ACTA ONCOLOGICA
2021, VOL. 60, NO. 6, 760–770
https://doi.org/10.1080/0284186X.2021.1900908

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ORIGINAL ARTICLE

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Genomic landscape of B-other acute lymphoblastic leukemia in an adult retrospective cohort with a focus on *BCR-ABL1*-like subtype

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ABSTRACT

Introduction: *BCR-ABL1*-like acute lymphoblastic leukemia (ALL) is a high-risk disease with a complex genomic background. Though extensively studied, data on the frequency and mutual associations of present mutations are still incomplete in adult patients. This retrospective study aims to map the genomic landscape of B-other ALL in a cohort of adult patients with a focus on the *BCR-ABL1*-like ALL subtype.

Methods: We analyzed bone marrow and peripheral blood samples of adult B-other ALL patients treated consecutively at three major Czech teaching hospitals. Samples were analyzed by cytogenetic methods, gene expression profiling, multiplex ligation-dependent probe amplification (MLPA), and next-generation sequencing (NGS).

Results: Fifty-eight B-other ALL patients (not *BCR-ABL1*, *KMT2A*-rearranged, *ETV6-RUNX1*, *TCF3-PBX1*, or iAMP21) were included in the study. Median follow-up was 23.8 months. Samples from 33 patients were available for a gene expression analysis, 48.9% identified as *BCR-ABL1*-like ALL. Of the *BCR-ABL1*-like ALL cases, 18.8% harbored *IGH-CRLF2* and 12.5% *P2RY8-CRLF2* fusion gene. We observed a higher MRD failure rate in *BCR-ABL1*-like than in non-*BCR-ABL1*-like ALL patients after the induction treatment (50.0 vs. 13.3%, p=.05). There was a trend to worse progression-free and overall survival in the *BCR-ABL1*-like group, though not statistically significant. Deletions in *IKZF1* gene were found in 31.3% of *BCR-ABL1*-like cases. Patients with concurrent *IKZF1* and *CDKN2A/B, PAX5* or PAR1 region deletions (*IKZF1*^{plus} profile) had significantly worse progression-free survival than those with sole *IKZF1* deletion or *IKZF1* wild-type (p=.02). NGS analysis was performed in 54 patients and identified 99 short variants in *TP53, JAK2, NRAS, PAX5, CREBBP, NF1, FLT3, ATM, KRAS, RUNX1*, and other genes. Seventy-five of these gene variants have not yet been described in B-cell precursor ALL to date.

Conclusion: This study widens existing knowledge of the *BCR-ABL1*-like and B-other ALL genomic landscape in the adult population, supports previous findings, and identifies a number of novel gene variants.

ARTICLE HISTORY
Received 18 October 2020
Accepted 5 March 2021

KEYWORDS
B-other acute lymphoblastic leukemia; adults; *BCR-ABL1*-like; *IKZF1*^{plus}; next-generation sequencing

Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous disease with a complex pattern of molecular changes, including aneuploidy, copy number variations (CNVs), gene mutations, and chromosomal rearrangements. These genetic alterations deregulate gene expression or result in the formation of fusion proteins with constitutively upregulated kinase activity [1,2]. ALL represents the most common pediatric malignancy with a long-term overall survival (OS) rate approaching 90% [3–5]. In stark contrast to the outstanding therapeutic results seen in children with ALL, treatment outcomes of adult patients remain unsatisfactory, with approximately half of patients lost despite the incorporation of modern pediatric-inspired intensive therapeutic protocols [6].

Besides well-established B-cell precursor acute lymphoblastic leukemia (BCP-ALL) subtypes with recurrent genetic aberrations (*BCR-ABL1*, *KMT2A* rearrangements, *ETV6-RUNX1*, *TCF3-PBX1*, iAMP21), gene expression profile studies have identified several novel B-other ALL subtypes in recent years, including *BCR-ABL1*-like ALL, ALL with *DUX4*, *ZNF384*, or *MEF2D* rearrangements, *ETV6-RUNX1*-like ALL or ALL with *PAX5* alterations [7–11]. *BCR-ABL1*-like ALL (or Philadelphia chromosome-like, Ph-like ALL) is one of the novel B-other ALL subtypes defined by unique gene

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expression profile and associated with a poor prognosis [12–19]. Despite the absence of t(9;22) translocation and *BCR-ABL1* fusion, the gene expression profile is similar to *BCR-ABL1*-positive ALL in this subtype.

The incidence of *BCR-ABL1*-like ALL constitutes up to 10–20% of childhood BCP-ALL, reaches a peak in adolescents and young adults (AYA) (20–42%) and then slowly decreases with increasing age [12,16,17,20–22]. Even *BCR-ABL1*-like ALL is not a homogeneous disease subtype, but comprises a diverse range of genetic alterations activating cytokine receptor genes and kinase signaling pathways, providing the rationale for the use of targeted therapies [20,23,24]. Based on the type of cytokine receptor lesion or kinase fusion gene, *BCR-ABL1*-like ALL can be divided into six distinct subgroups: (1) *CRLF2* rearrangements (*IGH-CRLF2* and *P2RY8-CRLF2*), (2) ABL-class gene (*ABL1/2*, *PDGFRB*, *CSF1R*) rearrangements, (3) *JAK2* and *EPOR* rearrangements, (4) other JAK/STAT pathway activating mutations or deletions (*JAK1/2/3, IL7R, SH2B3*), (5) Ras pathway activating mutations or deletions (*KRAS, NRAS, NF1, PTPN11*), and (6) other rare kinase aberrations (e.g., *FLT3, NTRK3, LYN*) [20,24]. Sequence mutations and deletions in the lymphoid transcription factor gene *IKZF1* are also frequently observed in patients with both *BCR-ABL1*-like and Philadelphia-positive ALL [13,16,17,20,21,25].

Here, we present the results of a study aimed at mapping the genomic landscape of B-other ALL in a cohort of adult patients focusing on *BCR-ABL1*-like ALL subtype, and assessing a prognostic impact of *BCR-ABL1*-like status in these patients. We used classical cytogenetic methods with fluorescence *in situ* hybridization (FISH), gene expression profiling, real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR), multiplex ligation-dependent probe amplification (MLPA) for CNVs analysis and a newly designed next-generation sequencing (NGS) panel developed for comprehensive diagnostics of lymphoid malignancies. The other goal of the study was to establish means and standards for fast routine *BCR-ABL1*-like profile testing in adult B-other ALL patients in Czechia.

