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Outcome of ABL-class acute lymphoblastic leukemia in children in the pre-tyrosine kinase inhibitor era; an international retrospective study of the Ponte di Legno group

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Contributors

The project was conceptualized by RP and MLdB. Patients' characteristiscs and clinical outcome data were collected by the participating study groups and were provided via the chairs of each study group. Data was centrally collected and curated by HADGK and MLdB. Statistical analysis and computing in R was performed by HADGK, MF and JMB. Data was interpretated by MLdB, GC, ML, AVM, RP. Manuscript was written by MLdB and revised by all co-authors. Final version was approved by all co-authors.

Declaration of interest

MLdB, AVM, HAdGK, JMB, MF, GE, TI, AY, RS, LDP, NK, KGR, AV, AA, MZ, SE, GCazzaniga, AB, MLL and RP declare no competing interests. GCario reports personal fees from Jazz Pharmaceuticals and Novartis outside the submitted work. SPH reports personal fees from Novartis, Amgen, and other from Amgen, outside the submitted work. CGM reports personal fees from Illumina and grants from Loxo Oncology during the conduct of the study, and grants from Abbvie and Pfizer and personal fees from Amgen outside the submitted work. MS reports grants from SHIRE, JazzPharma, Servier, SigmaTau, Amgen, and Novartis during the conduct of the study, and personal fees from SHIRE, Servier, and JazzPharma outside the submitted work.

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Summary

Background—ABL-class gene fusions other than *BCR-ABL1* have been detected in ~3% of children with newly diagnosed acute lymphoblastic leukemia (ALL) and may be targeted by tyrosine kinase inhibitors (TKIs). This international study establishes the baseline characteristics and outcome of ABL-class ALL patients in the pre-TKI era.

Methods—Patients' characteristics and outcome of 122 children (1–18 years) with ABL-class B-cell precursor ALL were retrospectively collected through the Ponte di Legno consortium. Patients were enrolled in pediatric trials between 2000 and 2018 and were not exposed to TKIs during their first-line protocols. Event-free (EFS) and overall survival (OS) were determined by Kaplan-Meier methodology, and the cumulative incidence of relapse (CIR) and treatment-related mortality by a competing risk model.

Findings—Outcome of all ABL-class cases at 5 years was 31.0% (standard deviation (SD) 4.6) for CIR, 59.1% (95%CI 50.5–69.1) for EFS and 76.1% (95%CI 68.6–84.5) for OS. ABL-class patients displayed a high frequency of poor prednisone response (28 of 57 patients, 49%) and *IKZF1* deletions (36 of 59 patients, 61%), but both features lacked prognostic value. MRD-levels at the end of induction therapy (EOI) were very high ($\geq 1\times10^{-2}$) in 66% of ABL-class cases (61 of 93 patients), and most prevalent detected in *ABL2* (6 of 7 patients, 86%) and *PDGFRB*-fusion (43 of 49 patients, 88%) cases. MRD-EOI $\geq 1\times10^{-2}$ was predictive of an unfavorable outcome among ABL-class patients (HR_{EFS} 3.33, 95%CI 1.46–7.56; p=0.0039. The 5-year EFS was 80% (95%CI 58.7–100) for *CSF1R* (n=10), 68.6% (95%CI 54.5–86.3) for *ABL1* (n=40), 52.9% (95%CI 41.5–67.5) for *PDGFRB* (n=64), and 37.5% (95%CI 15.3–91.7) for *ABL2* (n=8) fusion cases (p=0.059). Sixty-nine percent of relapses (25 of 36) occurred within 3 years after diagnosis. The 5-years CIR of patients who received hematopoietic stem cell transplantation (n=41; 17.8% SD 6.2) was lower compared to the non-transplanted group (n=43; 45.1% SD 8.4; p=0.013), but EFS and OS did not differ between the two groups.

Interpretation—Children with ABL-class B-ALL have a poor outcome on therapies without TKIs despite the use of high-risk chemotherapy regimens and frequent transplantation in first remission. This paper provides baseline outcome for evaluating the potential benefit of upfront TKI usage in ABL-class patients.

Keywords

ABL-class; tyrosine kinase inhibitors; ALL; clinical outcome; children

Introduction

Gene expression profiling studies have identified over 20 genetic subtypes of B-cell precursor acute lymphoblastic leukemia (B-ALL) in children. One particular group of interest because of a high risk of relapse is *BCR-ABL1*-like or Philadelphia chromosome (Ph)-like B-ALL. This group, first described in 2009, whilst negative for the *BCR-ABL1* fusion has a gene expression profile that mirrors that of *BCR-ABL1* positive ALL.[1,2] This *BCR-ABL1*-like/-Ph-like group is characterized by a high frequency of lesions involving ABL-class genes (12–18% of cases) as well as JAK-pathway genes (*JAK2, EPOR*), chemokine receptors (*CRLF2*) and/or MEK-ERK pathway genes.[3–5] The ABL-class group of genomic alterations consists mainly of in-frame fusions that join *ABL1*, *ABL2*, *CSF1R* and *PDGFRB,* among others, to genes normally expressed during B-cell development. The resulting chimeric proteins have profound tyrosine kinase activity in cells in which the ABL-class genes are usually not abundantly expressed, resulting in the activation of pathways involved in the survival and proliferation of immature lymphoid cells.[6,7]

The addition of tyrosine kinase inhibitors (TKIs) such as imatinib and dasatinib to chemotherapy-based therapies has significantly improved the outcome for children with newly diagnosed *BCR-ABL1* positive leukemia.[8–12] Similar to *BCR-ABL1*-positive ALL, ABL-class ALL is associated with high risk (HR) features of older age and higher white blood cell count at diagnosis and high minimal residual disease levels at the end of induction therapy (MRD EOI).[5,13] ABL-class fusions are found in much higher frequency (4%) in National Cancer Institute (NCI) high risk (HR) patients compared with 0.2% of standard risk patients.[14,15] Imatinib and dasatinib both have significant activity in pre-clinical models of ABL-class fusions which mirrors the results observed in *BCR-ABL1* positive pre-clinical models.[3,6,7] Given the molecular similarities to *BCR-ABL1*-positive ALL, there are strong grounds for assessing the potential benefit of TKIs in ABL-class patients. Several anecdotal reports have described excellent responses to additional TKI therapy but lacked information regarding long-term outcome.[6,16,17] Two recent reports highlight the potential efficacy of TKIs in patients but these are still hampered by small patient numbers and late introduction of TKI therapy.[13,18] The ABL-class cohort therefore remains a heterogenous group of patients with unverified baseline characteristics and outcome for each of the *ABL1*, *ABL2*, *CSF1R* and *PDGFRB* fusion types separately.

