Somatic variant profiling in chronic phase pediatric chronic myeloid leukemia

Clinical and cytogenetic risk factors play a crucial role in therapy stratification of chronic myeloid leukemia (CML). Pathogenic somatic variants beyond the *BCR::ABL1* fusion and the *ABL1* gene such as mutations in *ASXL1* have emerged as a genetic risk factor associated with inferior treatment response and outcome in adult CML.[1] Pediatric CML is characterized by distinctive clinical and genetic features.[2] However, there are few data on the mutational profile. The aim of this study was to establish the first somatic variant profile of a large and well-characterized cohort of pediatric patients diagnosed with CML in the chronic phase (CML-CP) and to assess clinical correlations.

Based on targeted next-generation sequencing covering 148 leukemia-associated genes, we analyzed 90 children and adolescents with CML-CP who had been enrolled in the German national CML-PAED registry at diagnosis. Fourteen individuals (16%) harbored at least one pathogenic somatic variant. The *ASXL1* gene was affected in 6 of these cases. Individuals with pathogenic somatic *ASXL1* variants presented with significantly higher initial platelet counts, and, generally, patients harboring a pathogenic somatic variant in *ASXL1* and other genes also showed a trend to inferior molecular response under tyrosine kinase inhibitor (TKI) treatment compared to patients without pathogenic variants.

Overall, we have uncovered fresh insights into the mutational landscape of childhood CML and identified the presence of pathogenic variants in addition to the *BCR::ABL1* fusion to be associated with differing hematologic and response characteristics. However, confirmation of the clinical significance of *ASLX1* and other somatic variants requires prospective data from larger numbers of cases with this rare disease in childhood.

All patients included in this study were diagnosed between 2006 and 2022 and enrolled in the German national CML-PAED-II trial and subsequent registry. The CML-PAED trial protocol was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethics boards of the medical faculties of the Technical University Dresden and the Friedrich-Alexander-Universität Erlangen-Nürnberg (EK282 122 006 and EK 236_18 B). The trial was registered at EUDRACT (2007-001339-69) and clinicaltrials.gov (NCT00445822).[3] Informed consent was obtained from the patients' legal representatives and, if applicable, the patients, after providing age-appropriate oral and written information. Diagnostic and response criteria were applied according to European LeukemiaNet (ELN) criteria.[4] Sequencing was performed using an IDT custom panel including 148 leukemia-associated genes / gene regions (*Online Supplementary Table S1*) according to the manufacturer´s instructions (Integrated DNA Technologies Inc., IA, USA). DNA samples isolated either from the patients' initial blood or from bone marrow were sequenced. Variants with a variant allele fraction (VAF) of ≥5% and a depth of ≥500 reads were included in the analysis. All detected variants were classified according to the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG).[5] Here, we only report alterations classified as likely pathogenic and pathogenic somatic variants. These will be further summarized and referred to as pathogenic somatic variants.

Characteristics of 90 pediatric patients diagnosed with CML-CP and included in this study are provided in Table 1. Age and sex distribution of patients, as well as the distribution of *BCR::ABL1* transcript types and cytogenetic categories, were representative for the overall cohort.[3] In 17 individuals (19%), pathogenic variants were identified in the initial sample. Follow-up material collected in molecular response (MR) 2 or better was subsequently analyzed in all of these cases. In 14 patients (16%), the pathogenic variants were undetectable in remission as proof of their somatic nature. In 3 patients, the variants were unchanged in remission and were, therefore, classified as germline. Consequently, at diagnosis of CML-CP, 14 out of 90 patients (16%) carried a total 15 pathogenic somatic variants (Figure 1A). The genes *ASXL1, ASXL2, BCOR, GATA2, IKZF1, KDM6A, KMT2D*, and *TET2* were affected (Figure 1B, *Online Supplementary Table S2*). Pathogenic somatic variants in *ASXL1* were the most frequent and were identified in 6 patients (43%), with one individual harboring two different *ASXL1* variants (Figure 1C).

Patients with pathogenic somatic variants in *ASXL1* and other genes showed a trend to higher initial leukocyte counts (Figure 2A). Initial platelet counts were significantly elevated in patients carrying pathogenic somatic *ASXL1* variants as compared to patients without pathogenic somatic variants in this analysis (P=0.027) (Figure 2B). Assessment of response kinetics exhibited a trend to a delayed cytogenetic response in the subgroup of patients with pathogenic somatic variants in the *ASXL1* gene (Figure 2D). Individuals with a pathogenic somatic variant in *ASXL1* and other genes also revealed a trend to inferior molecular response characteristics reaching major molecular response and deep molecular response at later time points than patients lacking pathogenic somatic variants (Figure 2E).