Methods

Patients and samples

In this retrospective study, we analyzed stored bone marrow and peripheral blood samples of adult BCP-ALL patients. The patients were consecutively treated from May 2007 to February 2020 at three major Czech university hospitals. All patients agreed to written informed consent of scientific use of their tissue in accordance with the Declaration of Helsinki. Samples for genetic analyses were collected at the time of diagnosis and samples for the minimal residual disease (MRD) assessment were collected in the 11th week of treatment before the first consolidation cycle. All B-other ALL patients with genetic and/or cell material stored in our biobanks were included in the study. For this study, we defined B-other ALL as the absence of *BCR-ABL1* (FISH and qRT-PCR), *KMT2A* rearrangements (FISH), *ETV6-RUNX1* (FISH), *TCF3-PBX1* (FISH), and iAMP21 (FISH). These recurrent genetic aberrations are routinely examined in all newly diagnosed BCP-ALL patients in our centers.

Ninety-two patients were initially included in the study. Due to a lack of sufficient DNA, RNA or cell samples, a cohort of 58 patients was available for the testing and further evaluation. The study flowchart is shown in Figure 1. The median follow-up duration of the cohort was 23.8 (0.0–131.8) months. A majority of the patients (77.6%) were treated by intensive regimens, all of which were pediatric or pediatric-inspired. Allogeneic or autologous stem cell transplantation (SCT) was performed in 43.1% of the patients. Detailed cohort characteristics are presented in Table 1.

BRC-ABL1-*like signature analysis*

BRC-ABL1-like cases were identified using a method based on the expression of 10 genes [26] with two minor modifications: 1 μg of total RNA was retrotranscribed using PrimeScript™ RT reagent Kit (Takara, Kyoto, Japan) according to the manufacturer's protocol and qRT-PCR was performed

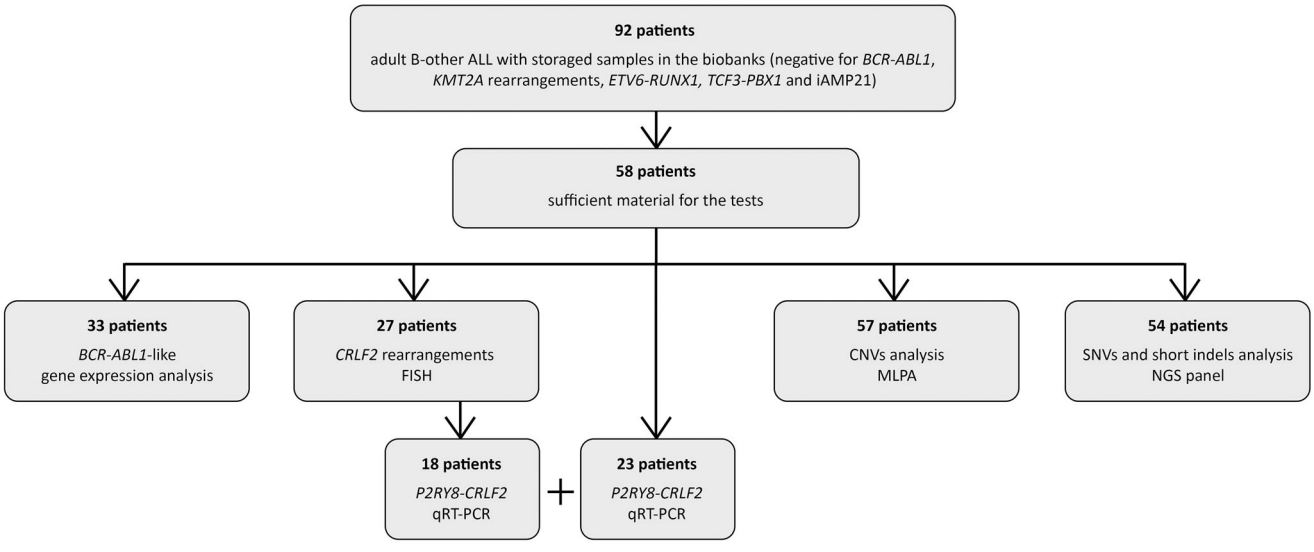

Figure 1. Study flowchart diagram.

Table 1. Study population characteristics.

Characteristics	*N*
Total patients	58
Age	
Median years (range)	44 (18–85)
AYA (15–39 years)	23 (39.7%)
Adults (40–54 years)	17 (29.3%)
Older adults (≥55 years)	18 (31.0%)
Sex	
Male	36 (62.1%)
Female	22 (37.9%)
Immunophenotype	
Pro-B	11 (19.0%)
Common-B	37 (63.8%)
Pre-B	5 (8.6%)
B (not specified)	5 (8.6%)
Treatment	
Intensive	45 (77.6%)
ALL CELL 2012 junior	38 (65.5%)
AIEOP-BFM 2009	3 (5.2%)
Blina-CELL	4 (6.9%)
Reduced/palliative	13 (22.4%)
ALL CELL 2012 elderly	8 (13.8%)
Others	5 (8.6%)
MRD week 11	
Negative	30 (51.7%)
Positive	19 (32.8%)
NA	9 (15.5%)
SCT	
No SCT	33 (56.9%)
Allogeneic in CR1	12 (20.7%)
Allogeneic after relapse	12 (20.7%)
Autologous	1 (1.7%)
Relapse	
No	24 (41.4%)
Yes	34 (58.6%)
Death	
No	34 (58.6%)
Yes	24 (41.4%)
Follow-up	
Median months (range)	23.8 (0.0–131.8)

AYA: adolescents and young adults; CR1: first complete remission; MRD: minimal residual disease; NA: not available; SCT: stem cell transplantation.
ALL CELL 2012 junior – modified GMALL 07/2003 [6]; AIEOP-BFM 2009 – EudraCT 2007-004270-43; Blina-CELL – ClinicalTrials.gov NCT04554485; ALL CELL 2012 elderly – EWALL backbone with intensified induction I and added PEG-asparaginase.

with a QuantStudio™ 12K Flex Real-time PCR System (Applied Biosystems, Foster City, CA, USA). Universal FastStart SYBR Green Master (Roche, Basel, Switzerland) and specific primers were used for qRT-PCR [26]. Expression levels of the target genes were normalized to the expression levels of the GAPDH constitutive gene and used for score computing [26]. Samples with a score of −0.3 or higher were identified as *BCR-ABL1*-like ALL.

