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ORIGINAL ARTICLE

Hematological, clinical, immunophenotypic characterization, and treatment outcomes of prognostically significant genetic subtypes of B-lineage acute lymphoblastic leukemia: A report of 1021 patients from India

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Abstract

Background: The published literature on hematological, clinical, flowcytometric-immunophenotyping, and minimal residual disease outcomes of the prognostically important genetic subtypes of acute lymphoblastic leukemia (ALL) is scarce from low-income countries. For newer classifications such as *BCR::ABL1*-like ALLs, the scarcity of patient-level data is even more pronounced.

Methods: The authors performed comprehensive detection of recurrent gene fusions and *BCR::ABL1*-like ALL cases followed by immunophenotypic profiling and obtained clinical outcome parameters for a large cohort ($n = 1021$) of patients from India. This cohort included a significant number of patients with *BCR::ABL1*-like ALL subtype and other genetic subtypes of ALL.

Results: Patients with *BCR::ABL1*-positive and *BCR::ABL1*-like ALL were significantly older, had male preponderance, and expressed a higher white blood cell count than *BCR::ABL1*-negative cases ($p < .05$). Logistic regression modeling of B-lineage-ALL (B-ALL) subtypes revealed that cluster of differentiation (CD)36 is a strong statistically significant predictive marker of *BCR::ABL1*-like ALL ($p < .05$). Furthermore, patients with *BCR::ABL1*-like ALLs show a significantly higher frequency of CD36 expression compared to *BCR::ABL1*-negative ALLs ($p < .05$). In terms of clinical symptoms, lymphadenopathy is a strong statistically significant predictive marker in *BCR::ABL1*-like ALLs compared to *BCR::ABL1*-negative ALL cases ($p < .05$). In terms of treatment outcomes, minimal residual disease (MRD) positivity in *BCR::ABL1*-positive ALL cases were statistically significant ($p < .05$), and *BCR::ABL1*-like ALL cases had high MRD-positivity as compared to *BCR::ABL1*-negative ALL cases but did not show statistical significance.

Conclusions: The findings evince the use of novel therapies and personalized treatment regimens to improve the overall survival of the newer incorporated

entities in B-ALLs. This is the first report characterizing the hematological, clinical, flowcytometric-immunophenotyping, and minimal residual disease outcomes of the prognostically significant subtypes of ALLs in patients from India.

Plain Language Summary

- Characterizing the hematological, clinical, flowcytometric-immunophenotyping, and minimal residual disease outcomes of the prognostically significant subtypes (n = 1021) of acute lymphoblastic leukemia (ALLs) in patients from India.
- We have made two independent logistic regression models of cluster of differentiation (CD) markers and clinical symptoms to differentiate prognostically significant subtypes of ALLs.
- Logistic regression analysis of CD markers revealed CD36 as a strong predictor in *BCR::ABL1*-like ALL cases compared to *BCR::ABL1*-negative ALL cases.
- Logistic regression analysis of clinical symptoms revealed lymphadenopathy significantly predicts *BCR::ABL1*-like ALLs ($p < .05$).
- In terms of treatment outcomes, *BCR::ABL1*-positive ALL had statistically significant minimal residual disease (MRD) ($p < .05$), and *BCR::ABL1*-like ALL cases had high MRD-positivity but did not show statistical significance as compared to *BCR::ABL1*-negative ALLs.

KEYWORDS
B-acute lymphoblastic leukemia (B-ALL), cluster of differentiation (CD) markers, flowcytometric-immunophenotyping (FCM-IP), high-risk ALLs, PGIMER in-house rapid and cost-effective classifier (PHi-RACE), recurrent fusion transcripts (RGFs)

INTRODUCTION

Acute lymphoblastic leukemia (ALL), one of the most common pediatric cancers, predominantly occurs in the pediatric age group of 2–10 years.[1–3] ALL remains the leading cause of morbidity in children and has worse clinical outcomes in adults.[4,5] The genetic landscape of ALL is highly heterogeneous and characterized by significant recurrent cytogenetic and/or molecular genetic abnormalities including *BCR::ABL1*, *ETV6::RUNX1*, *TCF3::PBX1* and *KMT2A-AFF1*, *DUX4*-rearrangement (~r), *MEF2D9*-r, *ZNF384*-r, PAX-r, *BCR::ABL1*-like, and genetic alterations in lymphoid transcription factors, most commonly *IKZF1* (IKAROS family zinc finger 1).[1] These structural and/or molecular variations, sequence mutations, and copy number variations disrupt the maturation of the lymphoid lineage, leading to uncontrolled cell growth and ultimately leading to leukemogenesis.[2,4–7]

Recently, several new genetic entities have been incorporated, as outlined above, into the biology of ALLs from high-income nations.[1] Detecting the high-risk genetic subtypes of ALL is still a pending task for low-income countries, especially *BCR::ABL1*-like ALL cases.[8,9] *BCR::ABL1*-like ALL is considered an entity in the 2022 World Health Organization classification of hematolymphoid neoplasms.[10,11] This entity is specified by a similar gene expression profile to that of Philadelphia (Ph)-positive ALL cases, without expressing *BCR::ABL1* fusion transcripts originating from Ph-chromosome and additionally associated with poorer clinical outcomes.[5–9,12–37]

An extensive literature search revealed the scarcity of published data on hematological, flowcytometric-immunophenotypic profile (FCM-IP), clinical characterization, and minimal residual disease (MRD) outcomes of prognostically important subtypes from low-income countries. In our previous studies, we have reported the incidence of 18.66% (184 of 986) *BCR::ABL1*-positive ALLs, 5.17% (51 of 986) *ETV6::RUNX1*-positive ALLs, 3.95% (39 of 986) *TCF3::PBX1*-positive ALLs, 1.21% (12 of 986) *KMT2A-AFF1*-positive ALLs, and 26.67% *BCR::ABL1*-like ALL cases of patients from India.[8,38] In this study, we characterized the hematological, immunophenotypic profile associated with aberrant myeloid markers, clinical characterization, and MRD outcomes of prognostically important subtypes of B-ALLs in patients from India (n = 1021). Evaluating hematological, immunophenotypic profile, clinical description, and MRD outcomes helps make alternate curative decisions in treating ALLs, especially for newly incorporated genetic subtypes.