According to reports in adult patients, myeloid-leukemia-associated mutations apart from the disease-driving *BCR::ABL1* fusion gene and resistance-mediating *ABL1* kinase domain variants are found in a considerable proportion of

newly diagnosed individuals with CML, most commonly affecting epigenetic modifier genes such as *ASXL1, DNMT3A*, and *TET2*.[6-8] Different retrospective analyses have suggested an inferior response to treatment with TKI in adult patients with CML harboring such lesions.[7,9] In large population studies, these variants have also been associated with age-related clonal hematopoiesis of indeterminate potential (CHIP) occurring in 10% of people >65 years of age but only 1% in people aged <50 years.[10-12] Pathogenic variants in the *ASXL1* gene were linked to worse MR and outcome in adults with CML.[1,13] Owing to the rarity of CML in childhood and adolescence, knowledge on pathogenic somatic variants in pediatric CML is still extremely limited. So far, there has only been one investigation into the mutational landscape in pediatric patients with CML. Ernst and colleagues assessed a cohort of 21 young CML patients including 16 pediatric cases <18 years of age. Four pediatric patients (25%) carried a pathogenic variant in the *ASXL1* gene, and the authors concluded that such variants were frequent in children and young adults with CML.[14]

Based on a total of 90 pediatric patients up to the age of 18 years diagnosed with CML-CP, we here present the first large systematic analysis of the variant profile in childhood CML. Notably, the proportion of patients with an *ASXL1* variant

Table 1. Characteristics of pediatric patients in chronic phase chronic myeloid leukemia included in this study.

Characteristics	Total N=90 N (%)	[a]Without pathogenic somatic variants N=76 N (%)	[b]With pathogenic somatic variant N=14 N (%)
Median age at diagnosis in years (range)	13 (1-18)	13 (1-18)	14 (1-7)
Sex			
Female	37 (41)	28 (37)	6 (43)
Male	53 (59)	48 (63)	8 (57)
Fusion transcript			
e13a2	30 (33.33)	28 (37)	2 (14)
e14a2	38 (42.22)	30 (39)	8 (58)
e13a2/e14a2	11 (12.22)	9 (12)	2 (14)
Unknown	11 (12.22)	9 (12)	2 (14)
Karyotype at diagnosis			
t(9;22)	86 (96)	72 (95)	14 (100)
Single	76 (84)	63 (83)	13 (93)
Variant translocation t(9;22)	7 (7)	7 (9)	0 (0)
Additional chromosome abnormalities	2 (2)	2 (3)	0 (0)
Complex karyotype	1 (1)	0 (0)	1 (7)
Unknown	4 (4)	4 (5)	0 (0)
Treatment response			
Median molecular response at 3 months (range)	5.28 (0.11-76)	4.25 (0.1142-76)	6.60 (0.5722-64)
Ratio *BCR::ABL1/ABL1*			
IS (optimal: ≤10%)	56 (62)	48 (63)	8 (57)
IS (warning: >10%)	26 (29)	21 (28)	5 (36)
Unknown	8 (9)	7 (9)	1 (7)
Median molecular response at 6 months (range)	0.4609 (0-43)	0.3400 (0-24)	1.2050 (0.0030-43)
Ratio *BCR::ABL1/ABL1*			
IS (optimal: ≤1%)	50 (56)	44 (58)	6 (43)
IS (warning: >1-10%)	22 (24)	19 (25)	3 (21.5)
IS (failure: >10%)	5 (6)	2 (3)	3 (21.5)
Unknown	13 (14)	11 (14)	2 (14)
Molecular response at 12 months, median (range)	0.0593 (0-3.8)	0.0580 (0-2.85)	0.3250 (0.0057-3.8)
Ratio *BCR::ABL1/ABL1*			
IS (optimal: ≤0.1%)	38 (42)	35 (46)	3 (21.5)
IS (warning: >0.1-1%)	16 (18)	14 (18)	2 (14)
IS (failure: >1%)	9 (10)	6 (8)	3 (21.5)
Unknown	27 (30)	21 (28)	6 (43)
Median major molecular response in months (range)	12 (4-48)	12 (4-48)	9 (6-24)
Unknown/not reached until today	26 (29)	21 (28%)	5 (36)
Median complete cytogenetic remission in months (range)	6 (2-20)	6 (2-20)	5 (2-17)
Unknown/not reached until today	24 (27)	20 (26)	4 (29)