CRLF2 *rearrangements analysis*

FISH analysis for *CRLF2* alterations was performed on the diagnostic patient sample cells stored in Carnoy fixative. The Dual Color Break-Apart CRLF2 Probe (ZytoVision, Bremerhaven, Germany) was used with the positive cells cut-off of 5%. A minimum of 200 nuclei were scored visually by two independent analysts. Results were captured using an automated Nikon Eclipse 80i fluorescence microscope (Nikon Instech, Tokyo, Japan) and images were analyzed and stored by imaging system Lucia Cytogenetics (Laboratory Imaging, Praha, Czech Republic). A negative FISH results for *CRLF2* alterations ruled out both *IGH-CRLF2* and *P2RY8-CRLF2* fusions.

P2RY8-CRLF2 *fusion gene detection*

The presence of *P2RY8-CRLF2* fusion transcript was verified by qRT-PCR. Reverse transcription of RNA to DNA was done using the SuperScript II Reverse Transcriptase kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Reactions were performed using primers (P2RY8_fw: 5′-CCTCTGAGCTCTCACCTGCTACT-3′, CRLF2_rv: 5′-AGCCTCCCAG CAGCCCGAC) and a probe (P_q: FAM-CTGCCGCTGCTTC-TAMRA) which were designed to flank the fusion breakpoint for real-time detection on a Light Cycler 480 (Roche, Basel, Switzerland) [27]. Subsequently, Sanger sequencing was performed to confirm and characterize the fusion breakpoints of the PAR1 region deletion.

Copy number variations analysis

CNVs were analyzed by MLPA using genomic DNA. We used the P335-B2 SALSA MLPA kit (MRC Holland, Amsterdam, Netherlands) according to the manufacturer's recommendations. The established MLPA kit included probes for *IKZF1*, *CDKN2A, CDKN2B, PAX5, ETV6, RB1, BTG1*, and the *BTG1* downstream region, *EBF1, JAK2, ZFY*, and the Xp22.33 region (PAR1 region, *SHOX* area, *CRLF2, CSF2RA, IL3RA*, and *P2RY8* genes). Two samples from healthy individuals were included in every analysis as well as negative controls. The MLPA products were run on the ABI 3500 Genetic analyzer (Life Technologies, Carlsbad, CA, USA). The MLPA data were analyzed using Coffalyser.net analysis software version 140721.1958 provided by the manufacturer using default settings.

Single nucleotide variations and short indels detection

Single nucleotide variations (SNVs) and short indels were identified by a newly implemented targeted NGS panel named LYNX. This custom panel represents a comprehensive tool for the analysis of a variety of genomic aberrations and markers in the most frequent lymphoid malignancies, such as chronic lymphocytic leukemia, ALL, diffuse large B-cell lymphoma, follicular lymphoma, and mantle cell lymphoma. The LYNX panel enables simultaneous detection of variants in 70 genes (Supplementary Table 1), genome-wide CNVs (over 6 Mb) and recurrent chromosomal aberrations (over 300 kb), common lymphoma-specific translocation, and the assessment of immunoglobulin (IG) and T-cell receptor (TR) gene rearrangements [28]. For the purpose of this study, we utilized data from gene variant analysis branch, where all coding exons and splice sites of 70 genes were explored with a sensitivity of 5% variant allele frequency (VAF). The library preparation was performed using capture-based SureSelect XT HS protocol (Agilent, Santa Clara, CA, USA) followed by sequencing on the Illumina NextSeq 500 instrument using Mid Output chemistry v2.5 (300 cycles). The DNA-seq data were analyzed using a validated in-house bioinformatics pipeline composed of freely available tools [28].

Detected variants were prioritized according to the following criteria for filtering: (1) a variant type of either missense, nonsense, in-frame, frameshift, or splicing variant, (2) >5% VAF with ≥5 variant reads, and (3) presence in population databases (1000 genomes, gnomAD) in <1% and/or presence in cancer mutation databases (COSMIC). Recurrent variants were manually inspected through the integrative genome viewer (IGV) [29] to distinguish assay-specific artifacts, which were subsequently filtered out. Commonly used variant effect predictors (Polyphen, SIFT, Mutation Taster, Align GVGD) were used together with searching in ClinVar and HGMD databases to establish the importance of detected somatic variants.

All SNVs and short indels were then categorized into four clinical significance groups according to the international standards and guidelines [30,31]: group 1 – pathogenic, group 2 – probably pathogenic, group 3 – variants of unknown significance, and group 4 – benign or likely benign. Paired healthy tissue samples for somatic/germline status assessment were not available for the study.

Statistical analysis

Acquired data were processed using STATISTICA 13 software (StatSoft/Tibco, Krakow, Poland). Survival analyses were carried out using the Kaplan–Meier survival estimator and the Gehan–Wilcoxon test. The null hypothesis was established as no difference in progression-free survival (PFS) and OS between assessed groups. PFS was defined as time duration from the diagnosis until progression or death, whichever occurred first, with censoring at the last follow-up for living patients. OS was defined as time duration from the diagnosis until death from any cause, with censoring at the last follow-up for living patients. The Fisher exact test was used to compare categorical variables, while the Mann–Whitney *U* test was applied for continuous variables. In all statistical analyses, *p* values <.05 were considered statistically significant.

Results

Clinical characteristics and BCR-ABL1-like signature analysis

In total, 58 patients with B-other ALL were included in the final study cohort. As expected, increasing age and reduced intensity treatment were associated with an inferior outcome; the 5-year OS rates for AYA, adults and older adults were 59.1%, 38.6%, and 32.4%, respectively, $p = .01$ (Figure 2(A)). The 5-year OS rates for patients treated with intensive and reduced/palliative protocols were 53.1% and 24.6%, respectively, $p = .001$ (Figure 2(B)).

