incidence of CD20-negative t(4;11)+ ALL in unselected series like ours (14.5%) could in part compensate the potential worsening effect of CD20-positive cases reported in series with lower incidence of t(4;11)+ ALL. However, even excluding the highly adverse cytogenetic subset of t(4;11)+ ALL which is typically CD20-negative, CD20 was not prognostically relevant in the context of the MRD-based prognostic analysis.

We showed for the first time how the molecular response to early chemotherapy at three distinct time points between weeks 10 and 22 and the related clinical outcome were similar among CD20-positive and CD20–negative patient subsets. Because MRD was the main predictor for relapse in multivariate analysis[6], this finding alone, obtained in a relatively large series of 89 patients evaluable for MRD and studied prospectively, argues against the independent prognostic value of CD20 expression in adult ALL, and documents how the analysis of CD20 clinical significance requires a concurrent MRD evaluation. As regards treatment, our data supports the view that allogeneic SCT may not be necessary as first-line therapy in CD20-positive/MRD-negative patients, although it remains an effective treatment modality for CD20-positive ALL.[11] Instead, transplantation results were rather poor in CD20-positive/MRD-positive patients. Since the CD20 and CD19 antigens (the latter being broadly expressed in B-lineage ALL) are useful therapeutic targets in CD20$^+$ ALL, as recently reported by Thomas *et al.*[12] and Topp *et al.*,[13] an additive therapeutic effect from rituximab and/or blinatumomab should be primarily assessed in patients with MRD-positive/CD20-positive ALL before an allogeneic SCT.

Authorship and Disclosures

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