The Ponte di Legno group consists of >20 established ALL study groups worldwide and was initiated to address outcome questions in rare subsets of newly diagnosed pediatric ALL patients for which individual study groups have only a limited number of cases. We undertook a retrospective study to investigate the clinical outcome of newly diagnosed ABL-class patients treated on first-line trials without TKIs. The results described in this paper will serve as reference to interpret the potential benefit of adding TKIs in the front-line treatment of children with ABL-class ALL.

Methods

Study design and participants

Newly diagnosed pediatric B-ALL cases (1–18 years of age) were retrospectively included in this study based on the presence of an ABL-class fusion, enrollment in a pediatric trial between 2000 and 2018, and no exposure to TKIs during their first-line protocol. Patient and outcome characteristics were collected from study groups belonging to the Ponte di Legno consortium using a standard case report form. In accordance with the declaration of Helsinki, written informed consent was obtained from parents or guardians, and the institutional review boards approved the use of patient data for research purposes. Patients were enrolled in pediatric trials between 3 October 2000 and 28 August 2018 and were not exposed to TKIs during their first-line protocols. Patients were categorized by NCI risk criteria into standard risk (SR) and high risk (HR; age ≥10 year and/or WBC $\geq 50\times10^9$/L at diagnosis). Minimal residual disease (MRD) testing was performed using IG/TCR PCR and/or flow-MRD depending on the protocol guidelines per study group. Results of these MRD monitoring methods are comparable according to previously published results.[19] Data of both detection methods were merged in this study defining 1% by flow-MRD as reflecting a value equivalent to 1×10^{-2} by IG/TCR MRD. In most cases MRD values were measured at the end of induction (EOI), although less commonly data were also collected at the end of consolidation (EOC). The EOI and EOC MRD time points are taken roughly 29–35 days and 77–80 days after start treatment, respectively, with minor differences between protocols. Response to a therapeutic window with 7 days of prednisone and one dose of intrathecal methotrexate was collected for BFM-based trials. A poor prednisone response (PPR) was defined by persistence of ≥1,000 blasts per μl peripheral blood on day 8 of treatment. Patients received risk-stratified treatment based on the criteria set by each treatment protocol. In the present study, patients treated with either standard, medium, or non-high risk arms of individual protocols were collectively assigned to the non-high risk (non-HR) treatment group and only patients receiving high risk therapy were assigned to the HR treatment group.

Procedures

The ABL-class cohort was defined by fusion genes involving *ABL1*, *ABL2*, *CSF1R* and *PDGFRB* gene loci. ABL-class cases were identified by the diagnostic and research laboratories of participating study groups. Patients were retrospectively tested, often as part of research interests to characterize the poor prognostic subset of Ph-like/*BCR-ABL1*-like ALL. B-ALL cases negative for prognostically relevant genetic lesions (*BCR-ABL1*, *KMT2A*-rearranged, *ETV6-RUNX1*, *TCF3-PBX1*, high hyperdiploidy) were subjected to total RNA sequencing, reverse transcriptase-PCR or fluorescence in situ hybridization (FISH) often prompted by cytogenetic or karyotypic evidence for abnormal chromosomal regions affecting 1q25 (*ABL2*), 5q13–34 (*CSF1R* and *PDGFRB*)[20] and 9q34 (*ABL1*), or by the gene expression signatures used to discover the Ph-like/*BCR-ABL1*-like cases.[1,3,4,21] Identification of ABL-class cases was dependent on sample availability and individual study groups' decisions to perform additional analyses. In the appendix p 1–2 the detection methods are described which were used by the Dutch Childhood Oncology Group (DCOG) as an example. Some cases included in this study have been presented in publications about

new fusion gene discovery (including *CRLF2*, *EPOR* and *JAK*-fusions) in *BCR-ABL1*-like/-Ph-like patients[5,6,20,22] and in a publication about outcome in a single protocol.[13]

In 59 (48.4%) of 122 cases, the presence of Ikaros (*IKZF1*) deletions (intragenic or fully deleted) was assessed by the multiplex ligation-dependent probe amplification (SALSA MLPA P202, MRC Holland). In a limited number of cases other B-cell development genes, including *PAX5* and *CDKN2A/2B* deletions were also analysed using the SALSA MLPA P335 assay. Data on *PAR1* and *ERG* status had limited availability by some study groups and *IKZF1*-plus status could be assessed according to Stanulla et al.[23] Instead, we compiled a derivative *IKZF1*-"plus" group of cases having a deletion in *IKZF1* with concomitant deletions in PAX5 and/or CDKN2A/2B, which largely (>85%) overlaps with the previously reported *IKZF1*-plus group.[23,24]

Outcomes

Complete remission (CR) was defined as <5% leukemic cells in the bone marrow and recovery of normal hematopoiesis, absence of peripheral blood leukemic cells and no evidence of disease at any other site. Early death was defined as death in induction prior to CR. Treatment related mortality (TRM) was defined as any death in first CR. Non-responders represented cases who failed to achieve CR after two courses of chemotherapy. Relapse was defined by disease recurrence after initial CR. The time between diagnosis and start of treatment is typically between 0 and 2 days.