Samples from 33 patients were available for the *BCR-ABL1*-like gene expression analysis. Sixteen (48.9%) of these cases reached the prediction score −0.3 or higher and therefore were classified as *BCR-ABL1*-like ALL. There was no statistically significant difference in age, sex, treatment intensity, or SCT number between the *BCR-ABL1*-like and non-*BCR-ABL1*-like ALL groups, although the patients in *BCR-ABL1*-like ALL group tended to be younger with the median of 37 (18–62) vs. 53 (18–67) years. Incidence of *BCR-ABL1*-like expression signature in AYA, adults and older adults was 9/13 (69.2%), 3/9 (33.3%), and 4/11 (36.4%), respectively. Distribution of particular *BCR-ABL1*-like ALL subtypes is shown in Figure 3(A).

Patients with *BCR-ABL1*-like ALL were more likely to remain MRD positive before the first consolidation cycle than patients with non-*BCR-ABL1*-like ALL (50.0 vs. 13.3%, $p = .05$). Nevertheless, this did not translate to decreased survival probability. There was a trend (though statistically insignificant) toward worse PFS and OS in the *BCR-ABL1*-like ALL vs. non-*BCR-ABL1*-like ALL group (Figure 2(C,D)).

CRLF2 fusion genes analysis

Material from 27 patients was available for a *CRLF2* rearrangements FISH analysis, identifying three (11.1%) *IGH-CRLF2* positive cases. Polymerase chain reaction for *P2RY8-CRLF2* fusion gene detection was performed in 18 of these 27 patients. Additional 23 patients were tested by qRT-PCR without FISH. A total of 50 patients were evaluable for the *P2RY8-CRLF2* fusion gene presence with four (8.0%) positive results (Figure 2).

Narrowing the cohort to only *BCR-ABL1*-like ALL cases ($N = 16$), two (12.5%) harbored *P2RY8-CRLF2* and three (18.8%) *IGH-CRLF2* fusion gene. Of the non-*BCR-ABL1*-like cases ($N = 17$), two (11.8%) were *P2RY8-CRLF2* positive and none *IGH-CRLF2* positive.

Copy number variations analysis

MLPA analysis was performed in 57 patients. Both monoallelic and biallelic exon deletions or the whole *IKZF1* gene deletions were found in 13 (22.8%) cases. Although statistically insignificant, we recognized a strong trend toward a higher incidence of *IKZF1* deletions in *BCR-ABL1*-like patients compared to non-*BCR-ABL1*-like ALL cases, 31.3 vs. 5.9% ($p = .06$). Deletions in genes associated with the *IKZF1*^{plus} profile were also detected: 14 (24.6%) in *CDKN2A/B* (four of them in cases with *IKZF1* deletions) and eight (14.0%) in *PAX5* (three of them in cases with *IKZF1* deletions) [27,32]. Overall, four (30.8%) patients with *IKZF1* deletions had also a deletion in some of the *IKZF1*^{plus} profile genes. Though PSF and OS difference between *IKZF1*-deleted and non-*IKZF1*-deleted groups was not statistically significant, PFS in *IKZF1*^{plus} genotype group was significantly worse than in groups with sole *IKZF1* deletion or *IKZF1* wild-type gene ($p = .02$) (Figure 2(E,F)). Gene duplications were identified in nine (15.8%) patients, frequently multiple genes duplications overlapping in one patient. Six of nine (66.7%) duplications occurred in cases with hyperdiploidy or complex karyotype changes. We also identified several other gene deletions and duplications among the analyzed samples. Summaries of MLPA analysis results are shown in Figure 4 and Supplementary Table 2. The incidence of CNVs in particular genes is shown in Figure 3(B).