[a]Without pathogenic somatic variant, i.e., no variants, (likely) pathogenic germline variants and variants classified as variant of unknown significance (VUS) according to the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG). [b]With pathogenic somatic variant, i.e., variants classified as likely pathogenic and pathogenic somatic variants according to the ACMG standards and guidelines. IS: International Score.

in our study was lower than in the analysis by Ernst *et al*. This can be explained by the fact that here we assessed an unselected population-based cohort. Therefore, and based on the significantly larger number of patients, our results are more likely to reflect the true incidence of pathogenic somatic *ASXL1* variants in pediatric CML.

The proportion of patients with pathogenic variants in our analysis was <20% and therefore lower than that described for adults with CML who harbor myeloid-leukemia-associated mutations in addition to *BCR::ABL1*; approximately 30% of cases at initial diagnosis.[6-8] This observation supports the hypothesis that a proportion of additional pathogenic variants found in adult patients with CML can not be disease-associated but are to be attributed to age-related CHIP, which is not present in pediatric individuals.[10-12] There was no difference in the actual spectrum of genes found to be mutated in this cohort of pediatric patients with those found in adult patients. Together with the data from adult cohorts[1,13] and the single pediatric report to date by Ernst *et al*.,[14] our observation that *ASXL1* was also the most fre-

