ARTICLE

ACUTE LYMPHOBLASTIC LEUKEMIA

Minimal residual disease in BCR::ABL1-positive acute lymphoblastic leukemia: different significance in typical ALL and in CML-like disease

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Recently, we defined "CML-like" subtype of BCR::ABL1-positive acute lymphoblastic leukemia (ALL), resembling lymphoid blast crisis of chronic myeloid leukemia (CML). Here we retrospectively analyzed prognostic relevance of minimal residual disease (MRD) and other features in 147 children with BCR::ABL1-positive ALL (diagnosed I/2000–IV/2021, treated according to EsPhALL ($n = 133$) or other ($n = 14$) protocols), using DNA-based monitoring of BCR::ABL1 genomic breakpoint and clonal immunoglobulin/T-cell receptor gene rearrangements. Although overall prognosis of CML-like ($n = 48$) and typical ALL ($n = 99$) was similar (5-year-EFS 60% and 49%, respectively; 5-year-OS 75% and 73%, respectively), typical ALL presented more relapses while CML-like patients more often died in the first remission. Prognostic role of MRD was significant in the typical ALL ($p = 0.0005$ in multivariate analysis for EFS). In contrast, in CML-like patients MRD was not significant (p values > 0.2) and inapplicable for therapy adjustment. Moreover, in the typical ALL, risk-prediction could be further improved by considering initial hyperleukocytosis. Early distinguishing typical BCR::ABL1-positive ALL and CML-like patients is essential to enable optimal treatment approach in upcoming protocols. For the typical ALL, tyrosine-kinase inhibitors and concurrent chemotherapy with risk-directed intensity should be recommended; in the CML-like disease, no relevant prognostic feature applicable for therapy tailoring was found so far.

Leukemia (2022) 36:2793–2801; https://doi.org/10.1038/s41375-022-01668-0

INTRODUCTION

BCR::ABL1 fusion gene is a hallmark of chronic myeloid leukemia (CML), and it is also found in acute lymphoblastic leukemia (ALL). In childhood, CML and BCR::ABL1-positive ALL are relatively rare and have similar incidence (close to one per million), increasing with age (particularly for CML) [1, 2].

By conventional diagnostic criteria BCR::ABL1-positive ALL is indistinguishable from CML in lymphoid blast crisis (LBC). However, while the treatment strategy for the BCR::ABL1-positive ALL is generally uniform (tyrosine-kinase inhibitors [TKI] with an intensive chemotherapy backbone), therapeutical approach to LBC-CML is less strictly defined. It is always based on TKI with variable intensity of concurrent chemotherapy, and usually heads towards stem cell transplantation [1, 3].

Approach to the minimal residual disease (MRD) monitoring varies according to diagnosis, age and local established practice. In CML, the Major-BCR::ABL1 fusion transcript (encoding p210 protein) is almost exclusively expressed and its levels are assessed by quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR); an absolute quantification of transcript copies is used and an "international scale" is employed, enabling to compare the MRD levels between different laboratories [4]. In ALL, quantification of clonal immunoglobulin (IG) and T-cell receptor (TR) genes rearrangements at the DNA level or monitoring of leukemia-associated immunophenotype by flow cytometry are usually considered a golden standard, particularly in children [5, 6]; however, in some adult ALL protocols, the qRT-PCR targeted to either Major- or minor-BCR::ABL1 fusion transcript (the latter,

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Received: 14 April 2022 Revised: 21 July 2022 Accepted: 22 July 2022
Published online: 6 August 2022

encoding p190 protein, being more prevalent in ALL) is used [7]. In ALL, the MRD level is usually assessed as relative to diagnosis, that is considered as a level of 100%. Although MRD clearance during early treatment stages is considered among the most important prognostic features in ALL in general [8], its prognostic impact in the BCR::ABL1-positive ALL is less clear, particularly in the era of TKI treatment [5].

In our previous work [9, 10], we used patient-specific genomic BCR::ABL1 fusions and IG/TR rearrangements for qPCR MRD monitoring (both at the DNA level, to eliminate drawbacks related to the high variability of BCR::ABL1 m-RNA expression both at diagnosis and during TKI treatment). We showed that in 20–25% of patients the MRD levels are discordant, with prolonged BCR::ABL1-positivity, and we demonstrated that in these cases the BCR::ABL1 fusion is present in a wider clone, involving myeloid cells, non-ALL B cells, and T cells. For similarity with LBC-CML we named these leukemias "CML-like" [9], distinct from"typical (BCR::ABL1-positive) ALL" (Fig. 1).

In the present study we analyzed MRD levels at early treatment timepoints using two DNA-based approaches (targeting IG/TR clonal rearrangements and BCR::ABL1 genomic fusion) in a large cohort of BCR::ABL1-positive childhood ALL. Based on concordance/discordance of the MRD levels we determined "typical ALL" and "CML-like" patients and we assessed predictive value of MRD and other features in these two subgroups.

METHODS

Patients and samples

This study included 147 patients diagnosed with BCR::ABL1-positive childhood ALL in the Czech Republic ($n = 29$), Germany ($n = 88$), and Italy ($n = 30$) between January 2000 and April 2021, and treated according to EsPhALL (2004, 2010 or 2017) [8, 11, 12]; ($n = 133$) or other ($n = 14$) protocols. Imatinib was used as TKI in the EsPhALL protocols, starting at day 33 (EsPhALL 2004) or day 15 (EsPhALL 2010, 2017). For details on patients and treatment protocols see Supplementary Tables 1, 2. Median follow-up was 58 months. Standard diagnostics (including cytogenetics/FISH and/or RT-PCR) were performed according to the practice of local diagnostic laboratories, and the minor-/Major-BCR::ABL1 fusion transcripts were identified. Half of the patients ($n = 70$) had sufficient material to be analyzed for the deletions of IKZF1 gene. Diagnostic and treatment procedures and protocols were approved by the local institutional review boards. Informed consent was obtained in accordance with the Declaration of Helsinki.