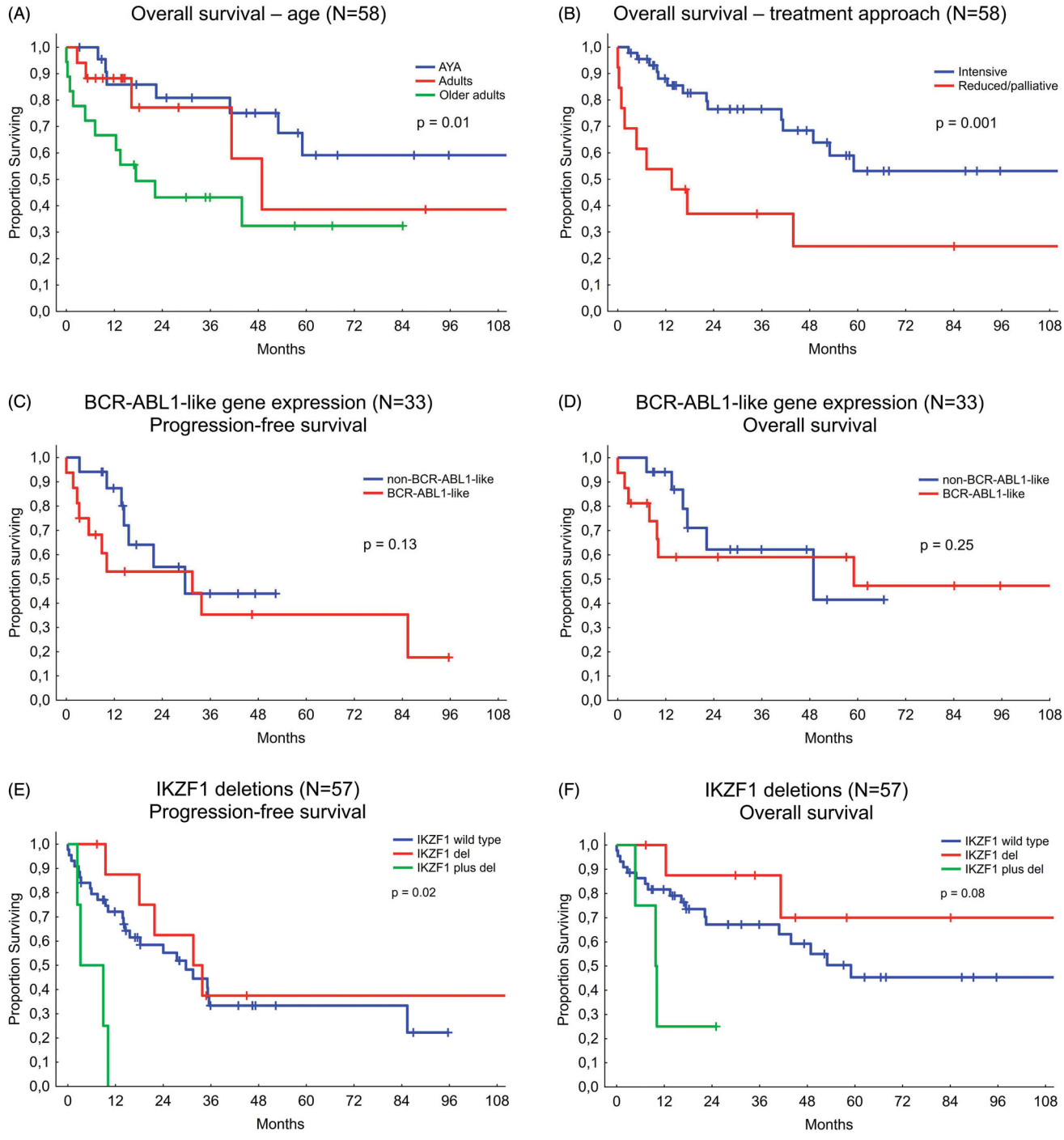

Figure 2. (A) Overall survival of the cohort based on the age at the time of diagnosis. (B) Overall survival of the cohort based on the treatment approach. (C) Progression-free survival and (D) overall survival based on *BCR-ABL1*-like ALL gene expression profiling, the method by Chiaretti et al. [26]. (E) Progression-free survival and (F) overall survival based on *IKZF1* deletion status: *IKZF1* wild-type, exon or whole *IKZF1* gene deletion and *IKZF1* deletion with adjacent deletion in some of *IKZF1*^{plus} profile genes (*CDKN2A/B, PAX5,* PAR1 region). AYA: adolescents and young adults.

Single nucleotide variations and short indel detection

The LYNX panel analysis focused on SNVs and short indels was performed in 54 patients. It identified a large number of SNVs and short indels in genes of JAK/STAT and Ras signaling pathways known to be associated with *BCR-ABL1*-like signature, namely *CRLF2, IL7R, FLT3, JAK2, KRAS, NRAS, PTPN11*, and *NF1* genes. Variants were also found in other genes associated with lymphoid malignancies, such as *TP53, KMT2A, IKZF1/2, PAX5, CDKN2B, BRAF, CERBBP, MYC*, or *PTEN*.

Forty-four (81.5%) patients harbored one or more SNVs or short indels. Total of 99 gene variants were identified, 39 (39.4%) of them with presumed pathogenic or probably pathogenic effect on phenotype. Twenty-one (21.2%) pathogenic or probably pathogenic SNVs and short indels in 18 patients were found in genes associated with *BCR-ABL1*-like ALL signature.

Moreover, 75 (75.8%) of total 99 variants have not yet been described in BCP-ALL, and 23 (23.2%) of them have not

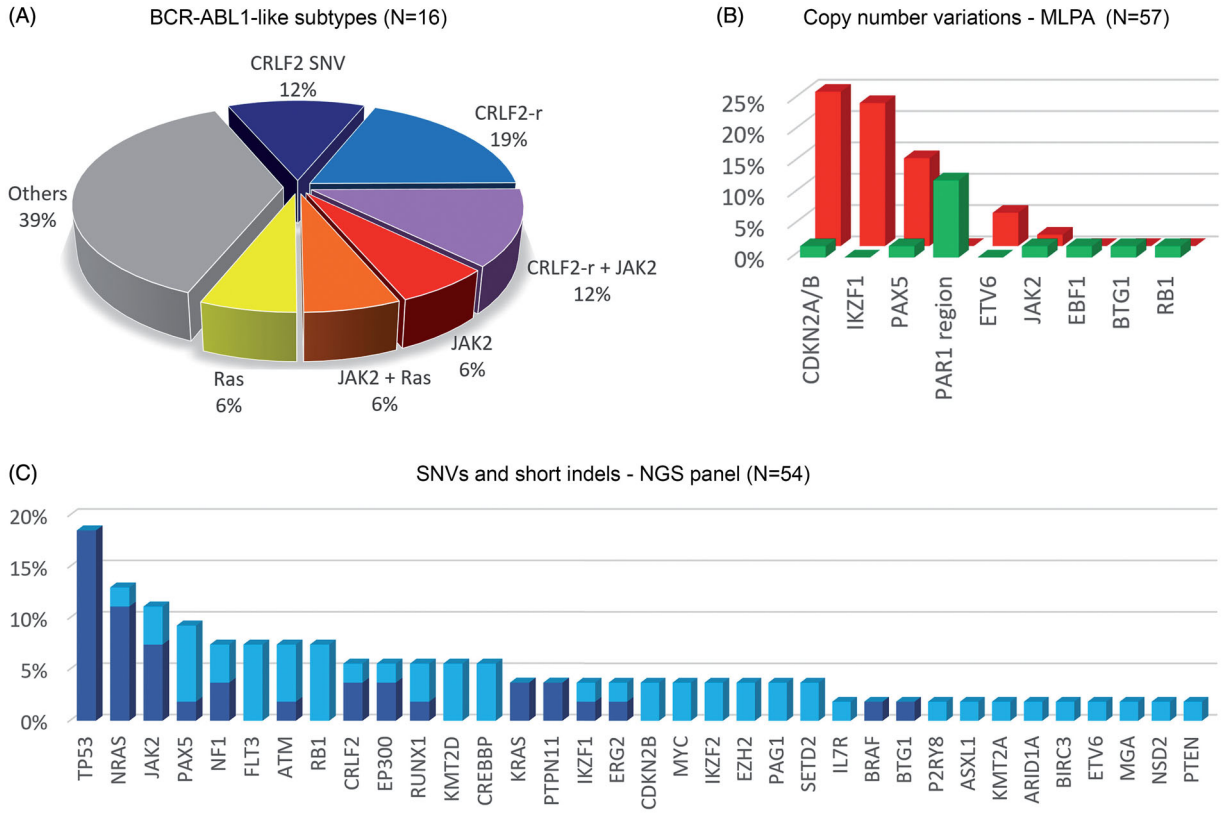

Figure 3. (A) Distribution of class-subtypes among the 16 detected *BCR-ABL1*-like ALL cases. (B) Incidence of CNVs in the cohort analyzed by MLPA. Deletions are shown in red, duplications in green. (C) Incidence of short gene variants in the cohort analyzed by the NGS panel. Pathogenic or probably pathogenic variants (groups 1 and 2) are shown in dark blue, variants of unknown significance and benign or likely benign variants (groups 3 and 4) are shown in light blue. CNVs: copy number variations; CRLF2 SNV: single nucleotide variants of CRLF2; CRLF2-r: CRLF2-rearrangements; MLPA: multiplex ligation-dependent probe amplification; NGS: next-generation sequencing; Ras: Ras kinase family single nucleotide variants (NRAS, KRAS).