MATERIALS AND METHODS

Patient selection and sample collection

The Department of Hematology of North Indian Tertiary Care Center, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, has provided the necessary

infrastructure and facilities for this research study. Institutional ethics committee (IEC) established by PGIMER approved this research study (vide no. INT/IEC/2017/191; 10.23.2017). Written consent was attained from all the participants, and the study was performed as per the Declaration of Helsinki (1975) (revised 2008). The study cohort included 1021 B-ALLs classified according to National Cancer Institute classification, as described in the Consolidated Standards of Reporting Trials Diagram (Figure 1). We have studied the clinical, hematological, FCM-IP, and MRD outcomes of prognostic significant genetic subtypes of B-ALL cases (n = 1021) including t(9:22)(*BCR::ABL1*), t(1:19)(*TCF3::PBX1*), t(12:21) (*ETV6::RUNX1*) and t(4:11)(*KMT2A::AFF1*), and *BCR::ABL1*-like ALL cases. The routine diagnostic workup of B-ALL patients in the Department of Hematology has been described in the Supplementary Methods.

FCM-IP

After morphological examination, peripheral blood/bone marrow samples were prepared to diagnose B-ALL cases using FACS-Navios (Beckman Coulter, California) and FACS-Canto II (BD Biosciences), and the data was acquired using Kaluza 2.1 and FACS Diva software 6.1.2.[38,39] The standardized primary panel of antibodies for diagnosing B-ALL cases is shown in Table S1. FCM-IP method has been described in the Supplementary Methods.

Recurrent gene fusion detection in B-ALLs

The total RNA was extracted from newly diagnosed B-ALL cases using Qiagen Mini-amp Blood RNA kit (Qiagen, Hilden, Germany). Furthermore, quantified RNA samples were subjected to iScript complementary DNA (cDNA) Synthesis Kit (Bio-Rad, Hercules, California) for cDNA synthesis. Last, cDNA (1 μg) was subjected to standardized multiplex reverse transcriptase–polymerase chain reaction (RT-PCR) to identify four recurrent gene fusions (RGFs) according to Pakakasama et al.[40,41] The primers of four RGFs are shown in Table S2 and the positive controls were provided by the Christian Medical College (Vellore, India) on request.

Identification of *BCR::ABL1*-like ALL cases

In recently published studies, we built a PGIMER in-house rapid and cost-effective classifier (PHi-RACE):PGIMER in-house rapid and cost-effective classifier using logistic predictive statistical computing for quick identification of *BCR::ABL1*-like ALL cases at diagnosis. With the PHi-RACE classifier, we have reported an incidence of 26.67% (143 of 536) *BCR::ABL1*-like ALLs from patients of Indian ethnicity.[8] The PHi-RACE classifier has been further described in the Supplementary Methods.

Logistic regression of CD markers and clinical symptoms across ALL subtypes

We have performed logistic regression on CD markers and clinical symptoms across ALL genetic subtypes using the glm function in R statistical software.[42,43] The logistic regression modeling of CD markers and clinical symptoms is further described in the Supplementary Methods.

MRD analysis

For performing the MRD analysis of adult and pediatric B-ALLs treated with modified Berlin-Frankfurt-Munich and Indian Childhood Collaborative Leukemia Group protocol at the end of induction therapy (EOI) (day 28),[44,45] we used standardized lyse-stain-wash method for MRD sample preparation.[46] MRD assay was standardized for a lower limit of quantification of 10^{-3} and considered positive at a threshold of >0.01% as per the institutional treatment protocol.[47,48] The MRD method is described in the Supplementary Methods.

Statistical analysis

Descriptive analysis has been presented as mean, median, and range. The Gaussian distribution of data was checked using Shapiro–Wilk test. For normally distributed data sets, Student t-test was used to compare two groups, whereas Mann-Whitney U test was performed for non-normally distributed data. Chi-square was used to compare categorical data. ANOVA was used for more than two-group comparisons. Binary logistic regression analysis of CD markers and clinical symptoms was performed on various genetic subtypes of ALLs using the glm function of R. All statistical tests we used in this study were two-tailed, with a significance level of $p < .05$ (represented as $^{*}p \leq .05$, $^{**}p \leq .01$, $^{***}p \leq .001$, $^{****}p \leq .0001$ in Table 1). All the statistical analyses were performed using licensed GraphPad Prism (v9.2) and R (v4.1).