Bone marrow samples for MRD monitoring were collected according to the treatment protocol. MRD levels in 364 bone marrow samples from three early treatment timepoints (day 15 of treatment [D15; $n = 86$]; end of induction IA, day 33 [TP1; $n = 136$]; end of consolidation IB, week 12 [TP2; $n = 142$]) were available. In 343/364 samples (94%) both targets (IG/TR and BCR::ABL1 genomic fusion) were assessed (D15, $n = 74$; TP1, $n = 128$; TP2, $n = 141$). In some patients, MRD levels in additional follow-up samples were taken into account for subtype assessment (typical ALL vs. CML-like).

IG/TR and BCR::ABL1 MRD quantification

Quantification of patient-specific IG/TR rearrangements was performed and interpreted according to the standards of the EuroMRD international network [6]. BCR::ABL1 genomic breakpoints were detected either by multiplex long-distance PCR [9] or by capture-based target enrichment sequencing using SureSelectXT technology with custom probes design (Agilent Technologies, Santa Clara, CA, USA). Primers amplifying the fusion sequence and fluorescein/tetramethylrhodamine-labeled probe (preferentially covering the breakpoint sequence) were designed for MRD monitoring.

The MRD levels were measured relative to the respective diagnostic sample, which was set to 1 (100%). For comparison of BCR::ABL1 vs. IG/TR MRD levels (including definition of CML-like patients), real measured MRD values were used. For categorization of MRD levels for survival analyses, the MRD level was rounded to the nearest log dilution (i.e., for example MRD 1×10^{-3} comprises all cases with the measured MRD level $\geq 5 \times 10^{-4}$ and $< 5 \times 10^{-3}$). For graphical presentation of MRD results, in accord with the EuroMRD international network convention, samples with non-quantifiable positivity (below quantitative range [QR]) were assigned an arbitrary level of 1×10^{-6}. Negative samples were assigned a level of 1×10^{-8}.

To establish a prognostic significance of MRD, the threshold $<10^{-3}$ vs. $\geq 10^{-3}$ was mostly used as a standard; however, due to high MRD levels particularly at the TP1, an alternative threshold $<10^{-2}$ vs. $\geq 10^{-2}$ was used to enable more reasonable distribution of patients (i.e., to prevent accumulation of vast majority of patients into one of the two groups) in some analyses.

Quantification of the ALB gene by qPCR was performed to measure the DNA concentration and normalize the MRD results.

Definition of discordant MRD and of CML-like disease

MRD results were scored as concordant when the variation between IG/TR and BCR::ABL1 levels was ≤1 log. Moreover, although this might artificially increase the number of concordant samples, we considered two samples concordant if (A) one target was quantifiable at a level <1 log above QR/sensitivity of the other target and the other target was non-quantifiably positive/negative, respectively; or (B) one target was non-quantifiably positive, whereas the other target was negative, and sensitivity of the former was higher, equal or <1 log lower than the sensitivity of the latter.

In accord with the original definition [9], patients with more than one discordant MRD sample were classified as CML-like. Moreover, mainly due to a low number of analyzed timepoints with measurable MRD in some cases, the definition of the CML-like disease was extended within this study and we considered as CML-like also patients with only one clearly discordant MRD sample with quantifiable BCR::ABL1 level higher by >1 log than IG/TR, if not followed by a sample with IG/TR level higher than BCR::ABL1 (see Results).

Statistical analysis

Event-free survival (EFS) and overall survival (OS) were calculated from diagnosis to first failure (death/relapse/second malignant neoplasm) or to death, respectively. Rates were calculated according to Kaplan–Meier and compared by log-rank test [13, 14]. Kaplan–Meier plots that compared transplantation with chemotherapy were adjusted to account for the waiting time to transplantation (with a landmark at median time to transplantation). Differences between MRD levels of two patient groups were analyzed by Mann–Whitney test, binary data were compared by chi-square or Fisher's exact test. For multivariate analysis, a Cox proportional hazards models was constructed for EFS and OS and the model selection was performed using the Akaike Information Criterion (AIC). The results of the best model (based on AIC) are presented.

RESULTS

Concordance of MRD approaches and subgroup assessment

We classified 36 patients as CML-like according to the original definition (>1 discordant MRD sample). Moreover, additional 12 patients with only one discordant sample were assigned to the CML-like subgroup based on the extended definition used within this study (see Methods). To prevent any possible bias caused by this extension, analyses concerning the CML-like subgroup were performed both with and without these 12 patients (see Supplementary Figures). Patients with concordant course of MRD who did not fulfill the extended CML-like criteria ($n = 99$) were classified as typical BCR::ABL1-positive ALL. Only 2/240 samples analyzed in these 99 patients had BCR::ABL1 level > 1 log higher than IG/TR. Both were D15 samples and in both patients, the subsequent sample showed quantifiable IG/TR and negative BCR::ABL1 substantiating their assignment to the typical ALL (Supplementary Fig. 1). Distribution of the Major-BCR::ABL1 variant (p210) was identical between the CML-like and typical ALL patients (9/48 = 19% and 17/99 = 17%, respectively).

The highest proportion of discordant samples in CML-like subtype was seen at TP1 (38/45; 84%). Of the 44 CML-like patients analyzed at both TP1 and TP2, 40 (91%) were discordant at least at one of the two timepoints, 26 (59%) were discordant at both (Fig. 2 and Supplementary Fig. 2A).