been indexed in online genetic databases to this date. The graphical summary of detected variants is shown in Figure 4 and their incidence in our cohort in Figure 3. Detailed NGS analysis results are attached in Supplementary Tables 3 and 4.

Discussion

The main aim of our study was to retrospectively map spectrum and frequency of genetic aberrations in AYA and adult B-other ALL patients with a focus on *BCR-ABL1*-like ALL subtype and to correlate the results with patients' survival outcomes. Though the genomic landscape of adult B-other and *BCR-ABL1*-like ALL has been already widely studied and described by many authors [16–21,33,34], there had not been such study performed in the cohort of Czech adult patients. A similar study was performed in an exome-wide and transcriptome-wide manner in Czech pediatric patients [27]. Our study did not cover other less frequent *BCR-ABL1*-like ALL subtypes, such as *ABL*-class, *JAK2*, and *EPOR* rearrangements.

The incidence of *BCR-ABL1*-like expression in our B-other ALL cohort was similar to the previous adult patient studies after the exclusion of cases with *BCR-ABL1*, *KMT2A* rearrangements, *ETV6-RUNX1*, *TCF3-PBX1*, and iAMP21 (19.7–55.7%) [16–21,26]. A survival detriment in *BCR-ABL1*-like vs. non-*BCR-ABL1*-like cases was not shown with sufficient statistical strength in our cohort. A combination of reasons could play a role in this observation. First, a small number of studied cases and relatively short follow-up of our cohort would attenuate a statistical strength of the observed survival difference between the *BCR-ABL1*-like and non-*BCR-ABL1*-like groups.

Second, most studies showing the dismal survival in adult *BCR-ABL1*-like ALL patients were performed in cohorts with a median age of 28–42 years [16,17,19–21,26]. However, the study of Tasian et al. analyzing a smaller cohort of older BCP-ALL patients with a median age of 43 years did not show the survival detriment of *BCR-ABL1*-like group with sufficient statistical strength [18]. In all these studies, the median age was always similar in both the *BCR-ABL1*-like and non-*BCR-ABL1*-like groups. The median age in our cohort was 44 years with a relatively distinctive, though not statistically significant difference between both groups; 37 years in *BCR-ABL1*-like and 53 years in non-*BCR-ABL1*-like group. Therefore, we could assume that the higher age of the whole cohort and the control group in particular could counteract an anticipated survival benefit of the non-*BCR-ABL1*-like group.

Finally, three (18.8%) of the *BCR-ABL1*-like patients were treated with a trial protocol in which the first induction phase of a pediatric-inspired chemotherapy backbone was replaced by one cycle of blinatumomab (NCT04554485). Our preliminary unpublished data show promising results in both early MRD clearance and OS in patients treated with this protocol. Several other clinical trials implementing

