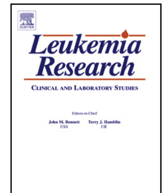
Leukemia Research 56 (2017) 44–51

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres



Genomic analysis of adult B-ALL identifies potential markers of shorter survival

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ARTICLE INFO

Article history:
Received 7 November 2016
Received in revised form 2 January 2017
Accepted 29 January 2017
Available online 3 February 2017

Keywords:
B-cell
Acute lymphoblastic leukemia
Genome-wide association study
Copy number abnormalities
CDKN2A
IKZF1

ABSTRACT

B lymphoblastic leukemia (B-ALL) in adults has a higher risk of relapse and lower long-term survival than pediatric B-ALL, but data regarding genetic prognostic biomarkers are much more limited for adult patients. We identified 70 adult B-ALL patients from three institutions and performed genome-wide analysis via single nucleotide polymorphism (SNP) arrays on DNA isolated from their initial diagnostic sample and, when available, relapse bone marrow specimens to identify recurring copy number alterations (CNA). As B-cell developmental genes play a crucial role in this leukemia, we assessed such for recurrent deletions in diagnostic and relapse samples. We confirmed previous findings that the most prevalent deletions of these genes occur in *CDKN2A*, *IKZF1*, and *PAX5*, with several others at lower frequencies. Of the 16 samples having paired diagnostic and relapse samples, 5 showed new deletions in these recurrent B-cell related genes and 8 showed abolishment. Deletion of *EBF1* heralded a significant negative prognostic impact on relapse free survival in univariate and multivariate analyses. The combination of both a *CDKN2A/B* deletion and an *IKZF1* alteration (26% of cases) also showed a trend toward predicting worse overall survival compared to having only one or neither of these deletions. These findings add to the understanding of genomic influences on this comparably understudied disease cohort that upon further validation may help identify patients who would benefit from upfront treatment intensification.
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http://dx.doi.org/10.1016/j.leukres.2017.01.034
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1. Introduction

B lymphoblastic leukemia (B-ALL) in adults is associated with a much worse prognosis [1] than pediatric B-ALL [2]. Longer term survival is dramatically higher in the pediatric B-ALL population with rates of 80–90% demonstrated in recent studies [3], contrasted with long-term survival rates of 30–40% in adults [4]. One reason for the disparity may be the differences between pediatric and adult treatment protocols with more intensive and longer treatment regimens for children. This has led to recent interest in treating adolescents and young adults with pediatric based regimens [5]. Furthermore, there is accumulating evidence that adult and pediatric B-ALL may have different underlying genomic alterations. On the cytogenetic level, recurrent genetic aberrations such as the translocation t(12;21)(p13;q22)/*ETV6-RUNX1* and a high hyperdiploidy state (51–67 chromosomes) have been associated with a better prognosis [6]. These occur more frequently in the pediatric population than in adults [7]. Conversely, low hypodiploidy/near-triploidy, complex karyotypes, and the translocation t(9;22)(q34;q11)/*BCR-ABL1* (Philadelphia chromosome) are more frequently observed in adult B-ALL patients compared to the pediatric population [8]. These cytogenetic abnormalities are associated with a worse prognosis [6]. In addition to differences in treatment protocols, these and other genetic differences between age groups may also contribute to the considerable disparity in prognosis.

Pediatric B-ALL has been well-characterized at the genomic level with genome wide single nucleotide polymorphism (SNP) arrays [9–11], but similar studies are limited in the adult population. Previous studies [12–14] performed in adults have identified recurrent deletions in genes known to be affected in pediatric B-ALL such as *CDKN2A, IKZF1, PAX5, ETV6, RB1, BTG1* and *EBF1*. The deletion of *IKZF1* has been linked to a poorer prognosis in pediatric B-ALL [15–18]; however, its clinical impact has not been well characterized in adult B-ALL [19–23]. *CDKN2A* deletions have also been described in adult B-ALL, but their prognostic impact has not been fully elucidated [19,23–26].

Utilization of genetic abnormalities for clinical risk stratification of pediatric B-ALL is well established. In adult B-ALL, only the presence of the Philadelphia chromosome and *MLL* rearrangements are commonly used for prognostic and therapeutic decision-making purposes. To improve these efforts, emphasis has been placed on identifying microdeletions that may have prognostic and/or predictive value. Validation of the findings of these prior genome wide studies of adult B-ALL and identification of novel genomic alterations may provide further insight into the pathophysiology of adult B-ALL, allowing for both improved risk-stratification and identification of therapeutic targets. To this end, we performed a genome wide SNP array analysis on 78 adult patients to characterize recurrent copy number alterations (CNA) in adult B-ALL patients. In addition to recurrent deletions, we also assessed recurrent copy number gains, some of which have not been previously reported in this population.

2. Methods

2.1. Patient selection

Eligibility criteria consisted of having a diagnosis of B-ALL from 1998 or later, being 18 years of age or greater at the time of diagnosis, and having an accessible formalin-fixed paraffin embedded (FFPE) bone marrow clot specimen available for molecular analysis.

