CD10-negative pre-B acute lymphoblastic leukemia (ALL): a distinct high-risk subgroup of adult ALL associated with a high frequency of MLL aberrations. Results of the German Multicenter Trials for Adult ALL (GMALL)

Running title: CD10-negative pre-B ALL

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ABSTRACT

Immunophenotyping disclosed CD10 negativity in 70 of 2,408 cases of B-lineage acute lymphoblastic leukemia (ALL), although other criteria followed classification of pre-B ALL (e.g., cytoplasmic immunoglobulin positivity). These blasts showed high myeloid antigen expression (60% CD65-positivity) and reacted with antibody 7.1 in 95% of the cases. MLL-AF4 fusion transcripts and/or an 11q23/MLL rearrangement were evident in 46/56 samples (82%). Although 83% of the patients achieved complete remission, the remission duration remained remarkably low: 141 days for MLL-rearrangement-positive and 245 days for MLL-rearrangement-negative CD10’ pre-B ALL. Thus the overall survival probability 3 years after diagnosis was 0.34 (± 0.20 SE) in MLL-rearrangement-negative versus 0.12 (± 0.06 SE) in MLL-rearrangement-positive CD10’ pre-B ALL.

Our data identify CD10’ cytoplasmic Ig-positive pre-B ALL as a rare (2.2%) but distinct immunosubtype of adult ALL that is characterized by a high MLL rearrangement rate and a worse outcome.

Introduction

Immunological subtyping and molecular genetic analysis enable risk-adapted treatment of acute lymphoblastic leukemia (ALL). One poor-prognosis subgroup of adult B-lineage precursor ALL is characterized by translocations involving the mixed lineage leukemia (MLL) gene on chromosome 11q23. These translocations occur in approximately 3% to 6% of all adult ALL and correlate with a younger age, a higher leukocyte count, and a CD19+/CD10-/cytoplasmic(cy)IgM− immunophenotype (pro-B ALL) coexpressing myeloid marker.1-3
Recent reports have shown that 11q23 translocations occur in up to 8% of T-ALL, which were characterized by a poor clinical outcome. Here we present evidence of another high-risk group with frequent MLL gene translocations involving 82% (46/56) of the distinct CD19+/CD10-/cyIgM+ pre-B ALL subset.

**Patients and Methods**

Dual-staining immunophenotyping of 3,168 newly diagnosed ALL specimens was performed with commercially available fluorochrome MoAb conjugates (Dako, Hamburg, Germany; BD Biosciences, San Jose, USA; Beckman-Coulter, Fullerton, USA) identifying pro-B ALL, TdT+/CD19+/CD10-/cyIgM-/surface(S)Ig-; c-ALL, TdT+/CD19+/CD10+/cyIgM-/SIg-; and pre-B ALL, TdT+/CD19+/CD10+/cyIgM+/SIg-. Ambiguous cases of CD10-positive B-lineage marker-positive blasts (5-15% CD10-positivity) were rechecked by a second antibody (BD Biosciences, Dako). Only consistently negative samples (<20% positive cells) were considered for CD10-negativity. Molecular detection of MLL-AF4 and BCR-ABL fusion as well as cytogenetic analysis were carried out as described elsewhere. FISH used directly labelled dual-color break apart MLL rearrangement probes (Vysis, Downers-Grove, USA). At least 200 nuclei were scored setting the cut-off value for false-positive nuclei at 10%. Patients were treated according the German Multicenter Adult ALL trials (GMALL), allocating MLL-AF4-positive patients to the high-risk arm. The median follow-up was 25.6 months (range, 1.8 to 70.4 months) for survivors. The protocols were reviewed and approved by institutional review boards at each of the participating sites and all patients signed informed consent prior to enrollment.
Results and Discussion

Prevalence of CD10^- pre-B ALL within B-lineage ALL

Immunophenotyping identified 382 pro-B ALL, 335 pre-B ALL and 1,621 c-ALL. In addition, 70 ALL specimens were positive for TdT, CD19, and cyILgM but did not express CD10 in an analysis with two antibodies. These samples were assigned to the unusual subtype of CD10^- pre-B ALL.