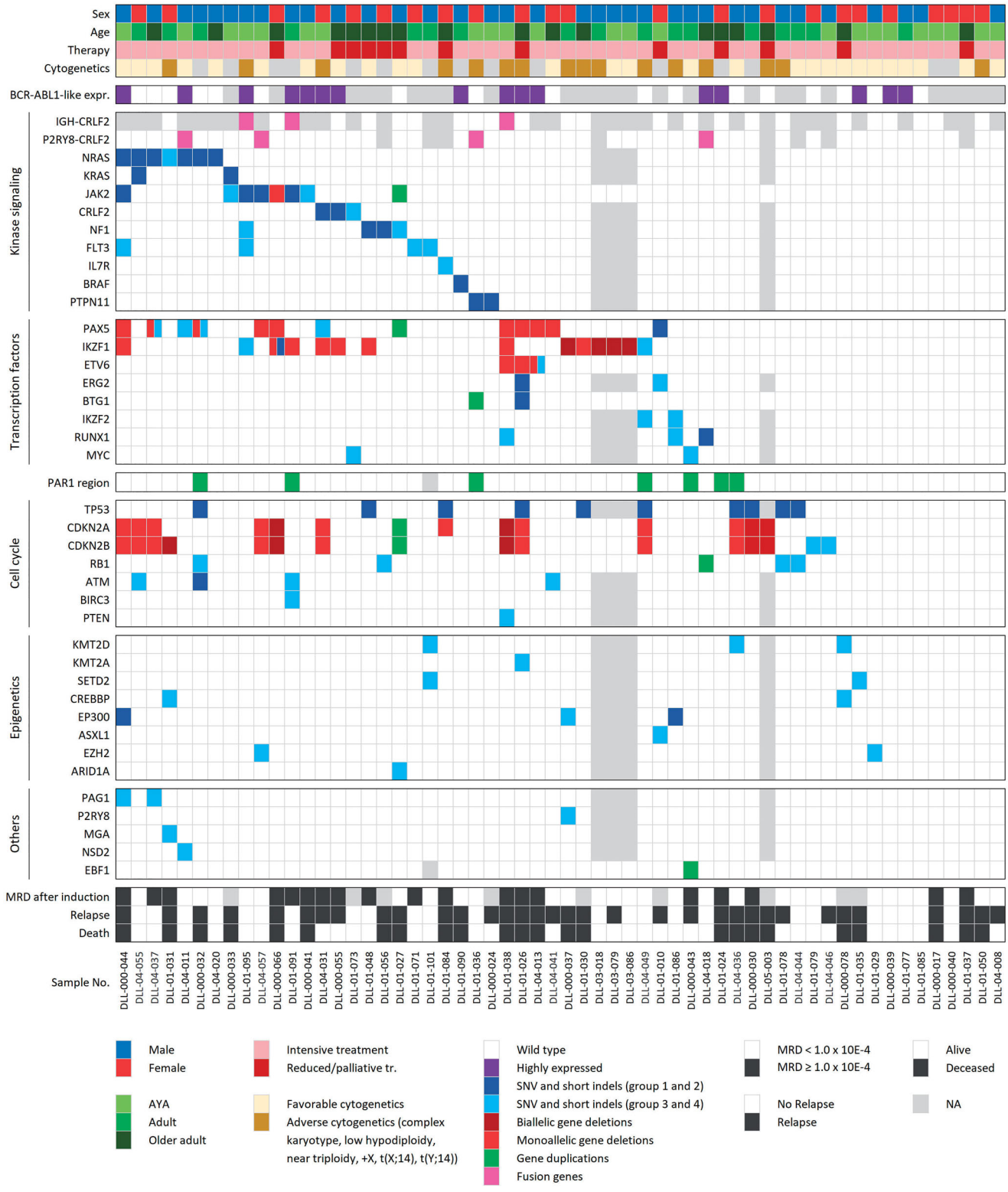

Figure 4. Graphical overview of the genetic aberrations identified in the study cohort. Data are shown for 58 BCP-ALL patients with available genetic material or cell samples. Gene aberrations are divided into functional categories. AYA: adolescents and young adults; MRD: minimal residual disease; NA: not available; SNV: single nucleotide variants.

blinatumomab and inotuzumab ozogamicin into the frontline therapy of Philadelphia-negative BCP-ALL have recently been completed or are still ongoing. The study by Kantarjian et al. [35] has been the only one published in detail to date; unfortunately, the paper does not address the impact of frontline targeted therapy on *BCR-ABL1*-like ALL subtype in particular. Other two completed trials have been published in summary only as congress abstracts yet [36,37]. Several other clinical trials with frontline blinatumomab or inotuzumab ozogamicin are currently ongoing (NCT03249870, NCT03150693,

NCT03480438, NCT03709719, NCT03367299, NCT04554485). Although the efficacy of monoclonal antibodies in frontline treatment of *BCR-ABL1*-like ALL subgroup is yet unclear, it is presumable that this novel treatment approach will improve survival of *BCR-ABL1*-like ALL patients compared to standard chemotherapy regimens. This could theoretically further diminish the survival difference between *BCR-ABL1*-like and non-*BCR-ABL1*-like groups in our own study.

Rearrangements of *CRLF2* were found in fewer *BCR-ABL1*-like cases than expected from previous adult studies (31.3 vs. 49.7–77.8%) [16–18,20,21]. This could be due to the small sample size as only few *CRLF2*-rearranged cases were detected. Additional mutations in *CRLF2*-rearranged patients of our cohort include deletions and SNVs in *IKZF1, PAX5, JAK2, CDKN2A/B*, and other genes (Figure 4) in concordance with previously published data [15–17,20,21,33,38]. While *IGH-CRLF2* fusion was detected exclusively in *BCR-ABL1*-like ALL patients, two *P2RY8-CRLF2* cases were detected among patients without *BCR-ABL1*-like gene expression profile. One of the possible explanations could be false negativity in accordance with 88.5% method sensitivity reported by the authors [26]. However, we assume that this finding was a consequence of the fact that *P2RY8-CRLF2* was present only in minor subclones in these patients, and therefore the low *CRLF2* expression exhibited a negative gene expression test result. A number of *P2RY8-CRLF2* cases with negative *BCR-ABL1*-like gene expression profile have been described before by other authors and comprise 20.1–26.5% of all *P2RY8-CRLF2* cases [16,20,35]. Interestingly, no *IGH-CRLF2* case with negative *BCR-ABL1*-like gene expression pattern has been described in those cohorts. We are generally cautious to mark this type of patients as *BCR-ABL1*-like because these minor *P2RY8-CRLF2* subclones may not unconditionally translate into worse survival rates and other typical clinical features of *BCR-ABL1*-like ALL. Both *P2RY8-CRLF2* non-*BCR-ABL1*-like patients in our cohort had a favorable disease course with complete molecular response and no relapse. Herold et al. reported also two similar patients with favorable outcome [16]. However, further data on the clinical outcome of these patients in larger cohorts are lacking. CNVs analysis identified a number of both monoallelic and biallelic deletions and several duplications in *IKZF1, PAX5, CDKN2A/B, ETV6, JAK2*, and other genes. These aberrations occurred more frequently in *BCR-ABL1*-like ALL cases in concordance with other studies [16,27,34]. With small cohort limitations in mind, we showed significantly dismal survival in patients with *IKZF1*^{plus} genotype (deletion in *IKZF1* with concurrent deletion in *PAX5, CDKN2A/B*, or PAR1 region) compared to patients with sole *IKZF1* deletion or *IKZF1* wild type. Though this phenomenon was described by Stanulla et al. and confirmed by Zaliova et al. in a pediatric BCP-ALL cohort [27,32], it have not yet been analyzed in the adult BCP-ALL to our knowledge. Further studies on larger cohorts are warranted to confirm the prognostic impact of *IKZF1*^{plus} profile in adult patients with BCP-ALL.

We believe the main contribution of our study lies in the results of targeted NGS analysis, since it is the first such study performed in the Czech adult B-other ALL patients. Majority of SNVs and short indels identified in our cohort has not yet been described in BCP-ALL, and many of them have not yet been listed in gene mutation databases. Among them, several novel gene variants were categorized as pathogenic or probably pathogenic, namely in the *TP53, NRAS*, and *JAK2* gene. The LYNX panel has recently been introduced into our laboratory practice and represents a versatile tool for the simultaneous analysis of genomic markers (gene variants, chromosomal aberrations, translocations, and IG/TR rearrangements) in lymphoid malignancies [28]. It was designed to meet specific demands at our workplace considering analysis turnaround time, comprehensive testing, expenses per sample, etc. On the other hand, we are aware that the LYNX panel is limited to genome areas where the genetic variations have been already described and is not primarily focused on BCP-ALL aberrations. However, the LYNX panel is suitable for the characterization of most SNVs and short indels associated with *BCR-ABL1*-like ALL phenotype with a high level of sensitivity and reproducibility, although it provides only confirmatory results.