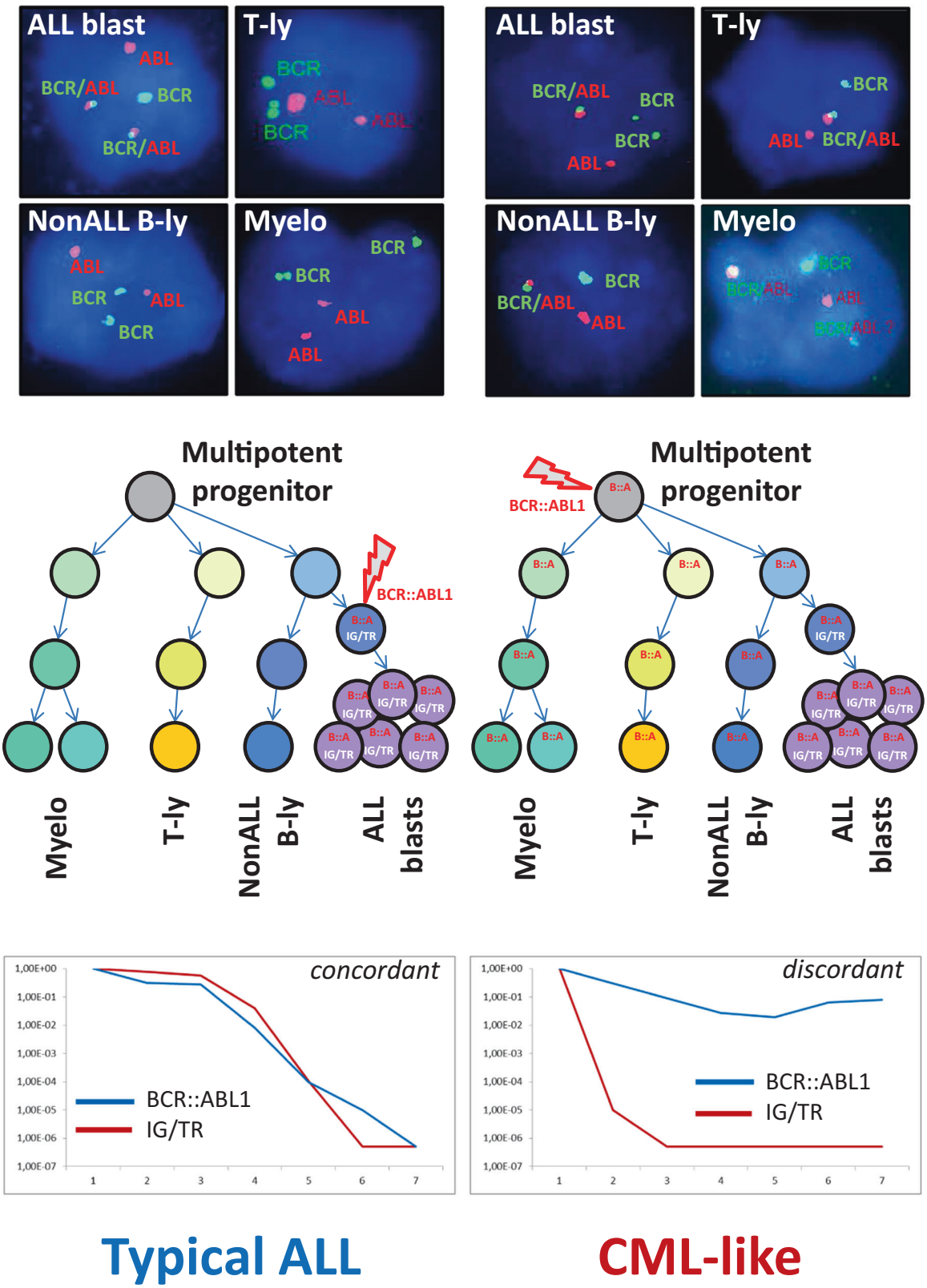

Fig. 1 Schematic illustration of key differences between "Typical ALL" and "CML-like" disease. Illustrative scheme showing the crucial differences between typical BCR::ABL1-positive ALL and CML-like disease in FISH, lineage/clonal involvement and MRD course measured by IG/TR and BCR::ABL1 DNA-based approach. Myelo myeloid lineage, T-ly T-lymphoid lineage, NonALL B-ly B-lymphoid lineage outside the ALL clone, B::A BCR::ABL1, IG/TR leukemia-specific immunoreceptor gene clonal rearrangement.

