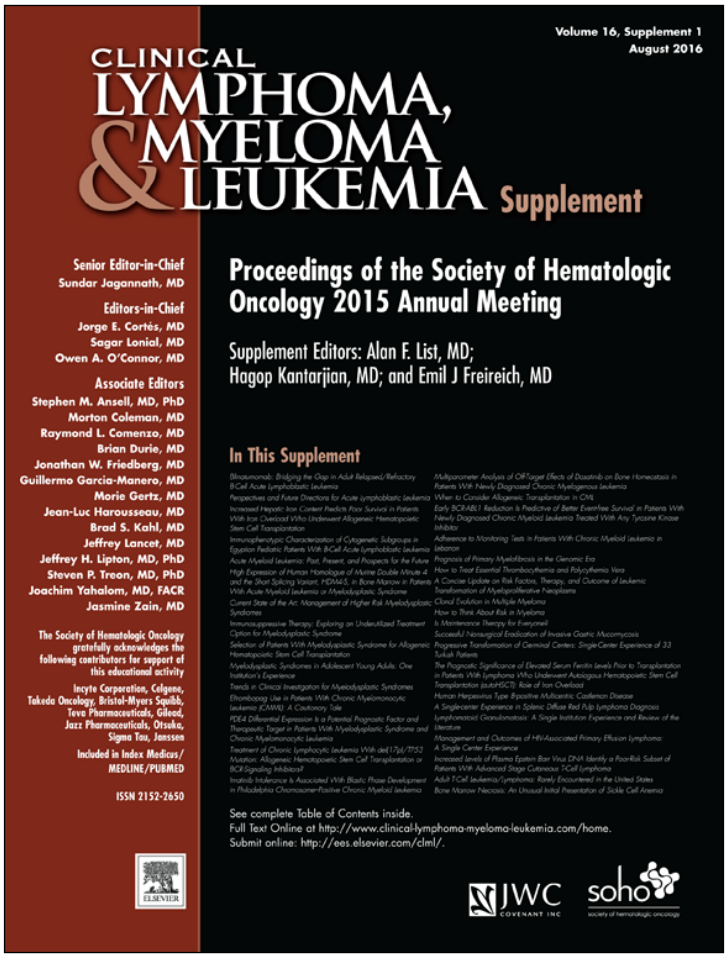


Immunophenotypic Characterization of Cytogenetic Subgroups in Egyptian Pediatric Patients With B-Cell Acute Lymphoblastic Leukemia

Shady Adnan Awad,[1] Mahmoud M. Kamel,[1] Mahmoud A. Ayoub,[1] Ahmed M. Kamel,[2] Essam H. Elnoshokaty,[1] Niveen El Hifnawi[1]

Abstract

ALL is the most common childhood malignancy and identification of prognostic factors is important for further improvement of the treatment outcome in this fatal disease. Cytogenetic changes and MRD are the most powerful prognostic factors in ALL. We identified significant correlations between some CD markers and cytogenetic subgroups which can be used in MRD monitoring and as potential therapy targets.

Background: Identification of prognostic factors in acute lymphoblastic leukemia (ALL) patients is important for stratifying patients into risk groups and tailoring treatment accordingly. Molecular and cytogenetic abnormalities are the most important prognostic factors. Minimal residual disease (MRD) is also an important predictor of relapse in ALL. However, the correlation of both prognostic variables has not been thoroughly studied. **Methods:** We investigated the correlation between defined cytogenetic abnormalities and selected new MRD markers (CD79b, CD123, and CD200) in 56 newly diagnosed Egyptian pediatric B-cell ALL patients. **Results:** CD123 found to be expressed in 45% of patients, CD200 in 80.3%, and CD79b in 67.9%. MRD analysis during treatment showed stable expression patterns of CD200. There was significant association of CD123 expression with the hyperdiploid ALL group ($P = .017$). Another association ($P = .029$) was found between CD79b negativity and the t(12;21) group. CD200 was widely expressed in all groups. **Conclusion:** There is a significant correlation between some markers, and certain ALL recurrent cytogenetic subgroups (CD123 and hyperdiploidy, CD79b negativity, and *ETV-RUNX1* group) have good prognostic value. CD200 can be used as MRD markers in ALL patients and can also can serve as therapy targets.