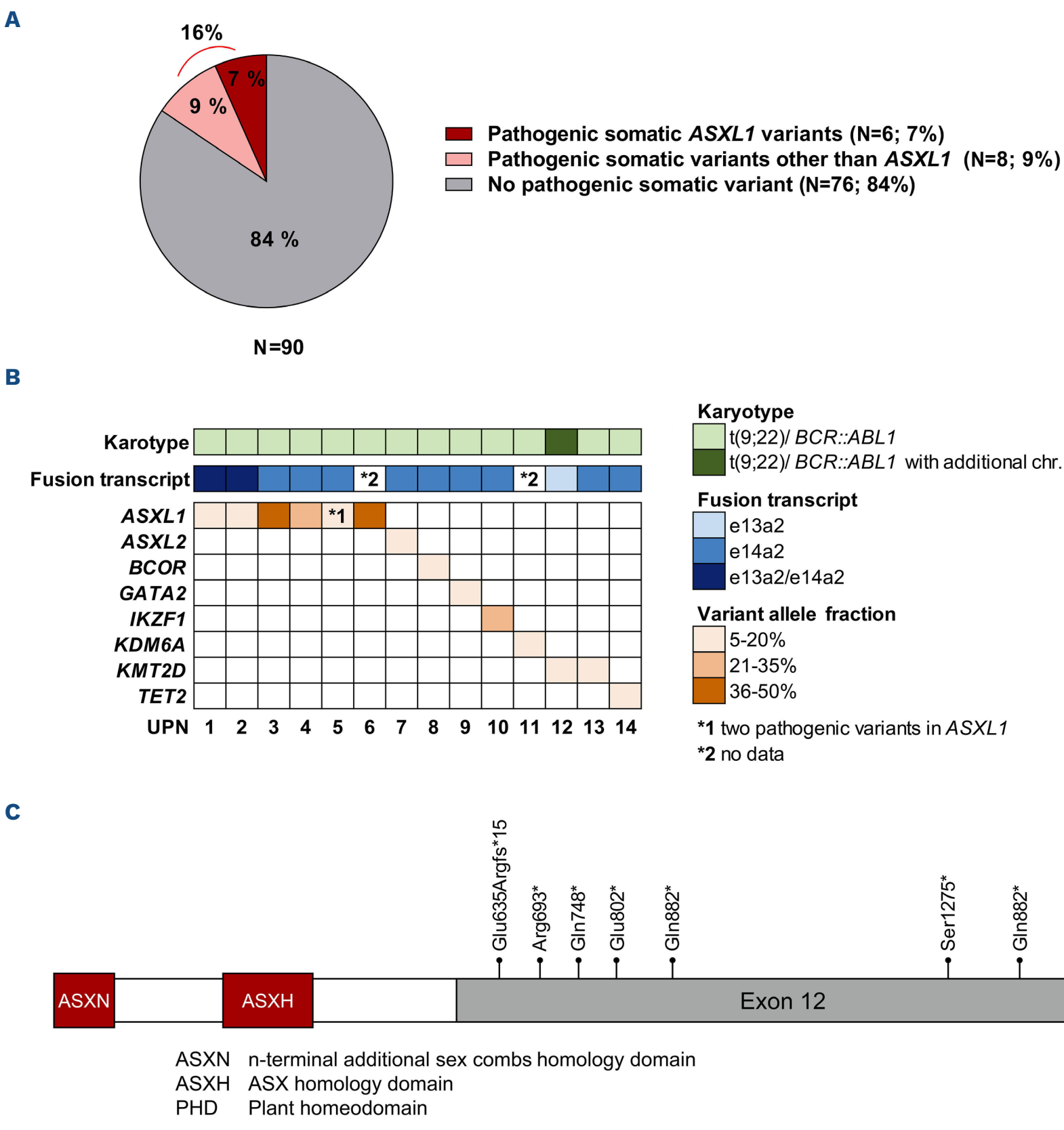

Figure 1. Pathogenic somatic variant landscape. (A) Proportional distribution of patients without pathogenic somatic variants (gray), patients with pathogenic somatic variants in genes other than *ASXL1* (light red), and patients with pathogenic somatic variants in *ASXL1* (dark red). (B) Pathogenic somatic variants detected in 14 individuals in 8 different genes (lower panel) shown in relation to karyotype and *BCR::ABL1* fusion transcript (upper panels). Graduation of variant allele frequency illustrated by color intensity. (C) Localization of pathogenic somatic variants in the *ASXL1* gene. chr.: chromosomal.

quently mutated gene in this pediatric study suggests that these gene mutations could be disease-related rather than age-related, and that they might have a functional role in the pathogenesis and progression of CML.

In this analysis, individuals harboring pathogenic somatic *ASXL1* variants exhibited higher initial leukocyte and platelet counts. These features could reflect a relative increase in proliferative capacity in this particular subgroup. Moreover, the group of patients carrying pathogenic somatic variants in *ASXL1* and other genes also showed a trend to a delayed molecular response as compared to patients lacking pathogenic variants in our screening. This observation suggests that the presence of a pathogenic somatic gene variant in addition to the *BCR::ABL1* fusion gene at diagnosis could represent an adverse prognostic factor associated with a delay in achieving a deep molecular response, thus requiring a prolonged intake of TKI. In children and adolescents, this may compromise growth (a particular TKI side effect in these age groups).[2] Based on data derived from adult patients showing a higher effectiveness of second-line TKI such as dasatinib compared to imatinib,[15] it appears conceivable that patients carrying such alterations could be candidates for an early switch to second-line TKI therapy; this would promote a fast and deep molecular response, thus enabling rapid withdrawal of treatment and, consequently, the lowest possible incidence of side effects. This current study for the first time provides a detailed insight into the variant profile of pediatric CML-CP. Con-