Whole-exome sequencing (WES), whole-genome sequencing (WGS), transcriptome sequencing, and SNP microarrays are performed in many large international hospitals to identify the class-type of *BCR-ABL1*-like ALL [20,21,27,33,34]. Notably, WGS represents an especially suitable yet expensive method for exploratory identification of new genetic aberrations. Another possibility is to employ available commercial NGS panels, e.g., amplicon capture-based panel Haloplex (Agilent, Santa Clara, CA, USA) or the comprehensive cancer panel Ion Ampliseq (Thermo Fisher Scientific, Waltham, MA, USA). Nevertheless, custom targeted NGS panels are frequently designed for specific purposes, such as combined RNA/DNA profiling in the FoundationOne Heme panel [39] or a comprehensive NGS assay for SNV, CNV, and translocations associated with ALL by Kim et al. [40]. Recently described a comprehensive custom NGS panel by Montaño et al. focused on BCP-ALL patients also offers new possibilities for improved stratification of BCP-ALL patients and more detailed analysis of *BCR-ABL1*-like ALL subgroup [41]. Although LYNX panel has only recently been introduced into our laboratory practice, our experience to date suggests that this targeted NGS panel represents a universal NGS tool for the simultaneous analysis of various genomic markers in lymphoid malignancies.

One of the aims of our study was to establish standards for fast routine *BCR-ABL1*-like profile testing of newly diagnosed adult B-other ALL patients in Czechia, including identification of the most common *BCR-ABL1*-like subtypes. Although through the recruitment of human and laboratory resources, such genetic testing of adult B-other ALL patients is feasible in Czechia, we believe that in the upcoming period it will not become a part of our routine clinical practice, for several reasons.

First, in our study, we did not observe the existence of a significant survival disadvantage for *BCR-ABL1*-like ALL patients. In our relatively small study, we detected that *BCR-ABL1*-like ALL patients have a tendency toward persistent MRD as observed previously in a German cohort [16]. Since

that the application of more intensive therapy, including SCT, is currently driven foremost by MRD response in each patient individually [6,42–45], we see no reason for routine precise genetic profiling and characterization of *BCR-ABL1*-like ALL patients.

Second, prospective randomized data on the efficacy of targetable *BCR-ABL1*-like-associated kinases inhibitors are still lacking [24,46–50]. Inhibition of ABL-class gene fusions (*ABL1/2, CSF1R, PDGFRB*) is one of the possible targeted treatment approaches, since approximately 9.8–14.0% of *BCR-ABL1*-like ALL patients harbor these genetic alterations [20,21,51]. Few case reports have shown successful use of ABL-directed tyrosine kinase inhibitors (TKIs) imatinib, dasatinib, and ponatinib in *PDGFRB*-rearranged or *ABL1*-rearranged (non-*BCR-ABL1*) ALL patients [52–56]. Two large prospective clinical trials are currently testing the efficacy of TKI in pediatric patients with ABL-class rearrangements (NCT02883049, NCT03117751) and two studies are ongoing in adult patients (NCT02143414, NCT02420717). A number of genetic alterations associated with *BCR-ABL1*-like profile result into the activation of JAK/STAT signaling pathway; namely mutations and rearrangements of genes *CRLF2, JAK1/2/3, IL7R, IL2RB, EPOR*, or *SH2B3* comprising 70.6–77.7% of *BCR-ABL1*-like ALL cases [18–21,51]. Several preclinical studies, case reports, and early phase clinical trials have shown the feasibility of adding *JAK2* inhibitors ruxolitinib, CHZ868 or AZD1480 to chemotherapy regimens in these patients [57–61]. Ongoing clinical trials in both pediatric and adult patients are investigating this therapeutic approach (NCT03117751, NCT02420717, NCT03571321, NCT02723994). Results of the listed clinical trials are highly warranted since off-label use of these drugs outside a trial setting remains problematic. Preclinical data or case reports have also been published on several other targeted drugs tested in *BCR-ABL1*-like ALL setting; e.g., Trk inhibitor larotrectinib, mTOR inhibitors sirolimus and everolimus, PI3K/mTOR inhibitor gedatolisib, *HSP90* inhibitor PU-H71, *SYK* inhibitors fostamatinib and PRT318, *MAP2K* inhibitor trametinib or Akt inhibitor MK2206 [62–67].

Finally, all available data on the dismal prognosis of *BCR-ABL1*-like ALL patients have risen from a setting of classic chemotherapeutic treatment protocols, adult or pediatric-inspired ones [15–21,26]. However, the vast majority of our patients is no longer treated by standard chemotherapeutic induction regimens since we have recently introduced anti-CD19 and anti-CD22 monoclonal antibodies into Philadelphia-negative BCP-ALL first-line treatment in Czechia (NCT04554485, NCT03249870). The impact of these novel monoclonal antibody-based frontline therapies on *BCR-ABL1*-like ALL is yet unclear and was already discussed in this paper. Based on the above, currently we see no rational reason for the introduction of *BCR-ABL1*-like profile testing in adult B-other ALL patients in routine clinical practice in Czechia. We assume that this testing will gain importance in the future as studies with novel drugs are completed. In conclusion, our study corroborates some of the previously published data on the genomic landscape of *BCR-ABL1*-like and B-other ALL, but also presents several new findings. We showed a survival detriment in adult BCP-ALL patients with the *IKZF1*^{plus} genetic profile. With the use of novel custom NGS panel, we identified a number of entirely novel SNVs and short indels in BCP-ALL not previously described. Although the clinical benefit of routine *BCR-ABL1*-like ALL testing in B-other ALL patients remains unclear from our point of view, we encourage others to perform such testing or sample banking in a prospective manner to facilitate similar analyses in future clinical settings.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Czech Leukemia Study Group for Life (CELL); the Technology Agency of the Czech Republic under the grant TACR TN01000013; the Ministry of Health of the Czech Republic under the project AZV NV19-03-00091 and the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 LQ1601 and the grant MUNI/A/1595/2020.

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