Statistical analysis

The Pearson $\chi 2$ and Kruskal-Wallis test were used to compare age, white blood cell counts and MRD levels between the four ABL-class fusion types. A competing risk model with relapse and death was employed to estimate the cumulative incidence of relapse (CIR) and the cumulative incidence of treatment-related mortality (TRM) from first diagnosis for cases who reached CR. The Gray's test was used to compare cumulative incidences between ABL-class patients. Kaplan-Meier (KM) methodology was used to estimate event-free survival (EFS) and overall survival (OS). EFS was defined as time from diagnosis to first event. Events were defined as non-response, early death, relapse, second malignancy and death in first remission. OS was estimated from diagnosis to date of death by any cause. Individuals without an event were censored at the last date of contact. EFS and OS KM-curves between ABL-class patients were statistically compared using the log-rank test and the 5-year survival percentage and standard error (SE) are given. To quantify the effect of risk factors on EFS, a Cox proportional hazard regression model was used to estimate the hazard ratio (HR) and the 95% confidence interval (CI). The effect of hematopoietic stem cell transplantation (HSCT) was investigated by a landmark approach to avoid immortal time bias. A waiting time to HSCT of 6 months was taken as landmark, and outcome events were only considered if occurring after the landmark. Analyses have been performed with SPSS version 25. All analyses concerning the competing risks model were performed in R software environment (version 3.2.2) with cmprsk package version 2.2–7. All plots for EFS, OS and CIR start at t=diagnosis.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

Newly diagnosed pediatric B-ALL patients (1–18 years of age) with ABL-class fusions enrolled in pediatric trials between 2000 and 2018 were eligible for this study, if they were not exposed to TKIs during their first-line protocols. Outcome characteristics of 122 patients were collected from 14 international study groups participating in the Ponte di Legno group (Figure 1). The breakdown per study group including NCI risk group is provided in appendix p 3. Data of 33 BFM/AIEOP patients was included in a prior single protocol study.[13] Two-thirds of the 122 patients (84) were diagnosed before 2010, from 3 October 2000 till 20 November 2009; the remaining 38 were diagnosed from 22 February 2000 till 28 August 2018. Since most study groups only selectively screened for ABL-class fusions, the frequency of ABL-class fusion types (see appendix p 6) may not represent a population-based distribution of lesions. The vast majority of ABL-class fusions involve *PDGFRB* (64 patients, 52%) and *ABL1* (40 patients, 33%), with a minority of cases having fusions involving *CSF1R* (10 patients, 8%) and *ABL2* (8 patients, 7%). Fourteen different fusion partner genes were found, and their frequencies per ABL fusion type are outlined in appendix p 4 and 6. *EBF1* was the predominant partner for *PDGFRB* (50/64 cases), *ZMIZ1* for *ABL1* (16/40), *SSBP2* for *CSF1R* (8/10), and *RCSD1* and *ZH3HAV1* for *ABL2* (each 4/8).

The ABL-class fusions were most often seen in patients classified as NCI high risk (93 of 121 patients, 77%), and were skewed towards NCI high risk for all four ABL-class fusion types. The mean age at diagnosis was 9.7±5.1 year and the mean presenting white blood cell count (WBC) was $97.9\pm114.8\times10^9$/L. Age and WBC varied between ABL-class types (Pearson $\chi 2$, p=0.0066 and p=0.079, respectively), with the highest means for *ABL2* fusion cases (Table 1; appendix p 7). Central nervous system involvement (CNS3) was detected at diagnosis in 4 of the 116 cases (3.4%) for which these data were available, including 1 *CSF1R* and 3 *PDGFRB* fusion cases. Testicular involvement was not observed in 44 males with available data. The male:female ratio was 1.7:1 and did not vary between ABL-class types (p=0.86; Table 1). In 36 (61%) of 59 cases tested for deletion status of *IKZF1*, *PAX5*, and *CDKN2A*/*B*, an *IKZF1* deletion was detected. In 13 (22%) of 59 patients only an *IKZF1* deletion was found and in 23 (39%) an *IKZF1* deletion plus a *PAX5* and/or a *CDKN2A*/*B* deletion. The frequency of *IKZF1* deletion was not significantly associated with certain ABL-class fusion types (Table 1). Neither an *IKZF1* deletion nor the *IKZF1*-"plus" genotype was associated with an unfavorable outcome compared to patients with wildtype *IKZF1* (appendix p 8).

The response to prednisone as a single systemic agent was assessed in 57 ABL-class patients, of whom 28 (49%) had a poor response (PPR; Table 1). The frequency of PPR varied between <10% for *CSF1R* (0 out of 4 patients) and *ABL1* fusion cases (1 out of 14 patients) and ≥60% for *ABL2* (3 out of 5 patients) and *PDGFRB* fusion cases (24 out of 34

patients; 71%, p=0.00015). A PPR in ABL-class cases was not predictive of an unfavorable clinical outcome (EFS p=0.35; appendix p 9). Non-HR and HR treatment was given to 28 (23%) and 93 (77%) of 121 ABL-class cases, respectively (Table 2). In the total cohort there was no significant difference in the EFS of non-HR (5-year 70.7%, 95%CI 54.3–92.1) and HR cases (5-year 55.4%, 95%CI 45.9–67.1; p=0.22; appendix p 10). Most *PDGFRB* fusion cases were NCI HR (52 of 64 patients, 81%) and had very high MRD levels at the end of induction. The few *PDGFRB* fusion cases who received non-HR treatment had a poor outcome; 6 out of 7 non-HR treated patients experienced an event compared to 24 out of 57 HR treated *PDGFRB*-fusion cases (appendix p 10; 5-year EFS of non-HR 28.6% (95%CI 8.9–92.2) and HR cases 56.5% (95%CI 44.5–71.8), p=0.032).

The 5-year EFS and OS of the total group of ABL-class patients was 59.1% (95%CI 50.5–69.1) and 76.1% (95%CI 68.6–84.5), respectively with median follow up of 6.7 years (interquartile range 3.9–9.4) for those without an event. Death in induction (early death) occurred in 3 (2.5%) and death in complete remission occurred in 7 (6%) patients. Two and a half percent (3 patients) were non-responders at the end of consolidation treatment (Table 2). These patients continued on protocol and reached CR; one remained in CR, one relapsed, and one patient died of HSCT-related toxicity. The 5-year cumulative incidence of relapse (CIR) of the total group was 31.0% (SD 4.6) (Figure 2A). In 25 (69%) of 36 cases relapse occurred within 3 years of diagnosis. Most relapses were found in the bone marrow (29 of 35; 83%; for 1 relapse location was not reported). Extramedullary relapse (mostly CNS) was seen either alone or combined with medullary relapse in 11 (31%) of the relapsed cases. Twenty of the 36 relapsed patients remained alive in second CR. Outcomes varied between ABL-class fusions. The 5-year EFS was 37.5% (95%CI 15.3–91.7) for *ABL2*, 52.9% (95%CI 41.5–67.5) for *PDGFRB,* 68.6% (95%CI 54.5–86.3) for *ABL1* and 80.0% (95%CI 58.7–100) for *CSF1R* fusion cases (Figure 2B; p=0.059). The corresponding 5-year CIR was 25.0% (SD 16.9) for *ABL2*, 29.6% (SD 8.2) for *ABL1*, 34.3% (SD 6.5) for *PDGFRB* and 20.0% (SD 13.4) for *CSF1R* fusion cases (Figure 2A; p=0.82). The 5-year OS varied between 37.5% (95%CI 15.3–91.7) for *ABL2* and ≥75% for the other fusion types (Figure 2C; p=0.0030). The highest Hazard Ratio was observed for *ABL2*-fusion cases compared to the remaining ABL-class cases (HR_{OS} 4.90, 95% CI 1.84–13.03, p=0.0015).