RESULTS

Patient characteristics

We studied 1021 newly diagnosed B-ALLs from the period of January 3, 2017 to January 6, 2022. The overall sex ratio was 1:0.61, with 635 males and 386 females. The median age of B-ALLs was 11 (range, 1–85 years). The hematological features, including complete blood count, showed median hemoglobin was 7.9 g/dL (range, 2.4–15.5 g/dL), median white blood cell count (WBC) was 12.2 (range, 0.3–576 × 10^9/L), median platelet count was 28 (range, 1.7–703 × 10^9/L), and blasts count was 90% (range, 20%–99%) in *B-ALLs*. The clinicobiological features of identified different genetic subtypes of B-

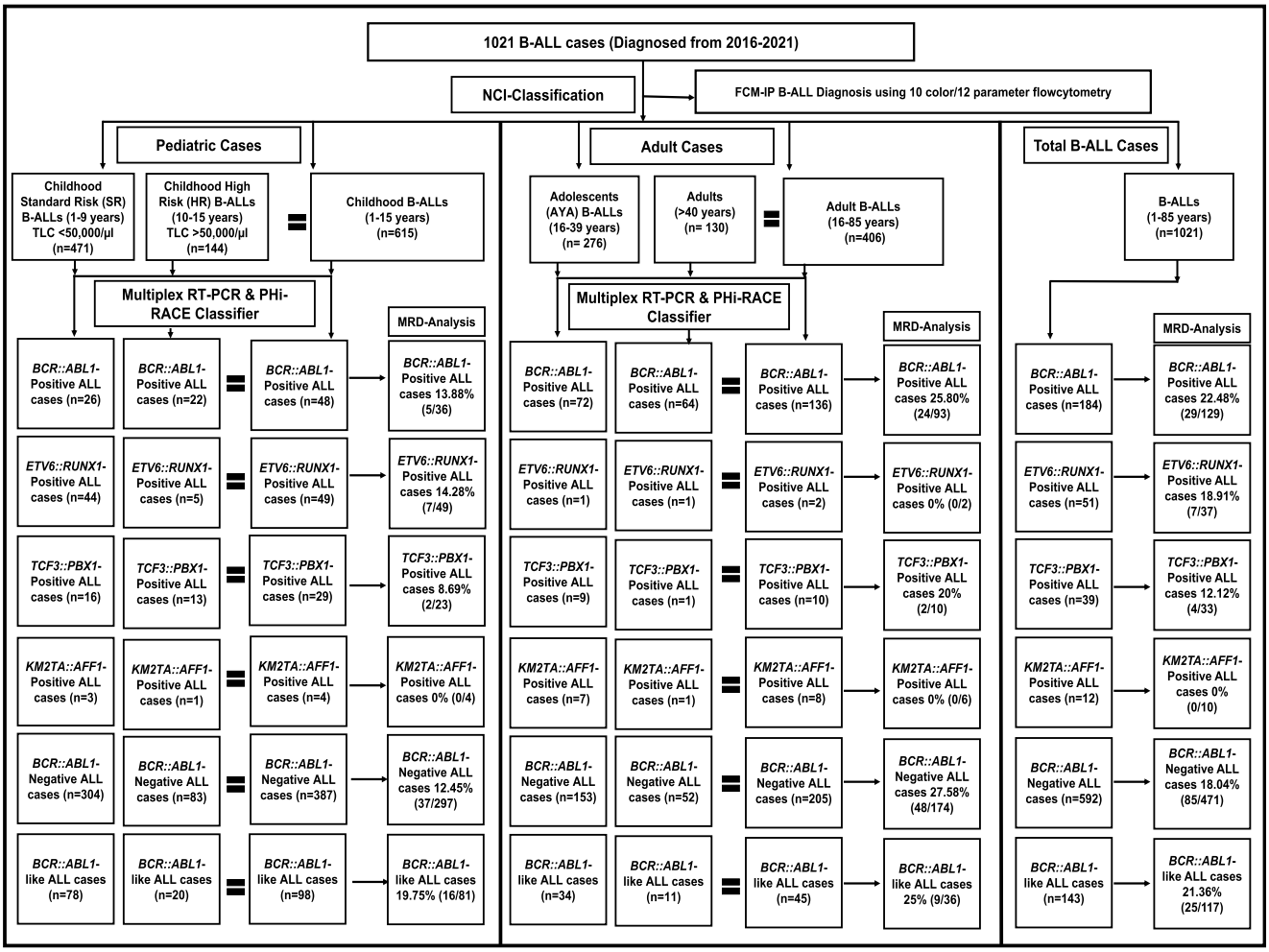

FIGURE 1 The study cohort included 1021 B-lineage acute lymphoblastic leukemia (B-ALL) cases diagnosed (January 3, 2017–January 6, 2022) according to flowcytometric-immunophenotyping. The B-ALL cases have been classified according to National Cancer Institute classification. Age 1–9 years, WBC count <50,000 per cubic millimeter at diagnosis = standard risk (SR) B-ALL cases (n = 471); age 10–15 years, WBC count >50,000 per cubic millimeter at diagnosis = high risk (HR) B-ALL cases (n = 144); age 16–39 years = adolescents and young adults (AYA) (n = 276); and age >40 years = adults (n = 130).

ALLs, including *BCR::ABL1*-negative ALLs (n = 592), *BCR::ABL1*-positive ALLs (n = 184), *ETV6::RUNX1* (n = 51), *TCF3::PBX1* (n = 39), *KM2TA::AFF1* (n = 12),[38] and *BCR::ABL1*-like ALLs (n = 143)[8] of ALLs are shown in Table 1 and Figure S1.

Flowcytometric-immunophenotype of various genetic subtypes of ALLs

The incidence of B-lineage surface and cytoplasmic CD markers expression including CD19, CD10, CD20, CD34, CD38, HLA-DR, TDT, CD58, CD36, CD123, CD81, CD86, cytoCD22, cytoCD79a, and CD45 was determined across various genetic subtypes of ALLs, as shown in Table S3 and Figure 2. We analyzed the frequency of expression of myeloid-associated markers (e.g., CD13, CD33, and CD117) across various genetic subtypes of ALLs. *BCR::ABL1*-positive and *ETV6::RUNX1*-positive ALL cases demonstrated a significantly higher frequency of CD13 and CD33 expression compared to other different genetic subtypes of ALL ($p < .05$). CD13 and CD33 expressing *BCR::ABL1*-positive and *ETV6::RUNX1*-positive ALL cases had male preponderance and significantly higher WBC compared with other genetic subtypes of ALLs. The expression of CD33 was higher in *BCR::ABL1*-like ALL cases but with no statistical significance compared to *BCR::ABL1*-negative ALL cases. In *KM2TA::AFF1*, we did not observe the expression of myeloid-associated markers. The comparison of myeloid-associated markers across various genetic subtypes of ALLs is shown in Table S4 and Figure 3. A representative *BCR::ABL1*-like ALL case shows the aberrant expression of myeloid-associated markers, as shown in Figure S2.