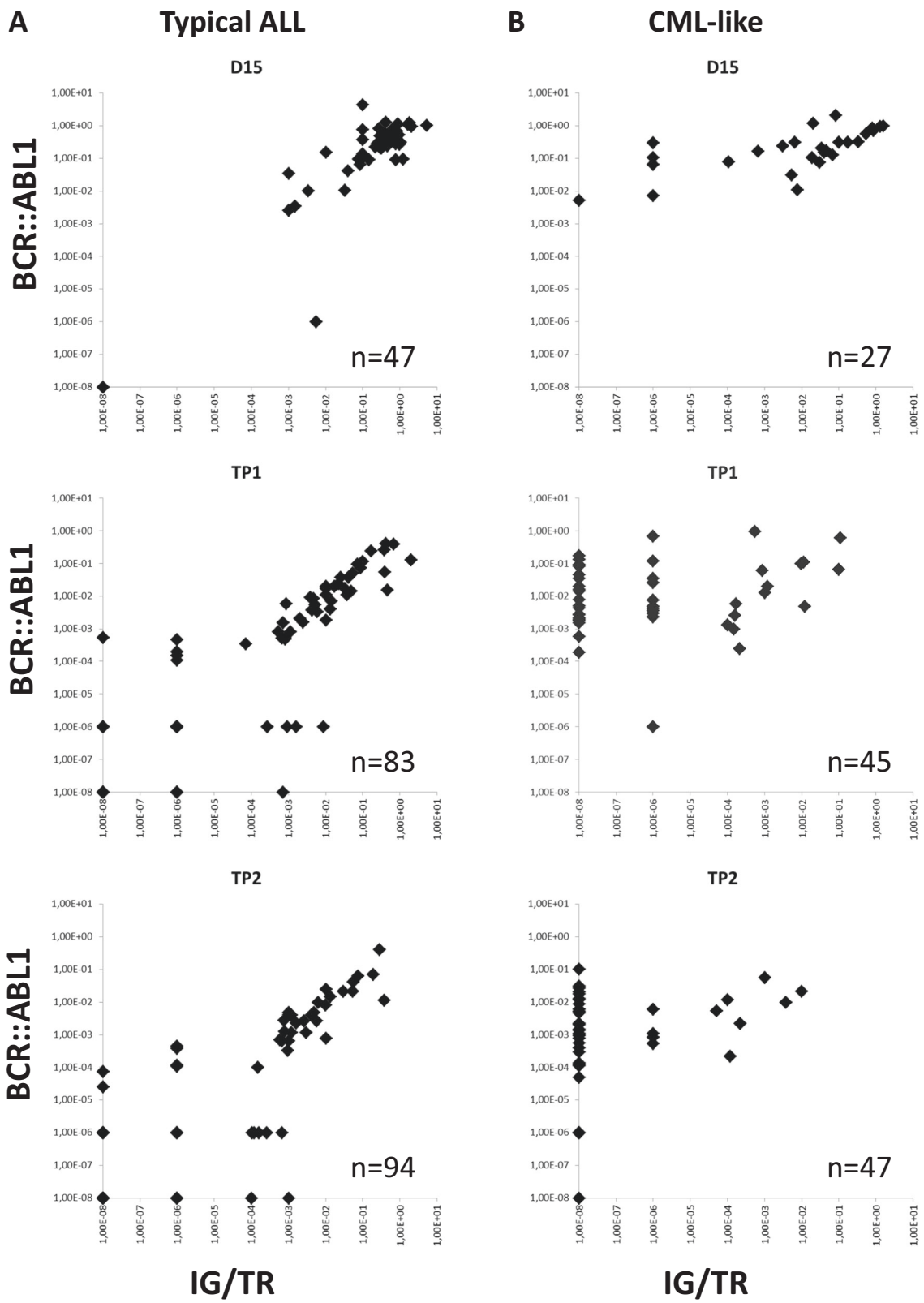

Fig. 2 Overall comparison of MRD levels by IG/TR and BCR::ABL1 DNA-based monitoring. MRD levels in 224 samples (D15, $n = 47$; TP1, $n = 83$; TP2, $n = 94$) of typical ALL patients (**A**) and 119 samples (D15, $n = 27$; TP1, $n = 45$; TP2, $n = 47$) of CML-like patients (**B**). Non-quantifiably positive samples are shown as having MRD level 10^{-6}; negative samples are shown as 10^{-8}. All patients included in the study had at least two samples analyzed by both targets, at least one of those being from TP1/TP2.

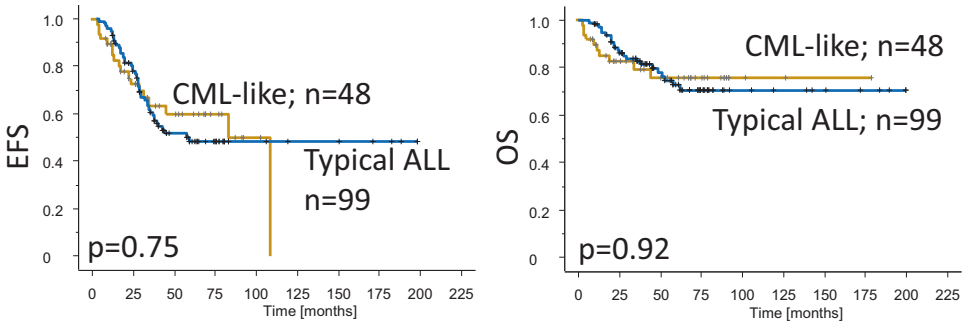

Fig. 3 Outcome of patients according to the subtype. EFS and OS of all patients according to subtype. CML-like in yellow, typical ALL in blue.

Overall prognosis according to subtype and the effect of treatment

Overall, the CML-like ($n = 48$) and typical ALL ($n = 99$) had similar prognosis (5-year-EFS 60 ± 8% and 49 ± 6%, respectively; 5-year-OS 75 ± 7% and 73 ± 5%, respectively) (Fig. 3 and Supplementary Fig. 2B). In accord with our previous data we still see a tendency to better EFS in transplanted CML-like patients (5-year-EFS 81 ± 10% vs. 47 ± 11%; $p = 0.06$; Supplementary Fig. 3A, B; Supplementary Table 3), however, the difference did not reach a significant level, possibly due to a low number of CR1 transplanted CML-like patients (15/48 of CML-like vs. 49/99 of typical ALL). Similarly, the CML-like patients transplanted in CR1 had a seemingly better outcome than CR1 transplanted typical ALL (3 events in 15 CML-like patients vs. 22 events in 49 typical ALL patients; Supplementary Fig. 3C, D), however, in the multivariate analysis (see below), the positive prognostic effect of transplantation was more pronounced in the typical ALL patients ($p = 0.055$ for EFS in CML-like and $p = 0.0003$ and $p = 0.028$ for EFS and OS, respectively, for typical ALL; see Supplementary Table 3).

The rate of deaths in CR1 was substantially higher in CML-like patients (6/48 = 13%; 1/6 after transplantation, vs. 3/99 = 3% of typical ALL; 2/3 after transplantation) ($p = 0.059$ for CR1 deaths in total, $p = 0.034$ for CR1 deaths in non-transplanted patients and $p = 0.011$ for CR1 deaths out of the total deaths). Median survival of the six CML-like patients and the three typical ALL patients lost in CR1 was 5 and 14 months, respectively.

The sites of relapse were similar in typical ALL and CML-like patients (21/11/10 and 7/3/2 of medullary/extramedullary/combined relapses, respectively). Interestingly, the CML-like patients did not show a markedly poor outcome after relapse (patients alive after relapse were 8/12 CML-like vs. 22/42 typical ALL).

MRD levels at TP1/TP2 and outcome

Typical ALL patients with very high MRD level at TP1 ($\geq 10^{-2}$) had worse EFS, with borderline statistical (non-)significance (BCR::ABL1 as target: 5-year-EFS 52 ± 7% vs. 32 ± 9%; $p = 0.18$; IG/TR as target: 5-year-EFS 56 ± 7% vs. 33 ± 8%; $p = 0.033$). In the CML-like subtype, no prognostic significance of any MRD level measured by any of the targets was found (Fig. 4A, Supplementary Fig. 2C).