Prevalence of MLL-AF4 transcripts and 11q23/MLL translocations in B-lineage ALL

A 50% prevalence of MLL-AF4 fusions (112/211 positive samples) was evident in pro-B ALL. All CD10^- pre-B ALL specimens were negative for MLL-AF4 fusion transcripts (n = 52 tested with RT-PCR) or an 11q23 translocation (n = 45 analyzed with FISH or cytogenetics). Similarly, c-ALL (CD10^-cyILgM^-) correlated with an exclusively MLL-AF4-negative (0/77 positive samples) and t(4;11)-negative (264 samples analyzed by chromosome banding) genotype.

In 57 CD10^- pre-B ALL sufficient cells were available for molecular or cytogenetic analysis. One patient was excluded since molecular genetic analysis was performed only at late relapse. All 56 evaluable CD10^- pre-B ALL were BCR-ABL-negative. Forty-two samples revealed MLL-rearrangement (RT-PCR 27, cytogenetics t(4;11) 3, t(11;19) 1, FISH 1, RT-PCR/cytogenetics 9, FISH/RT-PCR 1). Four samples were assigned MLL-rearrangement-positivity including one specimen with a MLL-AF4 fusion (RT-PCR) and a normal karyotype, and 3 samples with a MLL-deletion (n = 1) or a MLL-translocation detected with FISH and a MLL-AF4-negative RT-PCR
result. Ten patients were MLL-rearrangement-negative (RT-PCR 3, cytogenetics 1, FISH 1, FISH/RT-PCR 2, cytogenetics/RT-PCR 1, cytogenetics/FISH/RT-PCR 2).

**Features and outcome of CD10- pre-B ALL**

MLL-rearrangement-positive patients were characterized by a higher median white blood cell count (70150/µL versus 9200/µL; \( P = .0002 \)), and their blasts showed more frequently CD65 expression (67% versus 22%; \( P = .01 \)) (Table 1). The presence of neuroglial antigen 2 chondroitin sulfate proteoglycan (NG2) was indicative of an MLL-rearrangement in CD10- pre-B ALL (\( P = .02 \)).

Fourty CD10- pre-B ALL patients are evaluable for the treatment response and outcome. Seven additional patients were excluded from the GMALL trials due to age (n = 2), prior malignancies (n = 2), or other reasons (n = 3). Nine patients were not treated and documented within the GMALL trials.

A complete remission (CR) was achieved in 6 of 7 (86%) MLL-rearrangement-negative and in 27 of 33 (82%) MLL-rearrangement-positive patients (\( P = .06 \)). Five (18%) MLL-rearrangement-positive patients failed to respond to induction therapy, and one MLL-rearrangement-negative patient died within 56 days (early death). CR was maintained by 2 of 6 (33.3%) MLL-rearrangement-negative and 7 of 27 (26%) MLL-rearrangement-positive patients. Thus the probability of remission duration after 3 years was 0.37 (± 0.29 SE) in MLL-rearrangement-negative versus 0.28 (± 0.13 SE) in MLL-rearrangement-positive patients. Eight patients (7 MLL-rearrangement-positive) underwent stem cell transplantation in the 1st CR and 5 of these patients survived (all MLL-rearrangement-positive).
The presence of a CD10' pre-B ALL immunophenotype was indicative of a poor outcome (279 days; median overall survival 278 days in MLL-rearrangement-positive versus 354 days in MLL-rearrangement-negative patients). The overall survival probability 3 years after diagnosis accounted for 0.34 (± 0.20 SE) in MLL-rearrangement-negative versus 0.12 (± 0.06 SE) in MLL-rearrangement positive patients (Figure 1). For patients who achieved CR, the survival probability 3 years after diagnosis was 0.40 (± 0.22 SE) in MLL-rearrangement-negative versus 0.14 (± 0.08 SE) in MLL-rearrangement-positive CD10' pre-B ALL.