Clinical Lymphoma, Myeloma & Leukemia, Vol. 16, No. S1, S19-24 © 2016 Elsevier Inc. All rights reserved.
Keywords: ALL, CD123, CD200, CD79b, Hyperdiploidy, MRD

Introduction

Acute leukemia accounts for about one third of all childhood malignancy, of which acute lymphoblastic leukemia (ALL) is the most common type, accounting for about 80% of pediatric leukemia.[1] In Egypt, the annual incidence of ALL is about 4 cases per 100,000 children, accounting for about 20% of pediatric malignancies.[2,3] In recent reports, the overall survival of pediatric ALL is close to 90%, and about 80% experience long event-free survival.[4,5] The cornerstone of improved survival is the careful risk stratification of patients according to defined prognostic variables, then tailoring the treatment accordingly.[6,7]

Cytogenetic and molecular changes in ALL represent the most important, defined, and thoroughly studied prognostic variables; they are used in classification and risk stratification, though they do not entirely explain differences in treatment responses.[8-10] Also, currently, minimal residual disease (MRD) is the most powerful prognostic factor in ALL patients, even those with low-risk disease.[11,12] Few publications have studied the correlation between specific CD markers and certain cytogenetic groups, which needs to be further explored.[13,14]

The discovery of new cluster of differentiation (CD) markers of leukemia and its differential association with certain cytogenetic groups has many values, including increased resolution between leukemic and normal cells at diagnosis, characterization of different

[1]Department of Clinical Pathology
[2]Department of Pediatric Oncology, National Cancer Institute, Cairo University, Cairo, Egypt

Submitted: Feb 9, 2016; Accepted: Feb 9, 2016

Address for correspondence: Shady Adnan Awad, MD, PhD, Vuolukiventie 1b D89, Helsinki 00710, Finland
E-mail contact: Shady.awad@helsinki.fi

2152-2650/$ - see frontmatter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.clml.2016.02.032

cytogenetic ALL subgroups with a possible prognostic role of antigen expression and substratification within each group, and increased MRD sensitivity at follow-up. Furthermore, they may serve as sites of targeted therapy.[13-16]

In this study, we first classified 56 newly diagnosed pediatric ALL patients into subgroups on the basis of defined cytogenetic abnormalities. All patients were assessed for their expression profiles of three CD markers (CD123, CD79b, and CD200) at diagnosis and during follow-up visits for MRD. The aim of the study was to assess the association between these markers and defined cytogenetic abnormalities and other clinical and laboratory parameters, as well as to assess their stability in terms of MRD analysis.

Methods

Bone marrow samples were collected from 56 newly diagnosed consecutive B-cell ALL (B-ALL) patients who sought care at the pediatric oncology clinics of the National Cancer Institute, Cairo University. The diagnosis of B-ALL was established on the basis of the 2008 World Health Organization classification criteria.[17] Patient age was < 18 years (median, 5 years). Using European Group for Immunophenotypic characterization of Leukemias (EGIL) criteria,[18] patients were subclassified according to immunophenotypic criteria into three groups: pro-B-ALL, common type ALL (C-ALL), and pre-B-ALL.

This study was approved by the ethical committee review board of the National Cancer Institute, Cairo University, in accordance with the Helsinki guidelines for the protection of human subjects. Written informed consent was obtained from the parents of all participants.

Detection of Cytogenetic Abnormalities

Karyotyping (G banding) was performed in all samples. Interphase fluorescence in-situ hybridization (FISH) analysis to confirm changes and detect other changes was performed with locus-specific probes, double-color single fusion (Vysis, Downers Grove, IL, USA), for *ETV-RUNX1* and *BCR-ABL1* translocations. The sample was considered positive for translocation when ≥ 3% of blasts were positive for this translocation.

Polymerase Chain Reaction (PCR)-Based Panel

A PCR-based panel encompassing 5 important translocations, namely t(9;22)-P190, t(9;22)-P210, t(12;21), t(1;19), and t(4;11), was used to verify FISH results and to detect other changes. For copy number variation, in addition to routine karyotyping, DNA index analysis was used to the confirm the ploidy status of each sample.

CD123, CD79b, and CD200 Expression Profile

A flow cytometric evaluation of the immunophenotype of was analyzed in all 56 patients' diagnostic samples using multicolor flow cytometry (Coulter Epics XL, Navios; Beckman Coulter, Danvers, MA, USA). Navios software was applied for analysis. Additional tubes containing those markers (anti-CD79b PE monoclonal antibody from Beckman Coulter; anti-CD123 FITC and anti-CD200 PE monoclonal antibodies from Becton Dickinson, Franklin Lakes, NJ, USA) were added to the initial characterization panel at diagnosis and follow-up MRD monitoring. CD expression was considered positive when expressed on ≥ 20% of blast cells.

MRD Monitoring

Two panels were selected for each case according to antigen expression pattern at diagnosis. The selected panels were run at diagnosis and at days 14, 28, and 42 for all patients and also at 6 months and whenever progression was suspected. The CDs of interest were added to the routine MRD panel for B-ALL to verify their stability and potential use as MRD markers.

Statistical Analysis

Data were analyzed by GraphPad Prism 6.0f software (GraphPad Software, La Jolla, CA, USA). Numerical data were expressed as mean and standard deviation or median and range, as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For nonnormally distributed quantitative data, comparison between 2 groups was done by the Mann-Whitney test (nonparametric *t* test). The multivariant analysis of variance test was used to compare more than 2 quantitative groups. A P value of $< .05$ was considered significant.