Logistic regression model of clinical symptoms in six genetic subtypes of ALLs

The frequency of clinical symptoms manifested across various genetic subtypes of ALLs at diagnosis, including fever, bleeding, bony

TABLE 1 Clinicobiological features of various genetic subtypes of B-lineage ALLs.

Parameter	B-ALLs ($n = 1021$)	*BCR::ABL1*-negative ALLs ($n = 592$)	Significance	*BCR::ABL1*-positive ALLs ($n = 184$)	Significance	*ETV6::RUNX1*-positive ALLs ($n = 51$)	Significance	*TCF3::PBX1*-positive ALLs ($n = 39$)	Significance	*KMT2A::AFF1*-positive ALLs ($n = 12$)	Significance	*BCR::ABL1*-like ALLs ($n = 143$)	Significance
Age (years)	11 (1–85)	11 (1–85)	NS	31.50 (1–68)	****	5 (1–51)	****	11 (1–45)	NS	17.5 (1–44)	NS	14 (1–79)	NS
Median													
Range													
Males, % (*n*/*N*)	62.19 (635/1021)	65.03 (385/592)	NS	54.34 (100/184)	****	62.74 (32/51)	***	51.28 (20/39)	NS	41.66 (5/12)	NS	64.33 (92/143)	*
Females, % (*n*/*N*)	37.80 (386/1021)	20.27 (207/592)	NS	45.65 (84/184)	NS	37.25 (19/51)	NS	48.71 (19/39)	NS	58.33 (7/12)	NS	35.66 (51/143)	NS
Hb g/L	7.9 (2.4–15.5)	7.9 (2.4–15.5)	NS	8.0 (2.7–15)	NS	8.2 (3.3–15.2)	NS	7.6 (3.7–8.7)	NS	7.0 (4.3–13.3)	NS	8.1 (3–14)	NS
Mean (range)													
Platelet, $\times 10^9$/L	28 (1.7–703)	28 (1.9–703)	NS	29 (2–467)	NS	30 (9–181)	NS	23 (1.7–208)	NS	22 (10–113)	NS	28 (14–532)	NS
Median (range)													
TLC, $\times 10^9$/L	12.2 (0.3–576)	9.7 (0.3–396)	NS	41.0 (0.7–576)	****	8.4 (0.9–246)	NS	18.7 (1.7–301.6)	**	129.9 (2.2–450.2)	****	13 (0.4–318)	NS
Median (range)													
Blasts, % (*n*/*N*)	90 (20–99)	89 (20–99)	NS	90 (36–98)	NS	90 (68–97)	NS	90 (56–99)	NS	91.50 (69–98)	NS	87 (25–99)	NS

Note: Normally distributed data, *t*-test; non-normally distributed data, Mann-Whitney test.

Abbreviations: ALLs, acute lymphoblastic leukemias; Hb, hemoglobin; NS, not significant; PLT, platelet; TLC, total leukocyte count.

*$p < 0.05$.