At TP2, the typical ALL showed a negative prognostic impact of high MRD ($\geq 10^{-3}$) by both targets, for both EFS and OS (BCR::ABL1: 5-year-EFS 58 ± 7% vs. 33 ± 9%; $p = 0.015$ and 5-year-OS 83 ± 6% vs. 51 ± 10%; $p = 0.0012$; IG/TR: 5-year-EFS 57 ± 7% vs. 36 ± 9%; $p = 0.034$ and 5-year-OS 82 ± 6% vs. 53 ± 9%; $p = 0.0018$, respectively; EFS shown in Fig. 4B). Particularly, the typical ALL patients with very high MRD $\geq 10^{-2}$ (11/94 patients according to the BCR::ABL1 and 13/95 according to the IG/TR quantification; Fig. 4C) had a very poor prognosis with EFS < 20% and OS < 50%; neither of the targets showed prognostically relevant information in the CML-like subtype (Supplementary Fig. 2C).

Importantly, at least some of the patients with poor early response (MRD at TP1 $\geq 10^{-2}$) could have been successfully rescued by transplantation in the first complete remission (CR1) (Supplementary Fig. 4). Thus, to prevent a possible bias, we also analyzed only patients not transplanted in CR1 (Supplementary Fig. 5). The data show again a strong prognostic impact in the typical ALL and no significant value in the non-transplanted CML-like patients.

Notably, while CML-like patients expectedly had slower clearance of BCR::ABL1-positive cells, they showed a significantly faster clearance of MRD by IG/TR quantification than typical ALL patients ($p < 0.001$; Fig. 5).

Prognostic impact of other biological features

The incidence of IKZF1 deletion was significantly higher in the typical ALL compared to CML-like patients (32/42 [76%] vs. 12/28 [43%], respectively; $p = 0.006$). The IKZF1 status was shown to impact survival of imatinib-treated BCR::ABL1-positive ALL previously [15], however, in our cohort the impact on EFS or OS was not significant in any of the subgroups (all p values > 0.35). Distribution of NCI high-risk patients (age ≥ 10 years and/or WBC $\geq 50 \times 10^9$/l) was similar in the typical ALL and CML-like (71/99 [72%] and 36/48 [75%], respectively). While in the typical ALL patients the NCI risk tended to impact EFS (5-year-EFS 61 ± 10% vs. 44 ± 6%; $p = 0.12$) and OS (5-year-OS 89 ± 7% vs. 67 ± 6%; $p = 0.03$), there was no impact on prognosis in the CML-like patients ($p > 0.5$). Neither the BCR::ABL1 transcript type (minor vs. Major), the sex (female vs. male) nor the age at diagnosis (<10 years vs. ≥10 years) showed uneven distribution between groups nor any prognostic relevance.

Treatment protocol, namely the start of continuous TKI treatment, influenced MRD level at both TP1 and TP2 in the typical ALL patients, showing significantly lower MRD levels in patients with earlier (<1 month from diagnosis, mostly at D15) compared to later (>1 month, mostly D33) TKI start; no impact of TKI start on MRD levels was seen in CML-like patients. However, in both subtypes the patients with earlier start of the TKI tended to have better OS (5-year-OS 78 ± 9% vs. 67 ± 8%; $p = 0.082$ and 83 ± 8% vs. 60 ± 16%; $p = 0.066$ for typical ALL and CML-like, respectively; see Supplementary Fig. 6).

Diagnostic white blood cell count was similar in the typical ALL and CML-like patients (median 51.6 and 44.9×10^9/l, respectively; $p = 0.4$), as well as the number of patients with leukocytosis $\geq 50 \times 10^9$/l (51/99 [52%] and 23/48 [48%], respectively; $p = 0.7$). Impact of the hyperleukocytosis $\geq 50 \times 10^9$/l on outcome was highly significant in the typical ALL, but not in CML-like patients (Fig. 6A, Supplementary Fig. 2E). Moreover, the effect of diagnostic hyperleukocytosis in the typical ALL was apparent even in good responding patients who cleared their MRD before consolidation (Fig. 6B, Supplementary Fig. 7).

In multivariate analysis including WBC $\geq 50 \times 10^9$/l, age >10 years, sex, MRD level at TP2 (selected as stronger outcome predictor from the two MRD timepoints) and start of TKI or transplantation in CR1, the significant variables for EFS in the typical ALL were transplantation in CR1 ($p = 0.0003$), MRD at TP2 ($p = 0.0005$) and WBC ($p = 0.016$). In CML-like patients, none of the