MRD levels at the end of induction (EOI) were reported in 93 out of 122 cases. The EOI MRD levels were positive in 88 (95%) cases, $\geq 1\times10^{-4}$ in 77 (83%), and $\geq 1\times10^{-2}$ in 61 (66%) of the ABL-class cases, with notable variation between fusions (Table 2, Figure 3A). *ABL1* fusion patients had the most favorable MRD EOI response, i.e. 20 (69%) of 29 patients were $<1\times10^{-2}$ although only 11 (38%) were $<1\times10^{-4}$. The EOI MRD levels of $\geq 1\times10^{-2}$ were significantly associated with *ABL2* (6 of 7 patients, 86%) and *PDGFRB* (43 of 49 patients, 88%) fusions (Kruskal Wallis, p<0.0001). Levels of MRD were not affected by the different 14 fusion partners of the ABL-class genes (appendix p 11). From the 41 cases with available MRD data both at EOI and at end of consolidation (EOC), 7 (17%) had negative or non-quantifiable MRD at EOI and 16 (39%) at EOC (appendix p 12; Paired test p<0.0001). Because of the refractory nature of ABL-class ALL, we used a threshold of 1×10^{-2} to compare outcome. ABL-class patients with MRD EOI $\geq 1\times10^{-2}$ had an unfavorable 5-year EFS of 44.7% (95%CI 33.2–60.4) compared to 81.9% (95%CI 68.7–97.7) for those patients with MRD levels $<1\times10^{-2}$ (Figure 3B, p=0.0023) which, in

multivariate Cox models, appeared independent from NCI risk group and treatment arm (appendix p 5). The 5-year CIR corresponding to MRD EOI levels $<1\times10^{-2}$ and $\geq1\times10^{-2}$ were 18.1% (SD 7.5) and 41.1% (SD 7.0) respectively (Gray's test p=0.10; Figure 3C).

In total, 2 non-HR and 41 HR cases received a hematopoietic stem cell transplantation (HSCT) in first CR out of 115 cases for whom the HSCT status was reported. The median time to transplant was 6.7 months (interquartile range 5.8–7.9). The clinical outcome of 41 HR patients who received HSCT was compared to that of 43 HR patients without HSCT who survived at least 6 months from diagnosis without any event (landmark analysis). Sixteen events occurred after HSCT (7 relapses, 7 deaths in second CR and 2 second malignancies). The transplant-related mortality was high (7/41), and 6/7 of transplant-related deaths occurred in patients transplanted before 2010. In the group without HSCT, 18 events occurred, all relapses. The relapses and death in second CR (indicative of HSCT/treatment-related mortality) mainly occurred in the first 2 years following HSCT, after which the CIR and EFS curves stabilized. In contrast, relapses occurred up to 5-year after the landmark in the non-transplanted group (appendix p 13). The estimated CIR significantly differed between patients with HSCT (5-year 17.8%, SD 6.2) versus those without HSCT (5-year 45.1%, SD 8.4; P=0.013), but EFS and OS estimates did not significantly differ between the two groups (appendix p 13).

Discussion

This Ponte di Legno group study shows that the ABL-class subset of B-ALL, especially those with *ABL2* and *PDGFRB* fusions, are characterized by very high risk features, a high frequency of poor prednisone response (PPR), high MRD EOI levels and an unfavorable long-term outcomes. The 5-year estimates in this pre-TKI era are 31% CIR, 59% EFS and 76% OS. These outcome data are highly inferior compared to that of other children with newly diagnosed B-ALL treated with contemporary treatment protocols, i.e. <8% CIR, >85% EFS and >90% OS.[25,26] Our study also shows that the outcome varies between the four different types of ABL-class fusions with *ABL2* and *PDFGRB* fusion cases having the most unfavorable baseline characteristics of older age, high WBC and high MRD EOI levels.

ABL-class patients are characterized by older age and high WBC at diagnosis and 75% are classified as NCI-HR compared to 30–35% of the general pediatric ALL population.[26,27] The percentage of CNS involvement in ABL-class patients (3.4%) is low and comparable to the frequency seen in NCI HR cases of a large reference cohort (2.4%).[28] In contrast, ABL-class patients more frequently have a PPR compared to reference cohorts of newly diagnosed ALL (49% versus <10%, respectively).[25,26] Sixty-six percent of ABL-class patients have a very high and prognostically unfavorable MRD EOI level of $\geq1\times10^{-2}$ compared to less than 10% in reference cohorts.[25,26] Only 39% had negative/non-quantifiable MRD at the end of consolidation therapy, which is much lower than in other subsets of B-ALL, e.g. 77% in the AIEOP-BFM ALL 2000 trial.[26] Positive MRD at this late time-point is associated with a high risk of relapse and often used as an indication for HSCT. Considering the strong predictive role of MRD, a significant reduction in MRD levels in ABL-class patients during the first months of therapy is an important aim to reduce

the relapse risk and to reduce the intensity of treatment (e.g. by avoiding HSCT) and its associated complications and mortality.

The frequency of *IKZF1* deletions in ABL-class patients was high (61%) and comparable to the frequency in *BCR-ABL1* positive ALL (75%), both being much higher than the 15% in *BCR-ABL1* negative pediatric ALL.[2,4,29] We found that an *IKZF1* deletion with or without additional deletions in *PAX5*, *CDKN2A* or *CDKN2B* did not have additive prognostic value in contrast to the findings in non-ABL-class patients.[23] Hence, treatment should not be modified on the basis of an additional *IKZF1* deletion in ABL-class patients.