Results

Detection of Recurrent Cytogenetic Abnormalities

Full characterization of patients regarding age, sex, hematologic findings, and basic immunophenotypic criteria is presented in Supplemental Table 1 in the online version.

For detection of the defined prognostic recurrent cytogenetic abnormalities, karyotyping, DNA index, FISH, and PCR were performed.

Karyotyping was attempted in all patient samples; however, karyotyping was successful in only 21 subjects (37.5%). Twenty metaphases were analyzed to produce reliable results. Out of those 21 patients, only 10 had an abnormal karyotype, with 7 hyperdiploid (high hyperdiploid karyotype 51-56 chromosome) and 3 hypodiploid (44 chromosome). The rest had an apparently normal karyotype. No translocation could be proved from karyotyping (Figure 1).

DNA index (flow cytometry) analysis revealed that 10 (17.8%) had a high DNA index (> 1.16), which indicates a hyperdiploid karyotype. Six patients (10.7%) had a low DNA index (< 0.95), which is considered to be hypodiploidy. The rest of patients (40 patients, 71.5%) had a DNA index between these values, which is considered to be a normal DNA index.

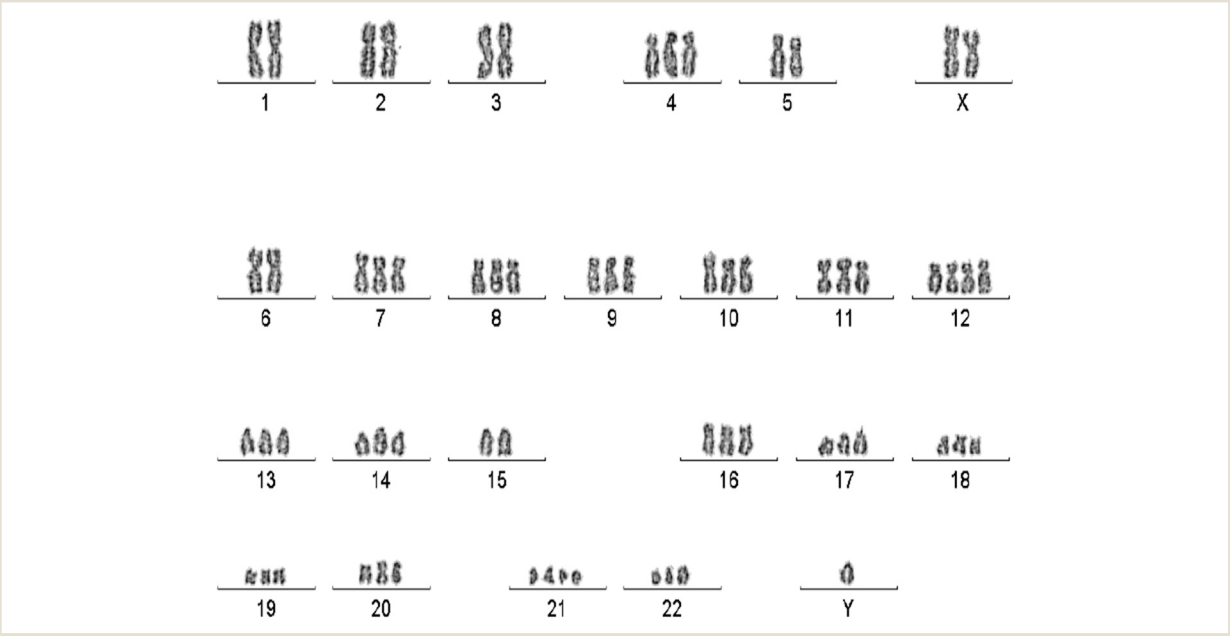
Interphase FISH to detect *ETV-RUNX1* and *BCR-ABL1* fusion genes was performed for all patients. Seven patients showed positive signals of t(12;21), representing 12.5% of the studied patients, and no patient was positive for *BCR-ABL1* translocation (Figure 2).

Molecular analysis results revealed that 4 patients (7.2%) were t(1;19) positive and 1 patient was t(4;11) positive. t(12;21) was found to be positive in the same 7 patients detected by FISH. No patient was positive for t(9;22) *BCR-ABL* translocation. Complex karyotype (combined genetic abnormalities in the same patient) was not detected in any patient.

Expression of Immunophenotyping (IPT) Markers in ALL Patients

Regarding the new markers, CD123 was found to be positive (expressed in > 20% of the blast population) in 25 patients (45%),

Figure 1 Karyotyping (G Banding) of Man With High Hyperdiploidy



Patient had 65 chromosomes, including trisomy of chromosomes 4, 7, 8, 9, 10, 11, 13, 14, 16, 17, 18, 19, 20, and 22; tetrasomy 12 and 21; and +X.

CD200 was found to be positive in 45 patients (80.3%), and CD79b was found to be positive in 38 patients (67.9%) (Table 1, Figure 2).