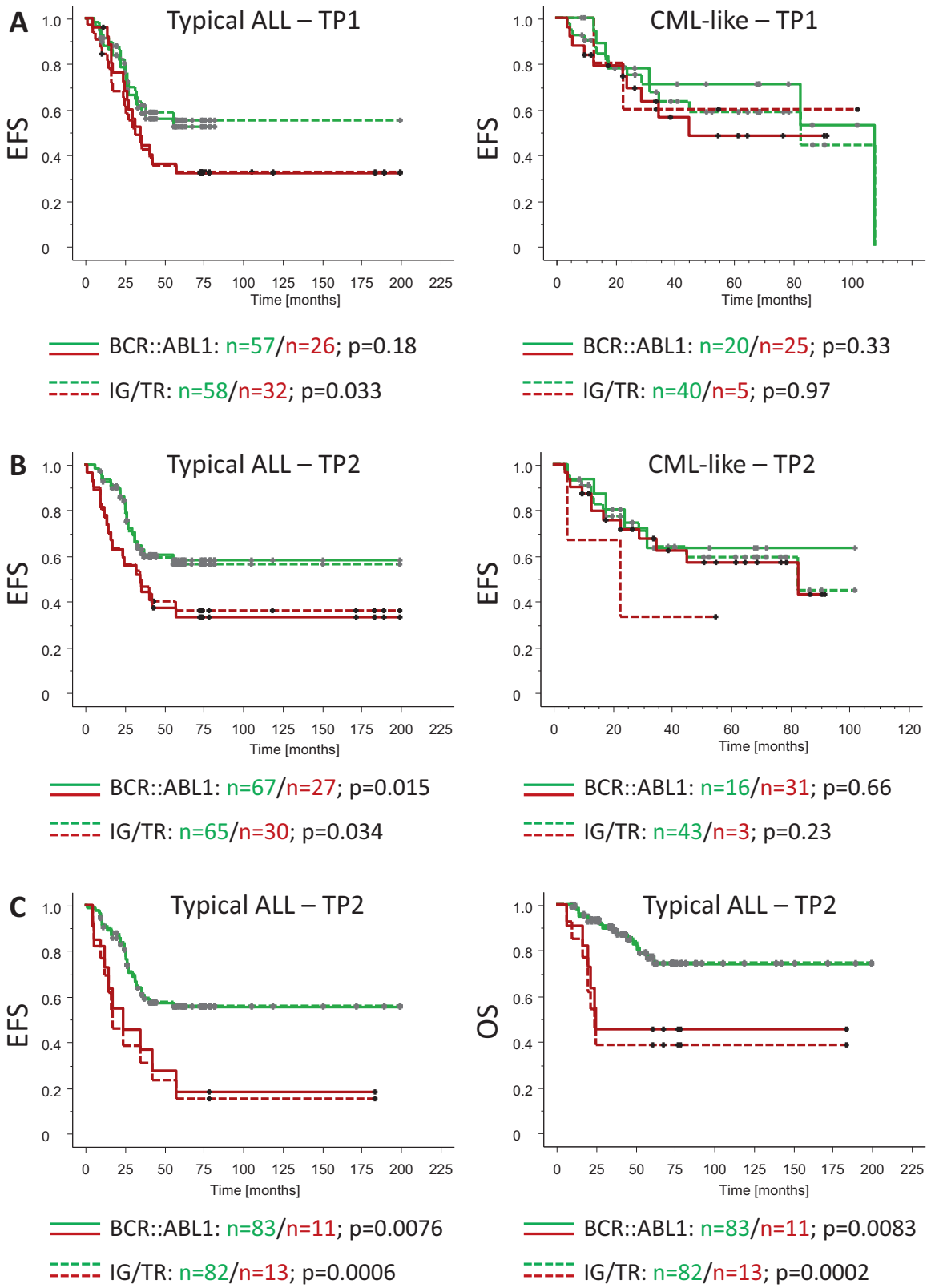

Fig. 4 Outcome according to MRD levels at TP1 and TP2. Survival curves of all patients according to subtype and MRD measured by BCR::ABL1 (full lines) and IG/TR (dashed lines). **A** EFS according to TP1 MRD < 10^{-2} (green) vs. ≥10^{-2} (red); (**B**) EFS according to TP2 MRD < 10^{-3} (green) vs. ≥10^{-3} (red); (**C**) EFS and OS of typical ALL with TP2 MRD < 10^{-2} (green) vs. ≥10^{-2} (red).

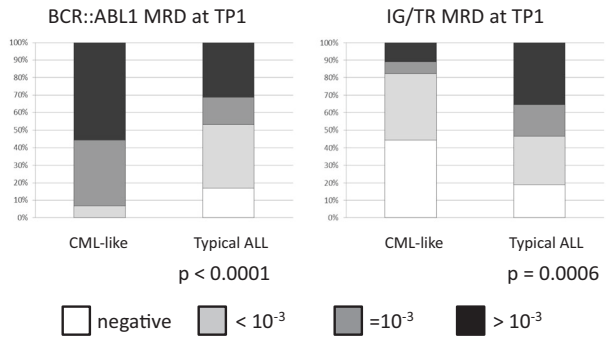

Fig. 5 MRD clearance at TP1 according to subtype and target. Percentage of patients with a particular MRD level in the CML-like and typical ALL; MRD levels divided into four categories: negative/ $<10^{-3}$/$=10^{-3}$/$>10^{-3}$.

variables reached p value < 0.05, the most significant was effect of transplantation ($p = 0.055$) (Supplementary Table 3).

DISCUSSION

In this study we aimed to assess the prognostic role of MRD clearance at early treatment timepoints in the BCR::ABL1-positive childhood ALL, and to show whether/how the MRD levels can be reasonably used for the therapy stratification and tailoring. Based on our previous work, defining two biologically distinct subtypes of BCR::ABL1-positive ALL (typical ALL and CML-like leukemia) [9, 10], we used for the MRD monitoring two concurrent approaches: quantification of IG/TR clonal rearrangements (generally considered as a golden standard in ALL) and of patient-specific genomic BCR::ABL1 fusion.

First of all, we focused on the MRD concordance/discordance to divide patients into typical ALL and CML-like subtypes. Ideally, definition of CML-like disease should be based on the proof of BCR::ABL1 fusion in myeloid or stem cell compartment. Unfortunately, in this study, we did not have routine access to viable diagnostic cells to perform cell sorting and BCR::ABL1 analysis in cell subpopulations. However, such analysis was performed in our original report and also in part of the patients included in this study and in all analyzed cases we demonstrated that evaluation of MRD concordance/discordance provides a reliable tool to distinguish between typical ALL and CML-like disease, as the results of subtype assessment by BCR::ABL1 detection in cell subpopulations and by MRD-based analysis were fully consistent (Fig. 1) [9]. However, there are cases in which the definition of CML-like vs. typical ALL is more challenging, particularly when the number of samples with measurable MRD levels is low. On the other hand, we cannot exclude the possibility that sporadic CML-like cases (with fast MRD clearance/less sensitive MRD targets/lack of samples from the early treatment timepoints) remained unrecognized.

The most informative timepoint for the subtype assessment was TP1 with discordant MRD in 84% of CML-like patients. Additional three CML-like patients were discordant at TP2; hence, 3 months from diagnosis, over 90% of CML-like patients could be successfully assessed by DNA-based MRD analysis.