**$p < 0.01$.

***$p < 0.001$.

****$p < 0.0001$.

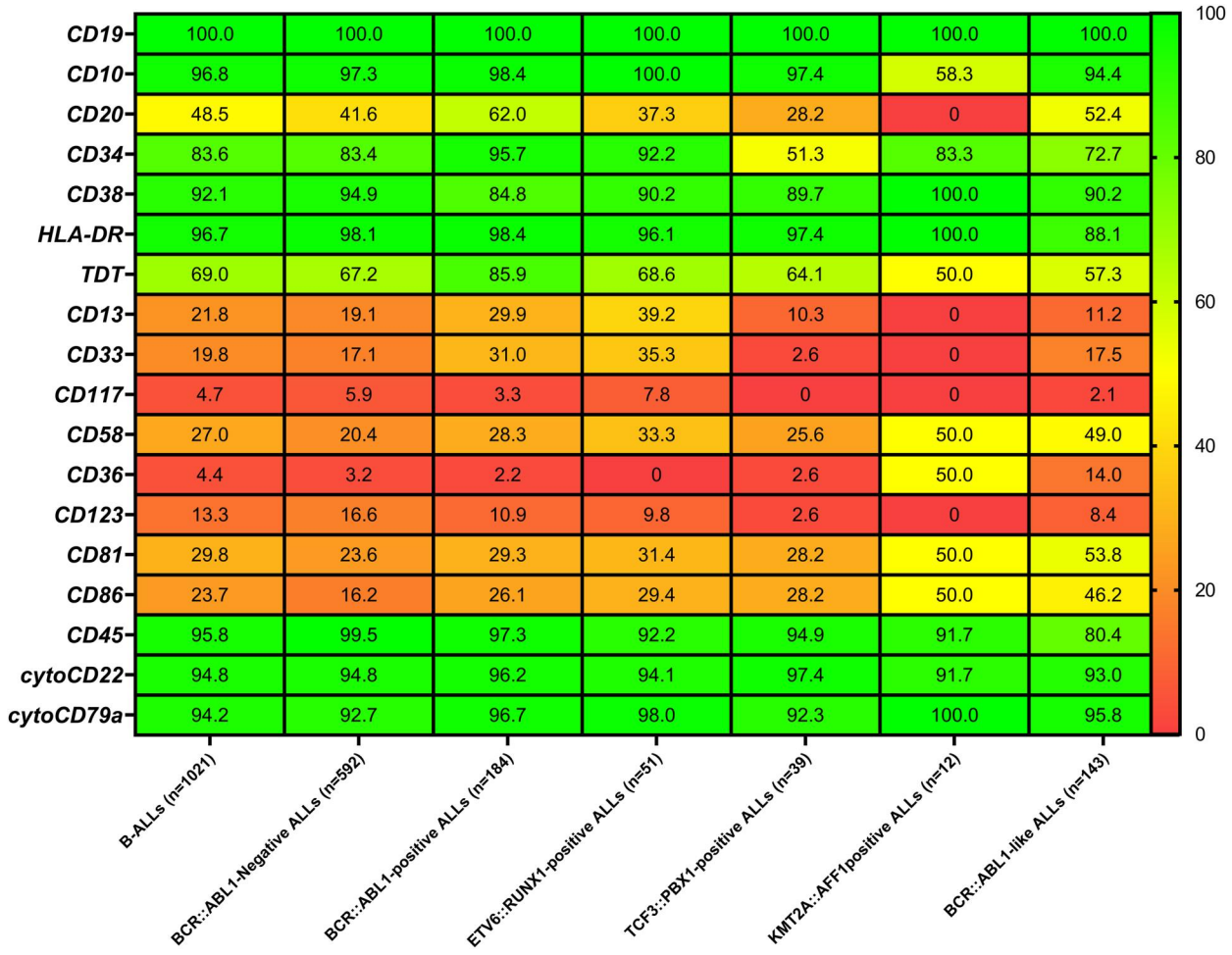

FIGURE 2 The % positivity of cluster of differentiation marker expression, including surface and cytoplasmic B-lineage markers in B-lineage acute lymphoblastic leukemias (B-ALLs), *BCR::ABL1*-negative ALL cases, *BCR::ABL1*-positive ALL cases, *ETV6::RUNX1*-positive ALL cases, *TCF3::PBX1*-positive ALL cases, and *BCR::ABL1*-like ALL genetic subtypes of ALLs.

tenderness, bone pains, ecchymosis, progressive pallor, lymphadenopathy, and organomegaly, including hepatomegaly and splenomegaly, is shown in Figure S3 and Table S5. Logistic regression analysis was performed in B-ALL subtypes (including *BCR::ABL1*-negative ALLs, *BCR::ABL1*-positive ALLs, *BCR::ABL1*-like ALLs, *ETV6::RUNX1*-positive ALLs, *KMT2A:AFF1*-positive ALLs, and *TCF3::PBX1*-positive ALLs) to evaluate the significant clinical symptom as predictor variables across genetic subtypes of B-ALLs. Likelihood ratio tests were significant for each of the six logistic regression models, indicating the models fit significantly better than empty models with just an intercept (i.e., a null model). However, modeling of the *KMT2A::AFF1*-positive ALL patients revealed very large standard errors for the coefficients, together with p values of 1, indicating that this subtype is not amenable to logistic regression employing clinical parameters as predictor variables and is removed from the summary tables and the figures for this analysis. Logistic regression analysis of the B-ALL subtypes revealed that lymphadenopathy shows a positive correlation in *BCR::ABL1*-like ALLs and *ETV6::RUNX1*-positive ALL cases and is negatively correlated in *BCR::ABL1*-negative ALL cases ($p < .05$). Fever shows a positive correlation in *ETV6::RUNX1* patients and is negatively correlated in *BCR::ABL1*-positive ALL cases ($p < .05$). Pallor shows a positive correlation in *BCR::ABL1*-like ALL cases and is negatively correlated in *BCR::ABL1*-negative ALLs ($p < .05$). Splenomegaly shows a negative correlation in *ETV6::RUNX1*-positive ALL cases ($p < .05$). We did not find any clinical symptoms that were statistically significant in *TCF3::PBX1*-positive ALL cases. The regression coefficients and measures of fit for the logistic regression models of clinical symptoms manifested across genetic subtypes of ALLs are shown in Table S6 and Figure 4.

Logistic regression model of CD markers in six genetic subtypes of ALLs

Logistic regression analysis of the B-ALL subtypes revealed that CD20, CD33, CD34, and TDT are statistically significant predictive markers of *BCR::ABL1*-positive ALLs, whereas CD38 and CD117 show a negative correlation with this subtype. Similarly, CD33, CD38, CD79a, CD117, CD123, and HLA-DR are statistically