Correlation of New CD Markers With Cytogenetic Groups

CD123. Comparison of CD123 expression between different cytogenetic groups revealed significant variation among these groups (chi-square test, $P = .032$). The CD123 overexpression incidence was highest in the hyperdiploid group (86%). When comparing the percentage of CD123-positive expression between the hyperdiploid group (8 of 10) and the rest of the patients (17 of 29), there was a significant difference (chi-square test, $P = .017$) (Figure 3).

CD200. Comparison of CD200 expression among different cytogenetic groups revealed that it was highly expressed in all groups without significant variation (chi-square test, $P = .92$). The highest percentage of CD200-positive expression was in the t(12;21) group (6 of 7 patients, 86%).

CD79b. Comparison of CD79b expression among different genetic groups had significant variation among these groups (chi-square test, $P = .048$). The CD79b-negative percentage was highest in the t(12;21) group (71%). When comparing the percentage of CD79b underexpression between the t(12;21) group (5 of 7) and the rest of the patients (13 of 49), there was a significant difference (chi-square test, $P = .029$) (Figure 3).

Comparisons were performed among patients positive and negative for CD123 and CD79b to ascertain the potential prognostic value of these markers, if any. No significant difference was found regarding selected parameters (age, hemoglobin, total leukocyte count, platelet, and immunophenotype).

MRD Monitoring

Follow-up of patients and bone marrow aspirate samples for MRD was performed for all living patients. Unfortunately, 3 patients (5.3%) died during the induction phase of treatment (days 11, 14, and 18); the rest of the patients were alive at the time of follow-up MRD. Forty-nine patients (87.5%) successfully had a D28 MRD level of < 0.01.

Three patients experienced suboptimal MRD by routine panel of 0.01, 0.05, and 0.1. These patients were followed up after receiving another induction cycle at day 42 of treatment, where MRD level became optimally < 0.01. A fourth patient never experienced remission (MRD was 5 at day 28). Another induction cycle was initiated, but the patient died at treatment week 12.

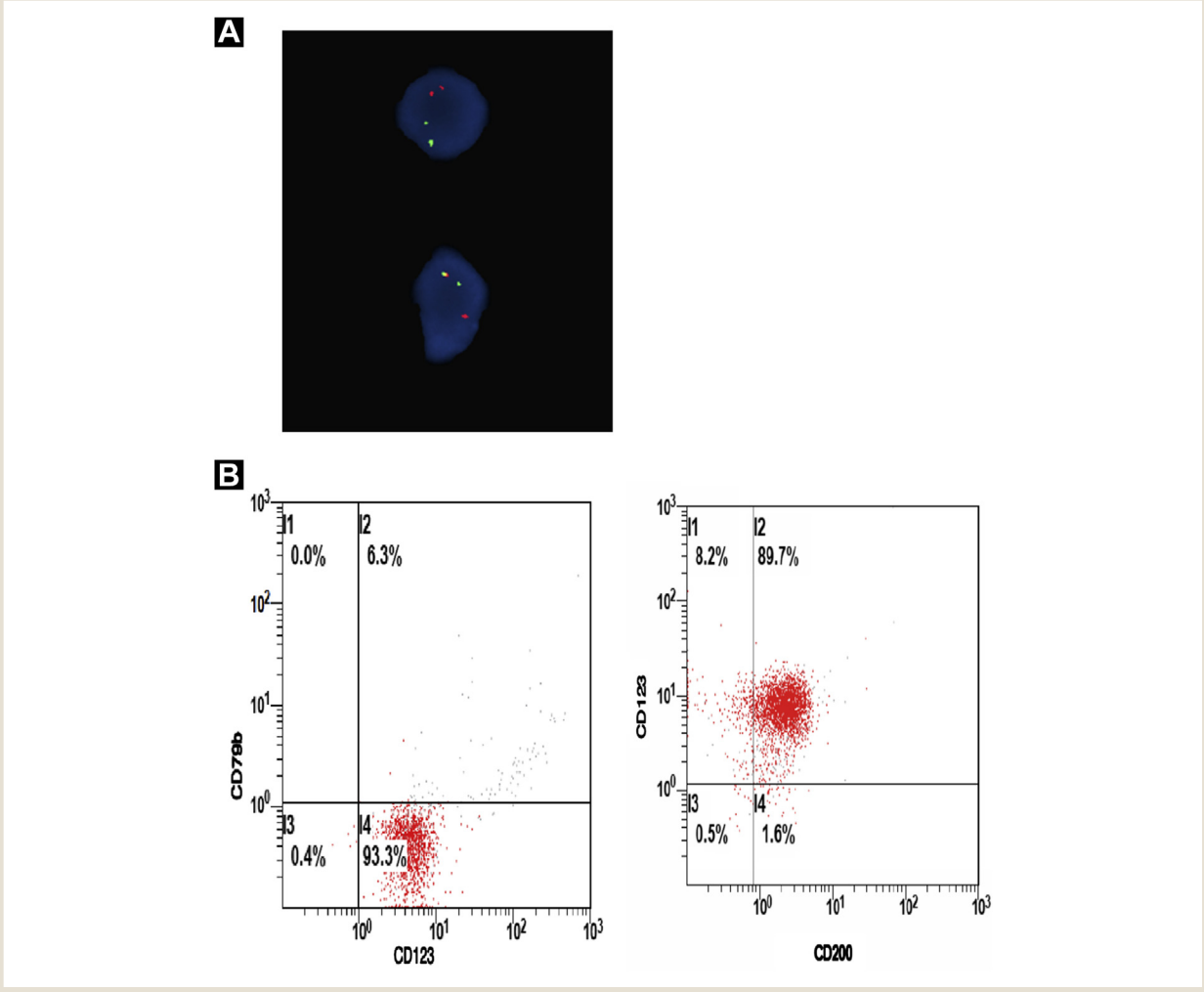
The routine panel for MRD (CD10, CD19, CD34, CD38, CD45, and CD58) was used to monitor MRD in the ALL patients. When comparing the results of MRD monitoring from this routine panel and from the incorporation of new markers, we found complete coordination between both results. Furthermore, in 2 of the 4 patients who did not experience optimal MRD response, the level of MRD detection increased by the incorporation of the new MRD markers by two to ten times (from 0.01 and 0.05 to 0.1).