While it is possible that higher risk patients were selected to undergo HSCT in first CR, we found that overall survival for ABL-class cases treated without TKI therapy was very similar with chemotherapy alone or HSCT in first CR. The reduced number of relapses following HSCT was counterbalanced by the number of treatment-related deaths. Similar results were observed in a recent study analyzing both TKI and non-TKI treated ABL-class patients enrolled in recent AIEOP-BFM trials.[13] In a landmark analysis (taking into account a 6 months waiting time to transplant), we noticed that relapses occurred over a longer timeframe of 5.5 years from diagnosis in the non-transplanted group, whereas the transplanted group suffered more often from early relapses (within 2.5 years of diagnosis). The occurrence of relapses early after HSCT is a known observation in ALL and indicates the failure of the conditioning regimen and the intended immune control by the engrafted immune cells. Early relapse rate in the transplanted group was not linked to a specific type of ABL-class fusion.

The data collected on ABL-class patients in this Ponte di Legno study was limited to the first-line treatment, up to the occurrence of the first event. The 122 patients were treated on >20 treatment protocols (1–22 patients each) between 2000 and 2018, which did not allow separate outcome analyses per protocol. MRD-guided risk stratification started to be used from 2000 onwards but was not implemented in all protocols in the same way. While for all but 29 patients MRD at the end of induction was available, only 41 patients also had MRD evaluated at the end of consolidation. Over the study period, incremental improvement in the overall survival of pediatric B-ALL was achieved, for example in the Netherlands from 86% (2000–04) to 91% (2005–09) and 93% (2010–15).[30] Two-thirds of the ABL-class patients were diagnosed before 2010, suggesting that there was a decline of recruited patients possibly due to the increased first-line use of TKIs in recent years. The transplant-related mortality was high, and 6/7 of transplant-related deaths occurred in patients transplanted before 2010. Further analysis of possible reasons for HSCT failure was limited by the fact that HSCT details including conditioning, donor type, stem cell source and pre-HSCT MRD were not available. The current study was not designed to evaluate the effect of HSCT in ABL class patients, and the number of patients was too low to draw conclusions on the effectiveness of HSCT in CR1 as an effective salvage. Similarly, no data were collected on the use of TKIs or immunotherapies as second line treatment in our study. Given the time period of this study, it is very unlikely that immunotherapies were used in first line therapy of these protocols.

The signaling pathways activated by ABL-class fusions strongly suggest that ABL-class patients may benefit from the addition of TKIs to combination chemotherapy. Preclinical studies conducted *in vitro* (Ba/F3 and Arf$^{-/-}$ cell lines), *ex vivo* (patients' cells) and *in vivo* (mouse models), all provide evidence that leukemic cells harboring ABL-class fusions are sensitive to different TKIs, including first (imatinib), second (e.g. dasatinib, bosutinib) and third generation (e.g. ponatinib) TKIs. TKIs were also efficacious in several case studies of children largely with refractory or relapsed ALL (appendix p 14 and 15, and references herein). Furthermore, the FRALLE group recently reported that all 8 MRD EOI positive ABL-class patients who received TKI achieved and remained in first complete remission for a prolonged time.[18] Similar, the AIEOP-BFM group recently reported that TKIs applied at different stages of therapy resulted in only one relapse among 13 children with ABL-class leukemia.[13] Together, these studies illustrate that TKIs can be beneficial to ABL-class patients. However, evidence for TKI efficacy in *CSF1R* fusion positive patients is lacking and limited to preclinical studies showing some sensitivity of *CSF1R*-fusion positive cells to TKIs.[8,31]

Targeting the ABL-class lesions by TKIs may be as effective as their use in children with *BCR-ABL1* positive ALL.[8,9,11,32] Most promising results have been obtained by giving TKIs continuously over a longer period in *BCR-ABL1*-positive ALL.[9,11] Given the baseline 5-year EFS of 59% observed in the present study including the use of alloSCT in a significant proportion of patients, TKI addition to upfront therapies may increase the long-term outcome also for ABL-class cases.

In conclusion, this Ponte di Legno study shows that without TKI the outcome of children with ABL-class B-cell precursor ALL is highly unfavorable compared to non-ABL-class ALL patients. The availability of TKIs that have established to be safe and effective when combined with chemotherapy in *BCR-ABL1* positive patients will fast track the use of TKI-containing combination therapies for ABL-class patients. This paper establishes the outcome standard to which these TKI-containing therapies will be compared.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Data sharing

Requests to receive de-identified study data can be submitted to the corresponding author and should include a description of the research question.

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Research in context

Evidence before this study

In the last decade, it has become clear that ABL-class gene fusions other than *BCR-ABL1* are detected in ~3% of children with acute lymphoblastic leukemia (ALL). Preclinical studies suggest that leukemic cells carrying ABL-class fusions can be targeted successfully by tyrosine kinase inhibitors (TKIs). The addition of TKIs to the therapy of *BCR-ABL1*-positive ALL has significantly improved the outcome but it is unknown whether this holds true for ALL with other ABL-class fusions. Moreover, the ABL-class fusion group is heterogeneous and includes patients with *ABL1*, *ABL2*, *CSF1R* and *PDGFRB* fusion types. The outcome for patients with these subtypes of ALL is not known because their occurrences are rare. A systematic search was not performed.

Added value of this study

This study was undertaken by the Ponte di Legno group. This group consists of >20 established ALL study groups worldwide and was initiated to address outcome questions in rare subsets of newly diagnosed pediatric ALL patients. We investigated the characteristics and outcome of children with ABL-class positive ALL treated on recent first-line trials without TKIs.

Implications of all the available evidence

The results described in this paper will serve as reference to interpret the potential benefit of adding TKIs in the front-line treatment of children with ABL-class positive ALL.