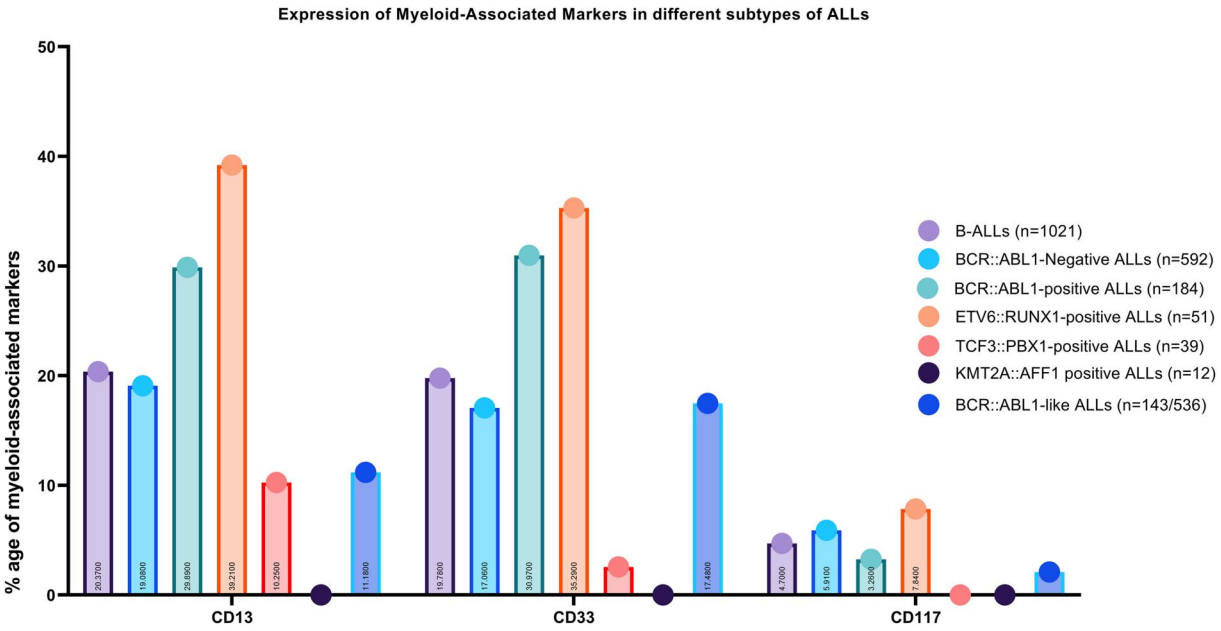

FIGURE 3 The expression of myeloid-associated markers in B-lineage acute lymphoblastic leukemias (B-ALLs), *BCR::ABL1*-negative ALL cases, *BCR::ABL1*-positive ALL cases, *ETV6::RUNX1*-positive ALL cases, *TCF3::PBX1*-positive ALL cases, and *BCR::ABL1*-like ALL genetic subtypes of ALLs.

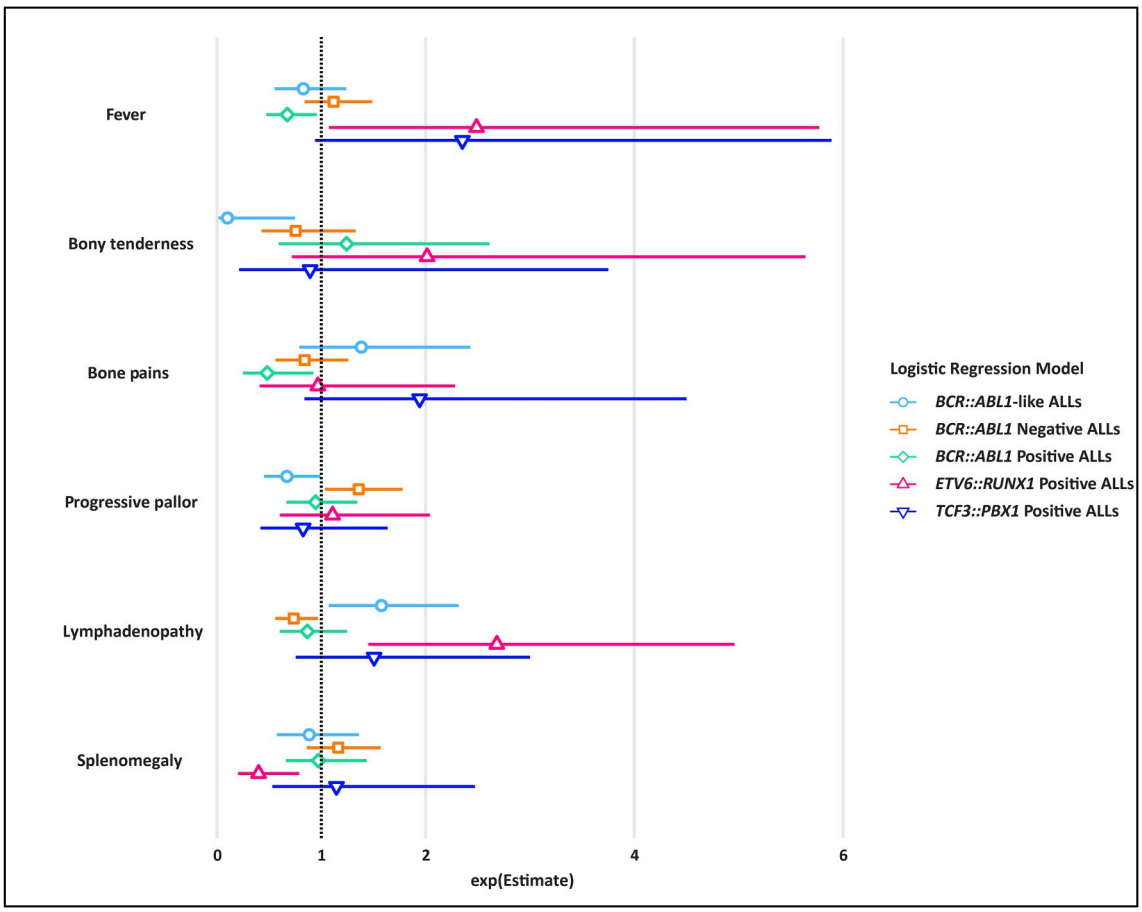

FIGURE 4 Forest plot of logistic regression coefficients obtained from logistic regression modeling using clinical parameters as predictor variables for the indicated acute lymphoblastic leukemia subtypes. The regression coefficients are plotted after exponential transformation of estimates obtained for predictor variables that showed significance ($p < .05$) in at least one of the plotted models.

significant positive predictors of *BCR::ABL1*-negative ALL subtype, whereas CD36, CD81, and CD86 are also negatively correlated with a positive diagnosis for this ALL subtype. For *BCR::ABL1*-like ALLs, CD36 expression is a strong predictor, whereas CD123, TDT, and HLA-DR expression significantly decreases the odds for the presence of this subtype. For the other ALL subtypes, the presence of CD13 is a positive predictor of *ETV6::RUNX1*-positive ALLs and CD10 is a strong negative predictor for *KMT2A:AFF1*-positive ALLs, whereas CD20 and CD34 are negative predictors of *TCF3::PBX1*-positive ALL subtype, as shown in Figure S4 and Table S7. Our logistic regression model shows that the CD36 markers strongly predict newly incorporated ALL subtypes (*BCR::ABL1*-like ALLs). The logistic regression model of six genetic subtypes of ALLs is shown in Table S8.