Notably, CD123, which was positive in 1 of these 4 patients; CD79b, which was positive in 3 cases; and CD200, which was positive in all cases, were stably expressed by blasts in MRD samples, thus denoting the stability of these markers as MRD markers.

Discussion

Many studies were performed to correlate the immunophenotyping criteria to the underlying cytogenetic abnormalities, which would help in characterizing these cytogenetic subgroups, highlight

Figure 2 t(12;21) Acute Lymphoblastic Leukemia (A) Interphase FISH for *ETV-RUNX1* Fusion Gene Shows Positive Signal (Bottom) and Negative Signal (Top). (B) Immunophenotyping Shows Blast Population Positively Expressing CD123 and CD200 (Right) and Negative for CD79b (Left)



Abbreviation: FISH = fluorescence in-situ hybridization.

the molecular pathways of defined ALL subtypes, and create new possibilities of targeted therapy.[12,14,15,19] Selection of the CD markers was either from routinely used diagnostic panels[12,19] or was based on gene expression analysis.[15] In this study, we selected 3 CD markers (CD123, CD200, and CD79b) on the basis of the promising results for their use as targets of therapy.[20-24]

Cytogenetic characterization of our patients showed percentages of different ALL recurrent cytogenetic subgroups similar to those reported in the literature, including large pivotal studies[5,9,25-28] and previous studies on Egyptian patients,[2,29] denoting the good representative features of the studied population.

Significant correlation was found between CD123 expression and hyperdiploid ALL compared to other ALL subgroups, which have been previously reported.[14,15] A possible explanation of this aberrant high expression comes from the fact that the X chromosome (containing the *IL3RA* gene) is one of the most common gained chromosomes in hyperdiploid ALL, which may lead to increased gene expression in the neoplastic cells.[30-32] This was also evident in 6 of 7 hyperdiploidy cases with an available karyotype.

Another significant correlation was found between CD79b-negative expression and *ETV-RUNX1* positive ALL.[14] In this previously published study, there was a significant correlation between CD200 expression and this cytogenetic group; however, we could not demonstrate such an association in our study, although it was partly similar in that CD200 expression was the highest in the *ETV-RUNX1* group. This can be partly explained by the wide positive expression of CD200 in almost all groups (80.3% of all patients), reflecting its potential role as a therapeutic target but not as a characterization marker. This high prevalence of CD200 expression has been noted in previous studies.[33,34]