2.2. Clinical data

Clinical data collected or calculated on this cohort included age, gender, hematologic laboratory values at presentation, bone marrow and peripheral blood blast percentage, cerebrospinal fluid (CSF) involvement, achievement of complete remission (CR), receipt of allogeneic transplant, cytogenetic profile, Philadelphia chromosome (Ph) status, event-free survival (EFS), relapse-free survival (RFS), and overall survival (OS). Cytogenetic data included standard karyotyping as well as fluorescence in situ hybridization (FISH) probe data at loci known to be involved in B-ALL. Philadelphia chromosome positivity (Ph+) required either identification of t(9;22)(q34.1;q11.2) by cytogenetic analysis, positive FISH probe detection of the *BCR-ABL1* fusion gene, or a *BCR-ABL1* fusion gene mRNA transcript detection by reverse transcriptase polymerase chain reaction (RT-PCR) in the peripheral blood or bone marrow.

2.3. Genetic analysis

Samples from 78 patients were collected. Seventy patients had samples available at the time of initial diagnosis of B-ALL. Samples at the time of relapsed disease were available in 24 patients. Eight of these patients did not have a sample at the time of initial diagnosis. Three patients had relapse samples at time of first and second relapse. DNA was isolated from FFPE bone marrow clots using the RecoverAll kit (Ambion) and assessed in 3 distinct batches with >20 samples in each batch (two batches utilized the OncoScan FFPE Express genome-wide SNP assay service from Affymetrix, OncoScan run at ARUP Laboratories for the third, Supplemental Table 1, Supplemental Table 2). This microarray platform utilizes molecular inversion probe (MIP) technology and has been validated on archived FFPE samples including those collected more than ten years prior to analysis. These MIP probes assess alleles at over 220,000 SNPs at selected locations distributed across the genome, with increased density within approximately 900 previously identified cancer genes [27]. Samples in each batch group were mostly unique to each of the three hospital cohorts of patients. Differences in some covariates (and in some survival measures) were present across the individuals in these batch groups (Supplementary Table 3) indicating some differences in the underlying cohorts. Hence these survival differences by batch group were accounted for in appropriate multivariate analyses.

Copy number alteration (CNA) data analysis was performed using Nexus Software V7 (BioDiscovery) and in-house coding. Both initial diagnosis and relapse sample cohorts were analyzed on a genome-wide aggregate basis for recurrent CNAs. The Nexus software used two algorithms, Genomic Identification of Significant Targets in Cancer (GISTIC) [28], and Significance Testing for Aberrant Copy number (STAC) [29], to identify recurrent CNAs that were most likely to have occurred in a non-random manner. The software also reported the incidence of CNA overlap at each locus based on the Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home) used for exclusion of normally occurring germline CNVs. In addition to gains and deletions, copy neutral loss of heterozygosity (CN-LOH) was also assessed with this software. However, differences in CN-LOH prevalence across the batch groups were unfortunately too variable to provide reliable estimates of this aspect in the genes assessed (data not shown). The most frequent CNAs called by the software were manually verified and additional manual calls were made when easily agreed upon by two investigators. This consisted of confirming that $\log_2$ ratios and B-allele frequencies exhibited aberration from the biparental disomy state at the probe level.

2.4. Statistical analysis

The proportion of samples with gene aberrations was assessed by processing batch to avoid potential confounding and to assess heterogeneity of event calls. Exact methods (Clopper-Pearson) were used to establish 95% confidence intervals. EFS was calculated ignoring transplant (as opposed to censoring at transplant when such occurred). Survival differences for OS, RFS, and EFS for single variables were assessed with Cox proportional hazards models and at times for comparison with the log-rank test. Kaplan-Meier statistics were used to estimate median survival. Concomitance of gene aberrations in each processing group was assessed with Fisher's exact test. Evidence for an effect across batch groups was assessed via Fisher's combined probability test (meta-analysis). Bonferroni adjustment for multiple comparisons was used in assessing concomitance of gene aberrations and covariates as well as calculation of the false discovery rate. Cox proportional hazards modeling was also used for multivariate analyses; variables significant in univariate analyses were tested in multivariate models. Distribution differences were assessed with Wilcoxon–Mann–Whitney's test. Two-sided p-values were reported for all statistical tests, and were calculated using SAS version 9.3 (SAS Institute, Cary, NC, USA).

EFS was defined as time from initial diagnosis to whichever event occurred first: relapse, death, or date of last follow-up. Complete remission (CR) required documentation of a bone marrow biopsy with cytogenetic analysis and flow cytometry when available. Patients with peripheral blood and/or CSF involvement at diagnosis required documentation of remission in the peripheral blood and/or CSF by cytology and flow cytometry when available in addition to bone marrow remission to qualify as a CR. For those patients achieving CR, RFS was calculated and defined as time from determined CR to relapse; events were censored at time of last known follow-up being relapse-free or time of death if such occurred during time of remission. OS was defined as time from initial diagnosis to death, with censoring at last-follow up.

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