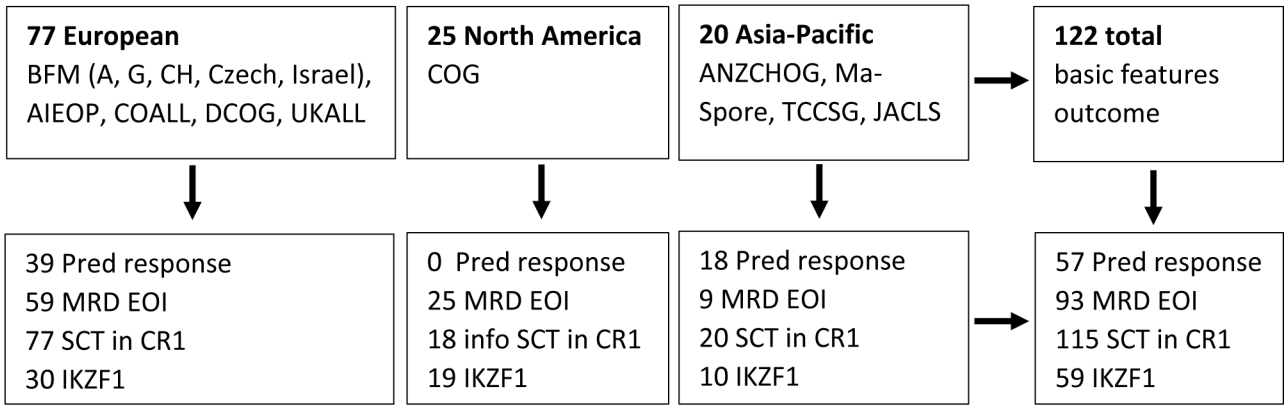
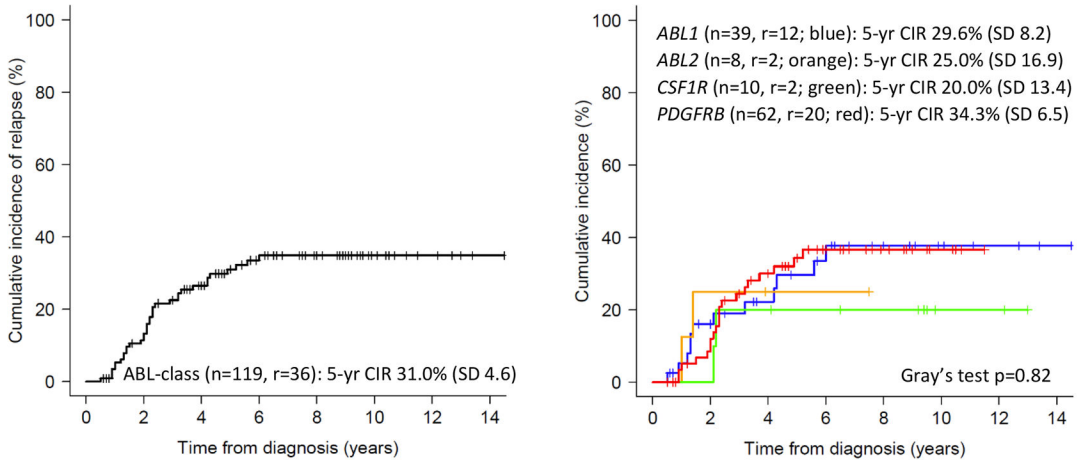


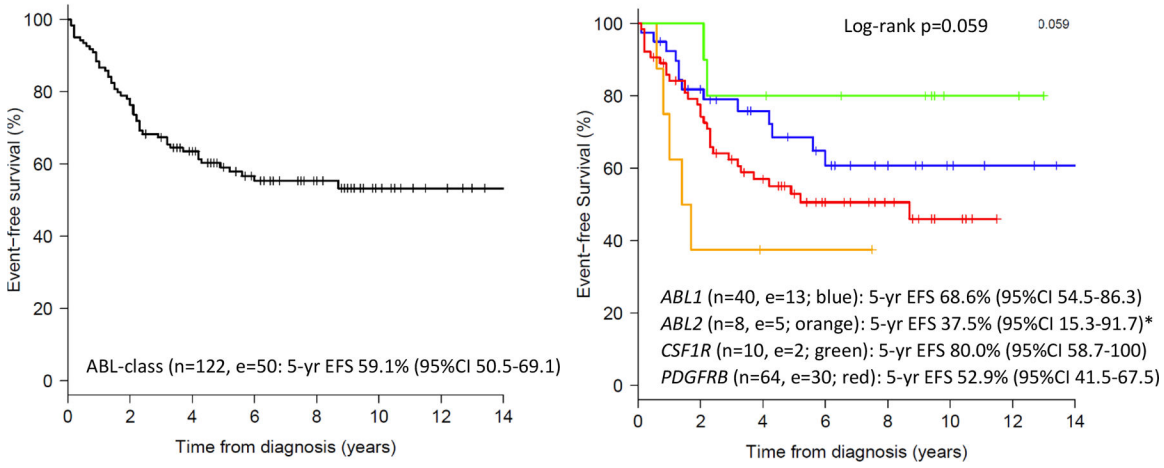
Figure 1: Cohort overview

The diagram shows the distribution of pre-TKI ABL class patients collected by the different study groups and the number of patients with information on prednisone response, end of induction minimal residual disease, stem cell transplant in first complete remission and IKZF1 deletion.