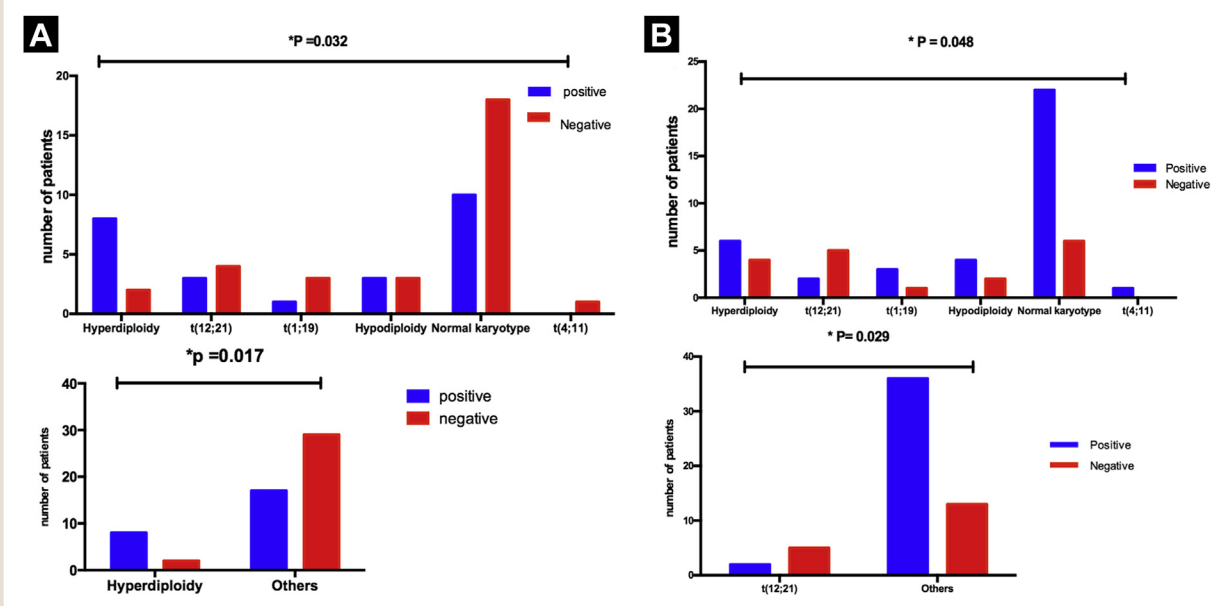
The stable expression profile of these markers and the perfect correlation of CD200 with MRD results using routine panels of even higher sensitivity indicated the potential use of these markers in MRD monitoring and follow-up, especially for CD200.

Table 1 Comparison Among ALL Cytogenetic Subgroups (n = 56)

Characteristic	Hyperdiploidy (n = 10)	t(12;21) (n = 7)	t(1;19) (n = 4)	Hypodiploidy (n = 6)	t(4;11) (n = 1)	Normal Karyotype (n = 28)	*P*
Age (years), mean ± SD	4 ± 1.3	3.5 ± 1	6.6 ± 4.6	9.2 ± 5.1	6 months	9.4 ± 5.3	.021
Hemoglobin (g/dL) (mean ± SD)	10.2 ± 1.3	10.1 ± 2.2	8.2 ± 1.5	8.3 ± 0.7	6.1	7.9 ± 2.3	.005
TLC ($\times 10^9$/L)							
Mean ± SD	21.8 ± 14.9	12.9 ± 5.4	38.7 ± 23.9	30.5 ± 18	120	36.4 ± 33.1	.122
Median (range)	18.20 (2.4-52.8)	14.40 (5.1-20.1)	32.80 (17.5-72.1)	28.05 (9.7-55.6)		25.5 (3.8-138)	
Platelets ($\times 10^9$/L)							
Mean ± SD	139.3 ± 108	177.4 ± 123	34 ± 15.1	56.5 ± 48	8	58.3 ± 84.6	.008
Median (range)	127 (9-386)	180 (35-400)	32 (18-54)	43 (12-147)		37 (4-442)	
Immunophenotype							<.001
C-ALL	7	5	0	2	0	19	
Pre-B-ALL	3	2	4	4	0	9	
Pro-B-ALL	0	0	0	0	1	0	
COG Risk Groups							<.001
Low	8	6	0	0	0	0	
Average	2	1	1	3	0	12	
High	0	0	3	2	0	12	
Very high	0	0	0	0	1	5	
New Markers							
CD123 positive	8 (80%)	3 (43%)	1 (25%)	3 (50%)	0	10 (36%)	.032
CD200 positive	8 (80%)	6 (86%)	3 (75%)	4 (67%)	1 (100%)	23 (82%)	.92
CD79b negative	4 (40%)	5 (71%)	1 (25%)	2 (33%)	0 (0%)	6 (21%)	.048

Abbreviations: ALL = acute lymphoblastic leukemia; B-ALL = B-cell ALL; C-ALL = common type ALL; COG = Children's Oncology Group; TLC = total leukocyte count.

Figure 3 Comparison Between Acute Lymphoblastic Leukemia Cytogenetic Subgroups Shown are Expression of (A) CD123 and (B) CD79b



Introduction of such specific and new markers to the routine panels would also allow monitoring of currently problematic leukemia, such as that lacking a traceable immunophenotype or that with immunophenotype shift.

In conclusion, we found a significant correlation between some markers and certain ALL recurrent cytogenetic subgroups (CD123 and hyperdiploidy, CD79b negativity, and *ETV-RUNX1*). This correlation may help in the further characterization of these groups and in understanding its underlying molecular pathways. Also, because they are associated with such favorable groups, these markers may have some prognostic values (although this was not quite statistically significant in this study). Using CD200 in MRD monitoring would increase the sensitivity and resolution of MRD. Interestingly, these markers may serve as potential therapeutic targets in ALL therapy.