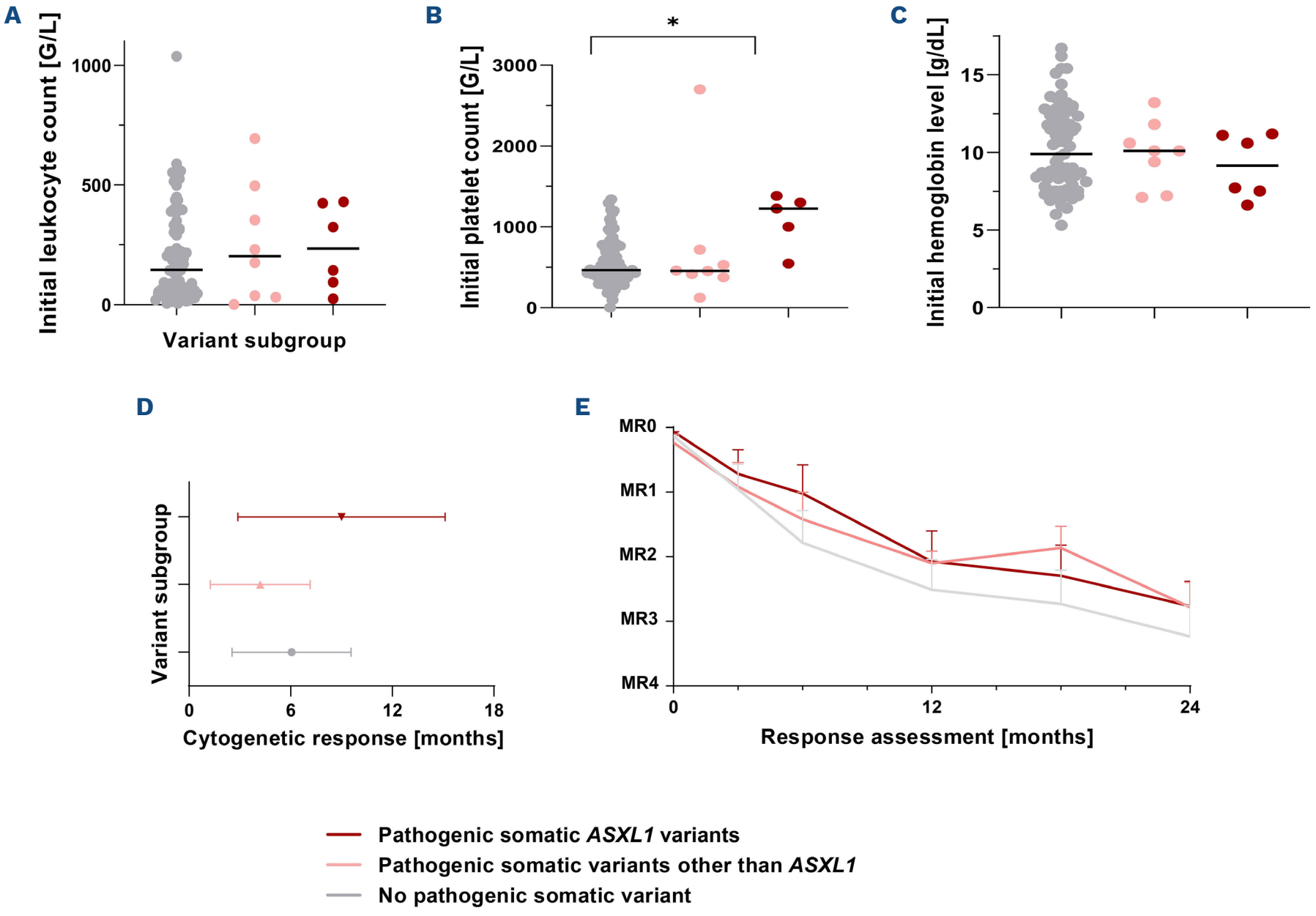

Figure 2. Initial hematologic parameters, time point of cytogenetic response and molecular response over time according to variant subgroup. (A) Initial leukocyte count in patients without pathogenic somatic variants (gray), patients with pathogenic somatic variants in genes other than *ASXL1* (light red), and patients with pathogenic somatic variants in *ASXL1* (dark red). (B) Initial platelet count in patients without pathogenic somatic variants (gray), patients with pathogenic somatic variants in genes other than *ASXL1* (light red), and patients with pathogenic somatic variants in *ASXL1* (dark red). (C) Initial hemoglobin level in patients without pathogenic somatic variants (gray), patients with pathogenic somatic variants in genes other than *ASXL1* (light red), and patients with pathogenic somatic variants in *ASXL1* (dark red). Unpaired one-way ANOVA was used for statistical comparison between the subgroups. (D) Time point of cytogenetic response in patients without pathogenic somatic variants (gray), patients with pathogenic somatic variants in genes other than *ASXL1* (light red), and patients with pathogenic somatic variants in *ASXL1* (dark red). (E) Initial *BCR::ABL1* transcript level and molecular response over time in patients without pathogenic somatic variants (grey), patients with pathogenic somatic variants in genes other than *ASXL1* (light red), and patients with pathogenic somatic variants in *ASXL1* (dark red).

sidering the possible prognostic role of the occurrence of pathogenic somatic variants beyond *BCR::ABL1*, it highlights the significance and the potential predictive value of variant profiling in pediatric CML. However, the number of pediatric patients with CML-CP assessed in this study is still limited. Therefore, joint international efforts enabling a deep and individual analysis of the potential role and relationship of additional genetic variants in pediatric CML are required.

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Disclosures

No conflicts of interest to disclose.

Contributions

YLB, GG and AK designed the research study. YLB, LG, TR, ES, SK, RS, SS and ZW performed the research. YLB, RN, TR, MS, MK, BS, MM, GG and AK analyzed the data. YLB, RN, MS, MK, BS, MM, GG and AK wrote the paper.

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Data-sharing statement

The data presented in this study are available only on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

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