(A) Cumulative incidence of relapse in ABL-class patients



(B) Event-free survival of ABL-class patients



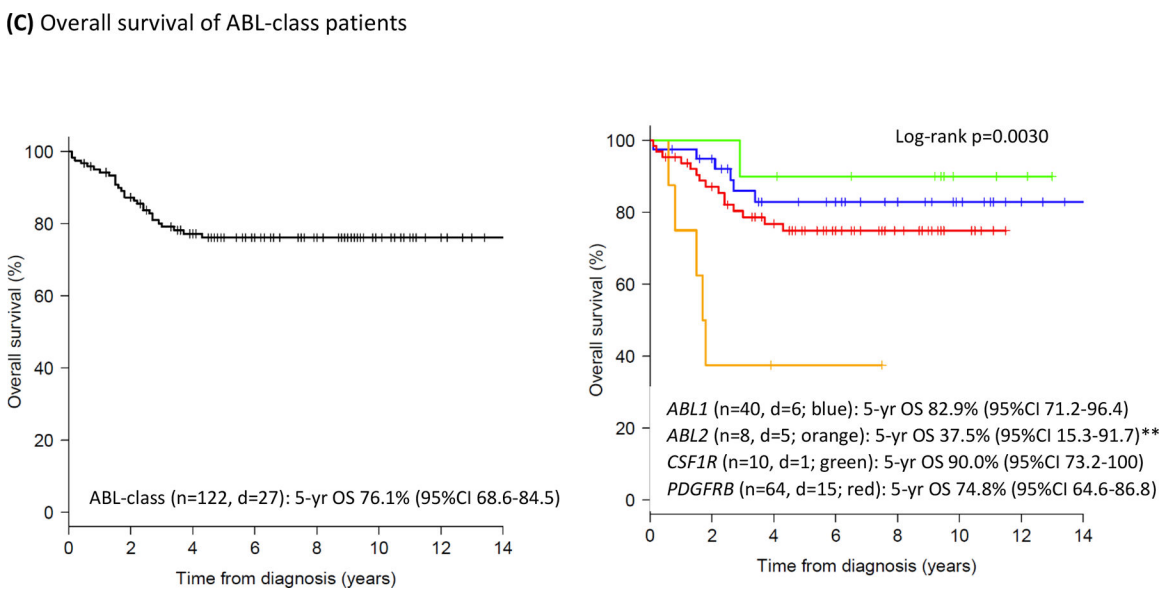


Figure 2: Outcome characteristics of children with ABL-class B-ALL in the pre-TKI era

(A) CIR curve of 119 ABL-class patients, excluding 3 early death cases (left panel) and per ABL fusion type (right panel), Gray's test p=0.82.

(B) EFS curve of 122 ABL-class patients (left panel) and per ABL fusion type (right panel), log-rank p=0.059. **ABL2*-fusion versus remaining ABL-class: HR 2.40, 95%CI 0.95–6.10, Cox p-value 0.064.

(C) OS curve of 122 ABL-class patients (left panel) and per ABL fusion type (right panel), log-rank p=0.0030. ***ABL2*-fusion versus remaining ABL-class: HR 4.90, 95%CI 1.84–13.03, Cox p-value 0.0015.

Color code ABL fusions: *ABL1*, blue; *ABL2*, orange; CSF1R, green; *PDGFRB*, red; n=number of patients in the analysis, r=relapse, e=event, d=death.

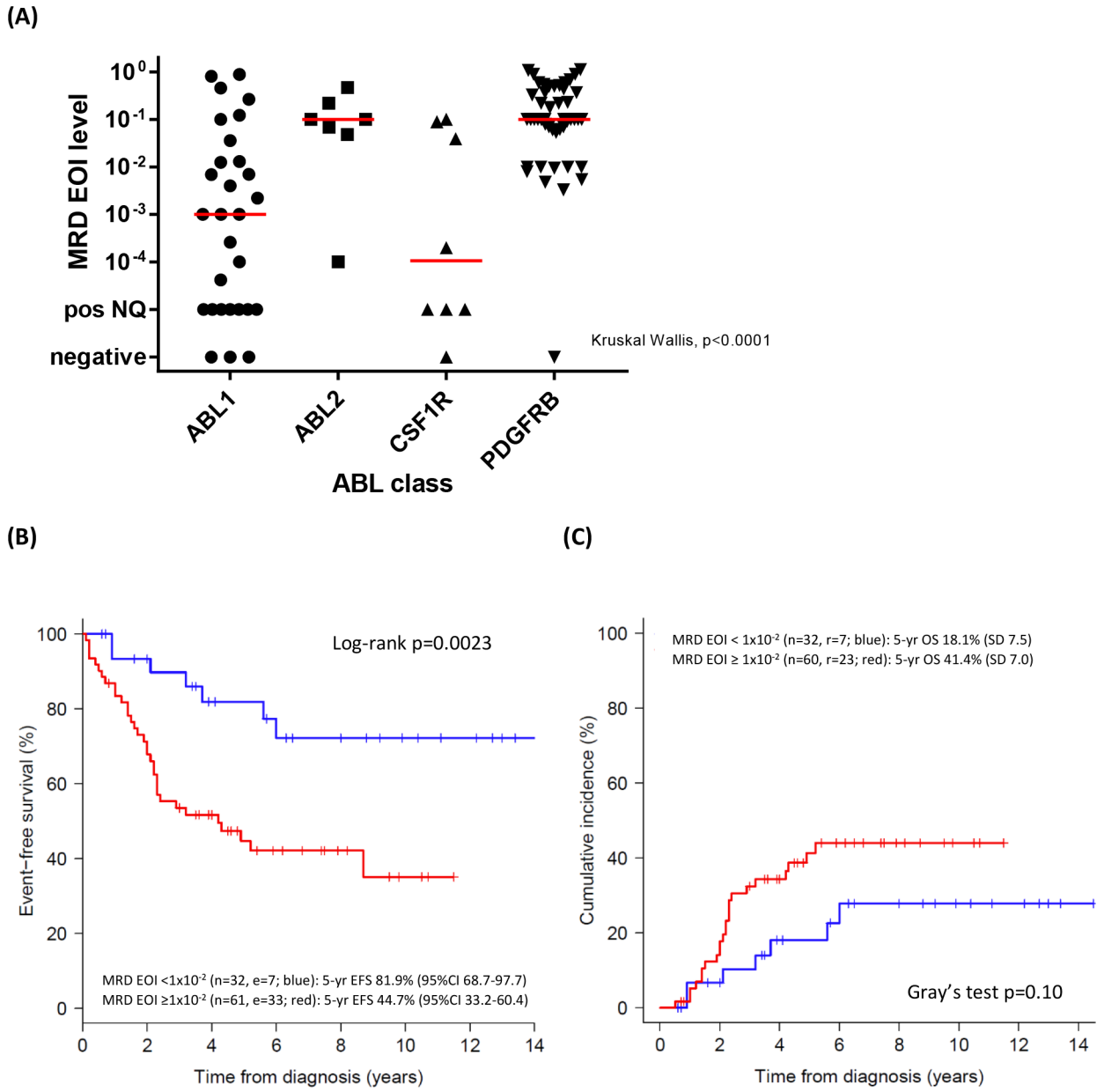


Figure 3: Minimal residual disease levels at the end of induction therapy in children with ABL-class B-ALL

(A) Absolute MRD levels at the end of induction (EOI) per ABL-class type. The red line indicates the median value per ABL-class type. Kruskal Wallis p<0.0001. *ABL1* and *CSF1R* MRD EOI levels are lower than those of *ABL2* (p=0.044 and p=0.041, respectively) and *PDGFRB* cases (p<0.0001 and p=0.0010, respectively).