Outcomes of identified patients with *BCR::ABL1*-like ALL signature

MRD (sensitivity of 0.001%) was evaluated using a 10 color/12 parameters flow cytometry at the EOI (after 4 weeks of treatment). The MRD data was available in 78.06% (797 of 1021) of B-ALL cases. The MRD positivity of 18.04% (85 of 471) in *BCR::ABL1*-negative ALLs, 21.36% (25 of 117) in *BCR::ABL1*-like ALLs, 22.48% (29 of 129) in *BCR::ABL1*-positive ALLs, 18.91% (7 of 37) *ETV6::RUNX1*-positive ALLs, 12.12% (4 of 33) *TCF3::PBX1*-positive ALLs, and 0% (0 of 10) *KM2TA::AFF1*-positive ALLs were compared across genetic subtype of ALLs, as shown in Table 2 and Figure 5. MRD outcome of *BCR::ABL1*-positive ALL cases shows a statistical significance ($p < .05$) compared to *BCR::ABL1*-negative ALL cases. In contrast to prior

TABLE 2 MRD outcomes of different genetic subtypes of ALLs at day 28.

Cases	Available MRD data, % (*n*/*N*)	MRD-positivity, % (*n*/*N*)	MRD-negativity, % (*n*/*N*)	Significance
B-ALLs ($n = 1021$)	78.06 (797/1021)	17.57 (140/797)	82.43 (657/797)	—
BCR::ABL1-negative ALLs ($n = 592$)	79.56 (471/592)	18.04 (85/471)	81.96 (386/471)	NS
BCR::ABL1-positive ALLs ($n = 184$)	70.10 (129/184)	22.48 (29/129)	77.51 (100/129)	**
ETV6::RUNX1-positive ALLs ($n = 51$)	72.51 (37/51)	18.91 (7/37)	81.09 (30/37)	NS
TCF3::PBX1-positive ALLs ($n = 39$)	84.61 (33/39)	12.12 (4/33)	87.88 (29/33)	NS
KMT2A::AFF1-positive ALLs ($n = 12$)	83.33 (10/12)	0 (0/10)	100 (10/10)	NS
BCR::ABL1-like ALLs ($n = 143$)	81.81 (117/143)	21.36 (25/117)	78.63 (92/117)	NS

Abbreviations: ALLs, acute lymphoblastic leukemias; MRD, minimal residual disease; NS, not significant.
**$p < 0.01$.

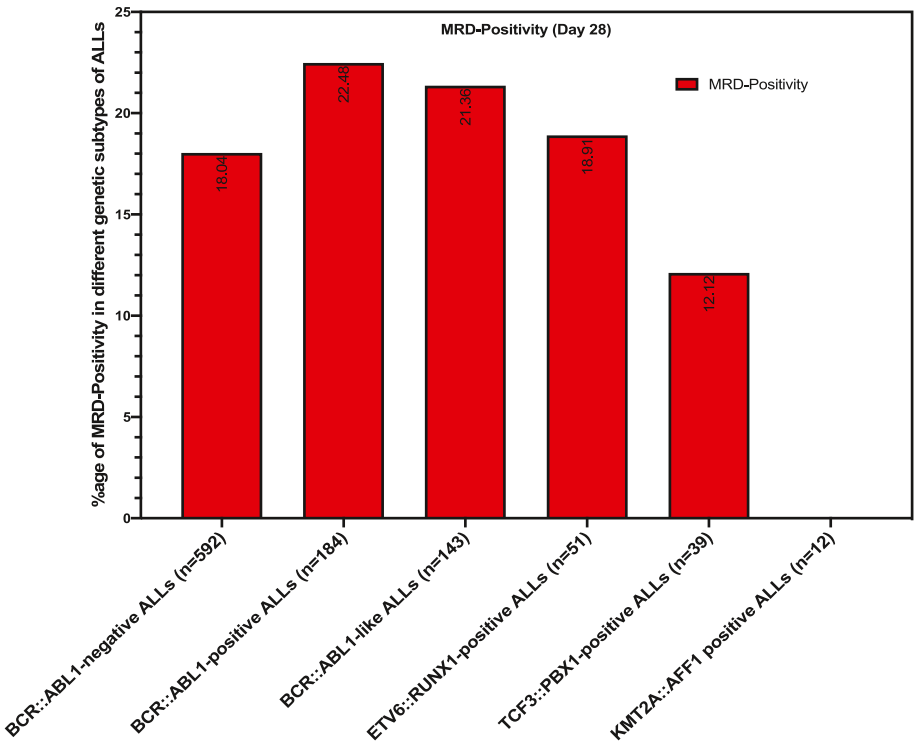

FIGURE 5 Minimal residual disease outcomes of different genetic subtypes of B-lineage acute lymphoblastic leukemias.

studies, there was no difference observed in the MRD-positivity of patients with *BCR::ABL1*-like ALL compared to *BCR::ABL1*-negative ALL cases.