Studies on immunological markers and other cell phenotype expressions have provided valuable clues for diagnosing and classifying ALL according to cell lineage affiliation and differentiation.1,14 Numerous reports have been published on chromosomal translocations and mutations in human leukemias (overview in Pui et al15) and have promoted the development of risk-adapted therapies.13

The results of our analysis underline the importance of a distinct CD10' pre-B ALL subtype characterized by a high prevalence of MLL rearrangements indicating involvement of a primary genetic event.16 Interestingly, 90% of the cases in which the blasts were tested reacted with antibody 7.1,7 adding NG2 expression to the main characteristics of MLL-rearrangement-positive CD10' IgM+ pre-B ALL (100% positivity).

Regarding the therapeutic outcome, a CR could be achieved in more than 80% of MLL-rearrangement-positive CD10' pre-B ALL, which goes beyond the results obtained for MLL-rearrangement-positive pro-B ALL in the GMALL trials 03/87 and 04/891 and correlates favorably with the CR rate given for standard-risk BCR-ABL-negative pre-B ALL and c-ALL patients in the GMALL 05/93 trial.6

With one exception, all MLL-rearrangement-positive patients with CD10' pre-B ALL
received high-dose cytosine-arabinoside induction therapy but were nevertheless found to have a clearly lower chance of maintaining CR than those with, for example, BCR-ABL-negative pre-B and c-ALL or even MLL-rearrangement-positive pro-B ALL, the latter comprising a well known therapeutic high-risk group. The adverse outcome is illustrated by a median overall survival rate within and below the range of values reported for BCR-ABL-positive ALL patients and may be influenced by the high WBC of most MLL-rearrangement-positive CD10− pre-B ALL.

CD10− cyIgM+ pre-B ALL identifies a distinct immunophenotypic entity of partly differentiated precursor B-lineage ALL with intracytoplasmic immunoglobulin expression. Recognition of this subtype is important, because it was observed in 17% of all pre-B ALL specimens and is characterized by a high prevalence of MLL-AF4 fusion transcripts. The overall survival was low in MLL-rearrangement-positive patients and although our data did not analyse different therapeutic options, stem cell transplantation in 1st CR might improve the results. On the other hand, for the small subgroup of MLL-rearrangement-negative CD10− cyIgM+ pre-B ALL the survival ranged within the treatment results achieved in standard risk adult ALL. Further molecular analysis should help to elucidate the status of CD10− pre-B ALL compared to other MLL-rearrangement-positive acute leukemias, which have been related to two distinct expression profiles and are thought to result from an MLL-translocation-induced arrest at an early stage of B-cell development.
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References


Table 1.

Clinical and immunological features of 56 CD10⁻ pre-B ALL

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Each column gives the number of patients with a specific feature; ns, not significant. The χ² test was used to compare the clinical parameters of MLL-rearrangement-positive and -negative patients. Median values were compared by the 2-sided Wilcoxon-Mann-Whitney test.

⁺, data missing for 1 patient.

++, data missing for 2 patients

++++, data missing for 36 patients.
Figure 1

Overall survival probability in *MLL*-rearrangement-positive versus *MLL*-rearrangement-negative CD10⁻ pre-B ALL.

Overall survival probability in 7 patients with *MLL*-rearrangement-negative CD10⁻ pre-B ALL (0.34 ± 0.20 SE) (top broken line) and in 33 with *MLL*-rearrangement-positive CD10⁻ pre-B ALL (0.12 ± 0.06 SE) (bottom solid line) after treatment in the GMALL studies 04/89, 05/93, 06/00, and 07/03. Median follow-up of 354 days for *MLL*-rearrangement-negative versus 278 days for *MLL*-rearrangement-positive patients.
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