(B) Event-free survival of ABL-class cases according to MRD EOI levels. MRD EOI $<1\times10^{-2}$, n= 32, blue line, 5-year EFS 81.9% (95%CI 68.7–97.7) and MRD EOI $\geq1\times10^{-2}$, n=61, red line, 5-year EFS 44.7% (95%CI 33.2–60.4), log-rank P=0.0023. Cox proportional HR 3.34, 95% CI 1.47–7.60, p=0.0039.

(C) Cumulative incidence of relapse according to MRD EOI levels. MRD EOI levels $<1\times10^{-2}$, n=32, blue, 5-year CIR 18.1% (SD 7.5) and MRD EOI levels $\geq1\times10^{-2}$, n=60, red, 5-year CIR 41.1% (SD 7.0), Gray's test p=0.10.

Table 1:

Patients characteristics and risk factors

Risk Factor	Category	ABL-class	ABL1	ABL2	CSF1R	PDGFRB	Pearson χ^2
		N (%)	N	N	N	N	
		122	40	8	10	64	
Sex	Male	76 (62%)	25	4	7	40	
	Female	46 (38%)	15	4	3	24	0.86 *
Age at diagnosis	mean±sd (years)	9.7±5.1	7.1±5.3	14±4.3	10.4±4.5	10.7±4.5	
	1–9 yr	54 (44%)	26	1	3	24	
	10–18 yr	68 (56%)	14	7	7	40	0.0066 *
WBC at diagnosis	mean±sd (counts ×10e9/L)	97.9±114.8	99.1±110.1	142.4±71.1	65.5±100.4	96.7±124	
	<50	62 (51%)	21	0	7	34	
	50–100	18 (15%)	4	2	1	11	
	≥100	41 (34%)	14	6	2	19	0.079 *
NCI risk	SR	28 (23%)	14	0	2	12	
	HR	93 (77%)	25	8	8	52	0.081 *
CNS involvement	no	112 (97%)	38	8	9	57	
	yes	4 (3%)	0	0	1	3	0.34 *
Testis involvement	no	44 (100%)	15	3	4	22	
	yes	0 (0%)	0	0	0	0	N/A
Ikaros status	IKZF1 wildtype	23(39%)	4	2	2	15	
	IKZF1 deleted	36 (61%)	12	2	5	17	0.44 *

Risk Factor	Category	ABL-class	ABL1	ABL2	CSF1R	PDGFRB	Pearson χ^2
	of which only IKZF1 deleted	13 (22%)	5	0	2	6	
	or IKZF1 and PAX5 and/or CDKN2A/2B deleted	23 (39%)	7	2	3	11	0.72 **

Percentage of cases per category out of total number of cases with registered information is given in parentheses.

* Pearson $\chi 2$ p-values are estimates because the number of cases is less than 5 for some variables.

** *IKZF1* "plus" versus *IKZF1* only.

N/A, Pearson $\chi 2$ not applicable since no patients with testis involvement were reported. Number of patients with missing data: WBC 1; NCI risk 1; CNS involvement 6; testis involvement 32.

Table 2:

Risk stratification and response of children with ABL-class positive B-ALL

Response criteria	Category	ABL-class	ABL1	ABL2	CSF1R	PDGFRB	Pearson χ^2
		N (%)	N	N	N	N	
		122	40	8	10	64	
Prednisone window response	PGR	29 (51%)	13	2	4	10	
	PPR	28 (49%)	1	3	0	24	0.00015 *
Treatment arm	non-HR	28 (23%)	16	1	4	7	
	HR	93 (77%)	23	7	6	57	0.0023 *
MRD EOI	negative	5 (5%)	3	0	1	1	
	$<10^{-4}$, including positive not quantifiable	11 (12%)	8	0	3	0	
	10^{-4} to 10^{-2}	16 (17%)	9	1	1	5	
	$\geq 10^{-2}$	61 (66%)	9	6	3	43	<0.0001 *
Type of events	no event, in CR	72 (59%)	27	3	8	34	
	total number of events	50 (41%)	13	5	2	30	N/A **
	early death	3 (2.5%)	1	0	0	2	
	non-responder	3 (2.5%)	0	0	0	3	
	relapse	35 (29%)	12	2	2	19	
	2nd malignancy	2 (1.5%)	0	0	0	2	
	death in 1st CR	7 (6%)	0	3	0	4	
HSCT in HR treated cases	no	43 (51%)	13	1	4	25	
(landmark 6 months)	in CR	25 (67%)	8	0	3	14	
	relapse	18 (33%)	5	1	1	11	
	yes	41 (49%)	8	2	2	29	N/A **

Response criteria	Category	ABL-class	ABL1	ABL2	CSF1R	PDGFRB	Pearson χ^2
	in 2nd CR	25 (61%)	4	1	1	19	
	total number of events after HSCT	16 (39%)	4	1	1	10	
	relapse	7 (17%)	1	1	1	4	
	2nd malignancy	2 (5%)	0	0	0	2	
	treatment-related mortality	7 (17%)	3	0	0	4	

PPR, prednisone poor response, defined by ≥1,000 blasts per μl of peripheral blood at day 8 of a therapeutic window with prednisone; PGR, prednisone good response, defined by <1,000 blasts per μl of peripheral blood at day 8. Percentage of cases in each category out of the total number of cases with registered information is given in parentheses. One exception: HSCT variable: the total number of patients in 2nd CR and those suffering from any event was set to 100%. Three non-responders (all *PDGFRB* fusion positive cases) also received HSCT, one relapsed, one suffered from treatment-related mortality and one achieved a 2nd CR. These 3 patients were included in the HSCT outcome analysis for whom time from landmark at 6 months to relapse, to death and last contact was used, respectively.

*Pearson χ2 p-values are estimates because the number of cases is less than 5 for some variables.

**N/A, Pearson χ2 not applicable since time-related occurrence of events. Number of patients with missing or no data: Prednisone window response 65; treatment arm 1; MRD EOI 29; HSCT 7.