Our results indicate that while in the typical ALL the prognostic significance of MRD clearance is very strong, there is only a weak (if any) association of outcome and MRD levels at early timepoints in the CML-like patients. This could at least partly explain the less clear role of MRD in undivided cohorts of BCR::ABL1-positive ALL, where the prognostic significance of MRD in typical ALL patients is blurred by the CML-like cases. Although in CML-like patients the MRD dynamics of the two targets is—by definition—very different, none of those provided useful prognostic information.

The significantly faster clearance of IG/TR MRD in CML-like compared to typical ALL patients (Fig. 5) suggests that in the CML-like patients, the "fully leukemic" ALL clone is more sensitive to the initial ALL-guided therapy, while the BCR::ABL1-positive non-ALL cells without the clonal IG/TR rearrangement are significantly more resistant to this treatment. The clinical and prognostic relevance of the BCR::ABL1-positive, IG/TR-negative residual cells is unclear. On one hand these "CML-like" cells possibly pose a risk of developing new "accelerated phase" or "blast crisis". On the other hand, in this study, all CML-like relapses with available data (8/11) harbored at least some of the original IG/TR rearrangements, thus were clonally related to the dominant diagnostic ALL clone and did not develop from the BCR::ABL1-positive, IG/TR-negative cell reservoir.

Historically, hyperleukocytosis $>50 \times 10^9$/l was considered a poor prognostic feature in ALL and is still reflected in some ALL treatment protocols [16]. However, in the recent studies, effect of leukocytosis has been mostly believed as being outweighed by MRD clearance and leukocytosis is usually not considered in therapy stratification. Our data show that in the typical ALL subtype the negative prognostic effect of initial hyperleukocytosis is very strong, even in patients with fast MRD clearance, and it remains significant in multivariate analysis [17]. This suggests that hyperleukocytosis should be considered as stratification criterion for typical ALL patients in future treatment protocols. In contrast, similarly to MRD levels, the effect of initial leukocyte count on prognosis in CML-like patients is negligible.

Current treatment protocols combining TKI and a chemotherapy backbone do not reflect the different biology of the CML-like disease. Our previous data suggested that CML-like patients might benefit rather from early transplantation. We were not able to unambiguously confirm this benefit in the present study, possibly due to the relatively low number of CR1 transplanted CML-like patients, which could reflect their apparent good early response, routinely measured by IG/TR quantification. Moreover, the relapsing CML-like patients did not show a poor survival after relapse, benefiting from transplantation in CR2. Although the data are based on 11 relapsed CML-like patients only, this fact makes the decision regarding CR1 transplantation even more complicated. On one hand, one could argue that CML-like patients should be treated with chemotherapy plus TKI, and those who relapse should be transplanted with a fair chance to be cured in CR2. On the other hand, treatment related mortality in CML-like patients was very high with highly intensive chemotherapy. Whether a reduction of toxicity (e.g., by replacing part of frontline chemotherapy by immunotherapy, targeting the fully leukemic B-cell clone) possibly followed by early transplantation (soon after IG/TR negativity is achieved) could avoid a significant proportion of treatment related deaths, should be considered and assessed in future clinical studies.

Moreover, there are additional open questions, particularly for the CML-like patients non-transplanted in CR1. Should — like in CML — the TKI be switched in CML-like with persistent BCR::ABL1 MRD, because of the suboptimal effect on the primordial clone? Should the TKI be continued after the maintenance treatment (analogously to CML)? To answer these questions, we need to measure BCR::ABL1 MRD prospectively in addition to IG/TR monitoring. Particularly in the CML-like cases with persistent BCR::ABL1 positivity, prolonged use of TKI should be considered and MRD continuously monitored over the entire therapy and beyond. For practical reasons, peripheral blood samples could be used for the monitoring after the end of intensive treatment.

Until we gather detailed biological data, going beyond the scope of this clinical study, the attractive question whether CML-like disease is in fact a regular CML in lymphoid blast crisis or whether there is a wider spectrum of BCR::ABL1-positive leukemias differing e.g., in cell of origin and biological characteristics, is rather an academic question. However, if we put the equal sign