Clinical Practice Points

- Cytogenetic and molecular changes in ALL represent the most important prognostic variables; they are used in classification and risk stratification. Also, currently, minimal residual disease (MRD) is the most powerful prognostic factor in ALL patients, even those with low-risk disease. Few publications have studied the correlation between specific CD markers and certain cytogenetic groups, which needs to be further explored.
- As a conclusion, we found a significant correlation between some markers and certain ALL' recurrent cytogenetic subgroups (CD123 and hyperdiploidy, CD79b negativity, and *ETV-RUNX1*). While the third studied marker (CD200) was widely expressed in the majority of cases.
- We also noticed stable expression profile of these markers and perfect correlation of CD200 with MRD results using routine panels, even more increased sensitivity, denote the potential use of these markers in MRD monitoring and follow up.
- These results may help in further characterization of these groups and in understanding its underlying molecular pathways.
- Interestingly, these markers may serve as potential therapeutic targets in ALL therapy.

Disclosure

The authors have stated that they have no conflict of interest.

Supplementary Data

A supplemental table accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.clml.2016.02.032.

References

1. Howlader N, Noone AM, Krapcho M, et al. *SEER Cancer Statistics Review, 1975-2011*. Bethesda, MD: National Cancer Institute; 1975. Available at: http://seer.cancer.gov/csr/1975_2011/, based on November 2013 SEER data submission, posted to the SEER Web site April 2014.
2. Shalaby RH, Ashaat N, Abdel Wahab N, et al. Bcl-2 expression and chromosomal abnormalities in childhood acute lymphoblastic leukemia. *Acad J Cancer Res* 2010; 3:35-43.
3. Alieldin N. Statistical report of hospital based population registry of National Cancer Institute, Cairo University (2002-2010). Available at: http://www.nci.cu.edu.eg/. Accessed: January 20, 2015.
4. Kotecha RS, Gottardo NG, Kees UR, et al. The evolution of clinical trials for infant acute lymphoblastic leukemia. *Blood Cancer J* 2014; 4:e200.
5. Woo JS, Alberti MO, Tirado CA. Childhood B-acute lymphoblastic leukemia: a genetic update. *Exp Hematol Oncol* 2014; 3:16.
6. Schmiegelow K, Forestier E, Hellebostad M, et al. Long term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. *Leukemia* 2010; 24:345-54.
7. Biondi A, Cazzaniga G. Novel clinical trials for pediatric leukemias: lessons learned from genomic analyses. *ASH Educ Program Book* 2013:612-9.
8. Nathan PC, Wasilewski-Masker K, Janzen LA. Long-term outcomes in survivors of childhood acute lymphoblastic leukemia. *Hematol Oncol Clin North Am* 2009; 23: 1065-82.
9. Moorman AV, Ensor HM, Richards SM, et al. Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. *Lancet Oncol* 2010; 11:429-38.
10. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. *Lancet* 2013; 381:1943-55.
11. Campana D. Minimal residual disease monitoring in childhood acute lymphoblastic leukemia. *Curr Opin Hematol* 2012; 19:313-8.
12. Weng XQ, Shen Y, Sheng Y, et al. Prognostic significance of monitoring leukemia-associated immunophenotypes by eight-color flow cytometry in adult B-acute lymphoblastic leukemia. *Blood Cancer J* 2013; 3:e133.
13. Borowitz MJ, Devidas M, Hunger SP, et al, Children's Oncology Group. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. *Blood* 2008; 111:5477-85.
14. Coustan-Smith E, Song G, Clark C, et al. New markers for minimal residual disease detection in acute lymphoblastic leukemia. *Blood* 2011; 117:6267-76.
15. Djokic M, Björklund E, Blennow E, et al. Overexpression of CD123 correlates with the hyperdiploid genotype in acute lymphoblastic leukemia. *Haematologica* 2009; 94:1016-9.
16. Testa U, Riccioni R, Militi S, et al. Elevated expression of IL-3Ralpha in acute myelogenous leukemia is associated with enhanced blast proliferation, increased cellularity, and poor prognosis. *Blood* 2002; 100:2980-8.
17. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 2009; 114:937-51.
18. Le Viseur C, Hotfilder M, Bomken S, et al. In childhood acute lymphoblastic leukemia, blasts at different stages of immunophenotypic maturation have stem cell properties. *Cancer Cell* 2008; 14:47-58.
19. Zheng J, Wang X, Hu Y, et al. A correlation study of immunophenotypic, cytogenetic, and clinical features of 180 AML patients in China. *Cytometry B Clin Cytom* 2008; 74:25-9.
20. Testa U, Pelosi E, Frankel A. CD 123 is a membrane biomarker and a therapeutic target in hematologic malignancies. *Biomark Res* 2014; 2:4.
21. Moreaux J, Veyrune JL, Reme T, et al. CD200: a putative therapeutic target in cancer. *Biochem Biophys Res Commun* 2008; 366:117-22.
22. Zheng B, Fuji RN, Elkins K, et al. In vivo effects of targeting CD79b with antibodies and antibody-drug conjugates. *Mol Cancer Ther* 2009; 8: 2937-46.
23. Pfeifer M, Zheng B, Erdmann T, et al. Anti-CD22 and anti-CD79B antibody drug conjugates are active in different molecular diffuse large B-cell lymphoma subtypes. *Leukemia* 2015; 29:1578-86.
24. Jahn L, Hombrink P, Hassan C, et al. Therapeutic targeting of the BCR-associated protein CD79b in a TCR-based approach is hampered by aberrant expression of CD79b. *Blood* 2015; 125:949-58.
25. Forestier E, Johansson B, Borgström G, et al, NOPHO Leukemia Cytogenetic Study Group. Cytogenetic findings in a population-based series of 787 childhood acute lymphoblastic leukemias from the Nordic countries. *Eur J Haematol* 2000; 64:194-200.
26. Schultz KR, Pullen DJ, Sather HN, et al. Risk- and response-based classification of childhood B-precursor acute lymphoblastic leukemia: a combined analysis of prognostic markers from the Pediatric Oncology Group (POG) and Children's Cancer Group (CCG). *Blood* 2007; 109:926-35.
27. Sharathkumar A, DeCamillo D, Bhambhani K, et al. Children with hyperdiploid but not triple trisomy (+4, +10, +17) acute lymphoblastic leukemia have an increased incidence of extramedullary relapse on current therapies: a single institution experience. *Am J Hematol* 2008; 83:34-40.
28. Felice MS, Gallego MS, Alonso CN, et al. Prognostic impact of t(1;19)/TCF3-PBX1 in childhood acute lymphoblastic leukemia in the context of Berlin–Frankfurt–Münster-based protocols. *Leuk Lymphoma* 2011; 52:1215-21.
29. Mousa S, Mostafa S, Shaheen I, et al. Detection of trisomy 4 and 10 in Egyptian pediatric patients with acute lymphoblastic leukemia. *Clin Lab* 2014; 60:609-14.
30. Heinonen K, Mahlamäki E, Riikonen P, et al. Acquired X-chromosome aneuploidy in children with acute lymphoblastic leukemia. *Med Pediatr Oncol* 1999; 32: 360-5.
31. Heerema NA, Raimondi SC, Anderson JR, et al. Specific extra chromosomes occur in a modal number dependent pattern in pediatric acute lymphoblastic leukemia. *Genes Chromosomes Cancer* 2007; 46:684-93.
32. Paulsson K, Forestier E, Lilljebjörn H, et al. Genetic landscape of high hyperdiploid childhood acute lymphoblastic leukemia. *Proc Natl Acad Sci U S A* 2010; 107:21719-24.
33. Dorfman DM, Shahsafaei A. CD200 (OX-2 membrane glycoprotein) expression in B cell–derived neoplasms. *Am J Clin Pathol* 2010; 134:726-33.
34. Alapat D, Coviello-Malle J, Owens R, et al. Diagnostic usefulness and prognostic impact of CD200 expression in lymphoid malignancies and plasma cell myeloma. *Am J Clin Pathol* 2012; 137:93-100.