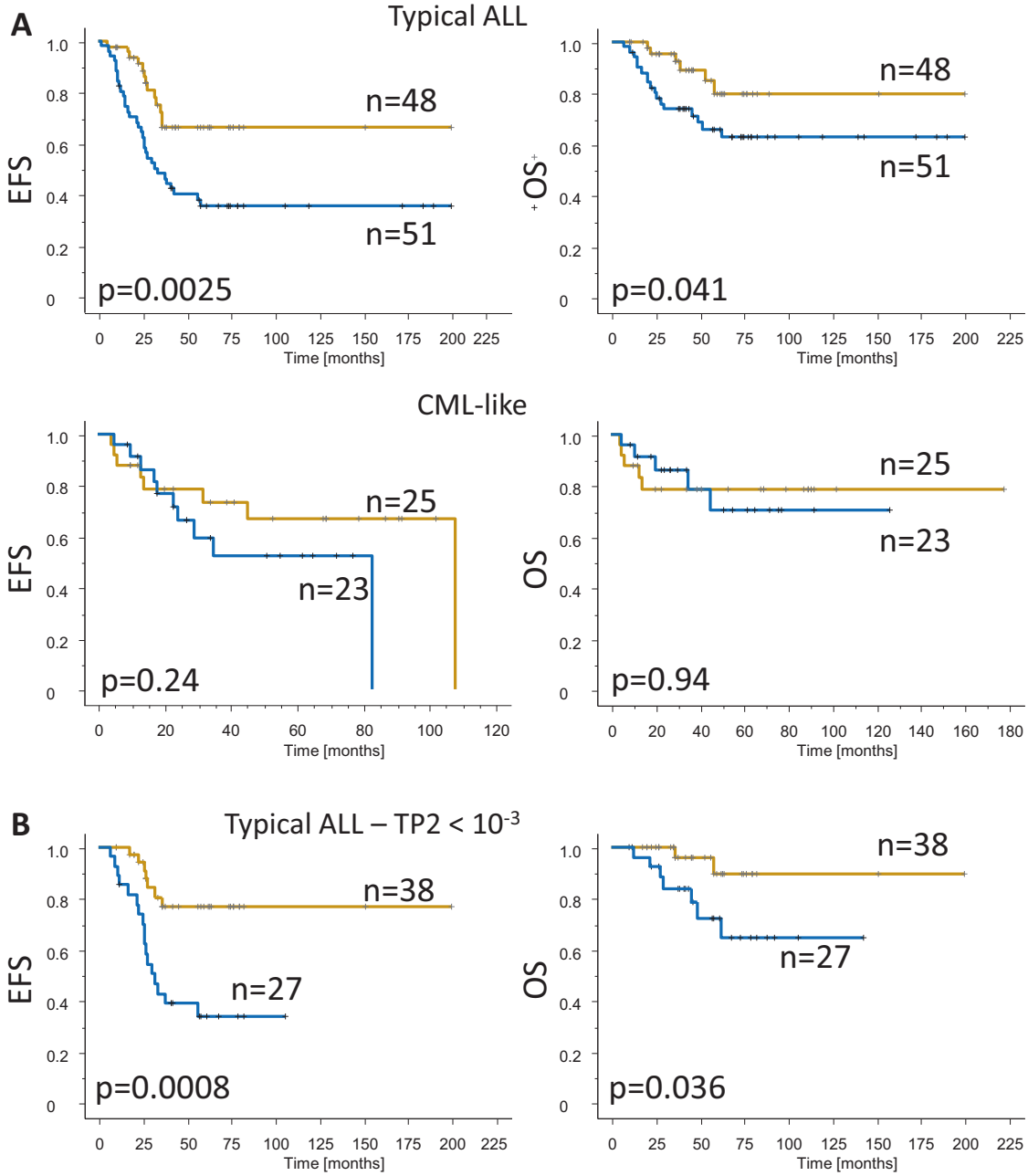

Fig. 6 Outcome of patients according to leukocytosis at diagnosis. A EFS and OS of typical ALL and CML-like patients according to leukocytosis at diagnosis; (**B**) EFS and OS of typical ALL patients with MRD by IG/TR at TP2 < 10^{-3}. Leukocytosis at diagnosis <50×10^9/l in yellow; leukocytosis at diagnosis ≥50×10^9/l in blue.

between the CML-like and CML, we must then re-define CML as a disease presenting — at least in childhood — very often in LBC and with the minor-BCR::ABL1/p190 fusion variant. Moreover, our data show that a significant part of these patients can be cured without transplantation (there were five toxic deaths in CR1, ten relapses [three relapsed patients died, seven are alive], and the remaining 17/32 non-transplanted CML-like patients are alive in CR1).

In conclusion, our data unequivocally confirm that although clinically presenting at diagnosis as one entity, there are two biologically distinct subtypes of BCR::ABL1-positive ALL — typical ALL and CML-like disease. The different biology is reflected in treatment response and although the overall survival rates of both subtypes are similar, the key reasons of treatment failure are different — toxicity of intensive ALL treatment in CML-like patients and relapses in typical ALL. Thus, identifying the disease subtype is essential to enable optimal treatment approach to each patient in upcoming protocols. Assessment of BCR::ABL1 presence in cell subpopulations, outside the ALL clone, would be an ideal approach to distinguish between typical ALL and CML-like patients already at diagnosis; however, this might be demanding to perform on a routine basis and thus parallel MRD monitoring using IG/TR and BCR::ABL1 at DNA level can be suggested as an alternative practical approach to identify vast majority of CML-like patients within the early treatment phases. Our results show a highly significant role of MRD clearance for outcome prediction in the typical ALL patients, substantiating its use for risk-based treatment stratification and tailoring. In contrast, the role of MRD in the early treatment timepoints in CML-like patients is mostly insignificant and inapplicable for therapy adjustment. Moreover,

we demonstrate that risk-prediction could be further improved by considering initial hyperleukocytosis as stratification marker, as it retains a strong prognostic value even in patients with good MRD clearance; however, this again applies to the typical ALL only, further underscoring the biological differences between typical ALL and CML-like disease. For the typical ALL, administration of TKI and concurrent chemotherapy with risk-directed intensity, which could be improved by using MRD and hyperleukocytosis in the stratification algorithm, seems to be reasonable strategy. On the other hand, we lack any relevant prognostic feature applicable for therapy tailoring in the CML-like disease, representing one-fourth to one-third of patients diagnosed as BCR::ABL1-positive ALL, and the best treatment approach for this subtype is still to be defined, including the role of transplantation in the first remission.

Note added in proof

At the time of processing of this manuscript, one of the CML-like patients (CZ_03) relapsed, 8 years from diagnosis, with identical BCR::ABL1 genomic breakpoint but completely different and unrelated IG/TR clonal rearrangements compared to original diagnosis.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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ACKNOWLEDGEMENTS

This study was supported by grants from the Czech Health Research Council (NU21-03-00128) and Charles University (UNCE 204012), by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00064203 (University Hospital Motol, Prague, Czech Republic), by the project National Institute for Cancer Research (Program EXCELES, ID Project No. LX22NPO5102)—Funded by the European Union—Next Generation EU, by Deutsche Krebshilfe (70112958), by the Italian Association for Cancer Research (AIRC) to AB (IG2017—20564) and G. Cazzaniga (IG2015—17593), and by the Comitato Maria Letizia Verga.

AUTHOR CONTRIBUTIONS

JZ, G.Cario, GCazzaniga, and MZ designed the study; LH, JK, AK, MB, LW, EF, JA, RK, CE, LB, MT, and JStu analyzed samples and provided data; MZ, PDL, MGV, VC, JSta, MS, AB, and JT provided diagnostic and clinical data; all authors participated on data integration, interpretation, and presentation; JZ and MZ wrote the draft; and all authors revised the draft and contributed to the final paper.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41375-022-01668-0.

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