Supplemental Table 1	Characteristics of 56 ALL Patients (n = 56)
Criterion	**Value**
Age (Years)	
Mean ± SD	7.1 ± 5.45
Median (range)	5 (6 months to 18 years)
Sex	
Male	36 (65%)
Female	20 (35%)
Hemoglobin (g/dL)	
Mean ± SD	8.3 ± 2.2
TLC ($\times 10^6$/L)	
Mean ± SD	35.3 ± 32.4
Median (range)	18.8 (2.4-155)
Platelets ($\times 10^6$/L)	
Mean ± SD	80.3 ± 105.1
Median (range)	40.5 (4-450)
PB Blast Percentage	
(Mean ± SD)	51.5 ± 33.3
Median (range)	65 (2-99)
BMA Blast Percentage	
(Mean ± SD)	90.2 ± 5.3
Immunophenotyping	
C-ALL	34 (60.7%)
Pre-B-ALL	21 (37.5%)
Pro-B-ALL	1 (1.8%)
New Markers (% Positive)	
CD123	25 (45%)
CD200	45 (80.3%)
CD79b	38 (67.9%)
Cytogenetic Fractions	
Hyperdiploidy	10 (17.8%)
t(12;21)	7 (12.5%)
t(1;19)	4 (7.2%)
Hypodiploidy	6 (10.7%)
t(4;11)	1 (1.8%)
Normal karyotype	28 (50%)

Abbreviations: ALL = acute lymphoblastic leukemia; B-ALL = B-cell ALL; BMA = bone marrow aspirate; C-ALL = common type ALL; PB = peripheral blood; TLC = total leukocyte count.