DISCUSSION

The genetic landscape of ALL is highly diverse due to the presence of >30 fusions identified so far.[1] In the present study, we observed a higher frequency of 18% *BCR::ABL1* fusion, followed by 4.9% *ETV6::RUNX1* fusion, 3.8% *TCF3::PBX1* fusion, 1.2% *KMT2A::AFF1* fusion, and 26.67% *BCR::ABL1*-like ALLs of all B-ALL patients. The authors from high-income and low-income countries reported the recurrent genetic abnormalities (RGA) frequencies including 0.6%–35% *BCR::ABL1*-positive ALLs, 0.8%–30.6% *ETV6::RUNX1*, 0.8%–6.2% *TCF3::PBX1*, 0.5%–19% *KMT2A-AFF1*, and 10%–33.1% *BCR::ABL1*-like ALL cases.[6,7,12-15,17,18,20,28,31,32,34,37,41,49-61] The overall frequencies of RGAs and *BCR::ABL1*-like ALL cases reported in the literature from low-income and high-income countries among B-ALL patients (from India as well as other countries) are summarized in Table 3. We also studied aberrant myeloid marker expression frequencies in B-ALL patient subgroups. Overall, we observed the expression of CD13 in 21.8% of the B-ALL patients, CD33 in 19.7% of the B-ALL patients, and CD117 in 4.7% of the B-ALL patients. The authors from high-income and low-income countries reported the aberrant expression of myeloid marker expression including 10.5%–54.5% CD13, 2.6%–89% CD33, and 0%–26.2% CD117.[38,39,60,62–75] These differences in frequencies of expression of various myeloid markers might be attributed to the inherent genetic differences among ethnic subpopulations, technical factors such as lack of uniformity in using

TABLE 3 Frequencies of recurrent genetic abnormalities and *BCR::ABL1*-like ALLs in B-ALL patients from low-income and high-income countries.

Publication (country of origin)	No. of B-ALL patients	t(9;22) (*BCR::ABL1*), %	t(12;21) (*ETV6::RUNX1*), %	t(1:19) (*TCF3::PBX1*), %	t(4:11) (*KM2TA::AFF1*), %	*BCR::ABL1*-like B-ALL, %
Mancini 2004 (Italy)[57]	325	31.3	NR	2.1	7.3	NR
Moorman 2007 (US)[58]	1373	19	NR	3	7	NR
Den Boer 2009 (Dutch)[17]	107	1	22	2	4	15
Bhatia 2012 (India)[41]	95	13.6	11.6	2.1	3.1	NR
Chen 2012 (China)[59]	1346	16.2	10.87	3.05	3.05	NR
Roberts 2014 (US)[6]	1725	6.3	11	6.2	5.3	15.3
Chopra 2015 (India)[50]	271	15.1	5.9	5.5	1.1	NR
Fasan 2015 (Germany)[32]	132	NR	NR	NR	NR	10
Den Boer 2015 (Dutch)[18]	127	33	NR	2.36	11	17
Jain 2017 (US)[12]	148	31	NR	NR	9.5	33.1
Herold 2017 (Germany)[14]	207	35	1	1	19	13
Heatley 2017 (Australia)[20]	245	3.2	30.61	NR	NR	11.7
Reshmi 2017 (US)[15]	1389	3.3	0.8	NR	NR	24.6
Roberts 2017 (US)[13]	798	23.2	1	2.9	15.4	24.3
Chen 2018 (China)[51]	2479	10.25	7.54	4.7	1.41	NR
Roberts 2018 (US)[31]	1023	0.6	6	NR	NR	20.1
Chiaretti 2018 (Italy)[27]	142	NR	NR	NR	NR	19.7
Tsaur 2018 (Russia)[34]	147	6.8	15.6	3.4	NR	10.88
Gupta 2019 (India)[52]	273	8.1	5.5	NR	NR	NR
Park 2020 (Korea)[56]	637	5.3	2.35	0.8	0.5	NR
Shen 2021 (China)[55]	291	3.1	20.4	4.5	5	NR
Chiaretti 2021 (Italy)[37]	88	NR	NR	NR	NR	31.8
Chen 2021 (China)[54]	1000	13.5	4.3	3.0	2.5	NR
Sharma 2022 (India)[39]	595	21	10.6	4.2	3	11.4
Present study 2023 (India)	1021	18	4.9	3.8	1.2	26.67

Abbreviations: ALL, acute lymphoblastic leukemia; B-ALL, B-lineage ALL; NR, Not Reported; US, United States.

monoclonal antibody clone type, and differences in flow cytometry methodology, processing, and data analysis.[74,76] Table 4 compares the reported frequencies of myeloid marker expression in B-ALL patients. Among genetic subgroups, there were some exciting observations encountered in our study. We found a statistically significant difference in the expression of CD13 and CD33 among the *BCR::ABL1*-positive (29.8% and 30.9%) and *ETV6::RUNX1*-positive (39.2% and 35.3%) B-ALL patients. Both subgroups expressed higher frequencies of these myeloid markers compared to other B-ALL subtypes, with a male predominance and significantly higher WBC. The published literature has also highlighted an increased CD13 and CD33 expression prevalence in *BCR::ABL1*-positive (10%–42.5% and 20%–36.3%, respectively)[38,77] and *ETV6::RUNX1*-positive B-ALLs (73.3% and 46.6%, respectively).[38,52] At least one of these two myeloid markers has been found to be expressed in 95% and 91% of *BCR::ABL1*-positive B-ALLs and *ETV6::RUNX1*-positive B-ALLs, respectively.[74]

Logistic regression modeling using CD markers as predictors of B-ALL genetic subsets revealed remarkable findings. Increased cross-lineage myeloid marker expression (such as CD33) in *BCR::ABL1*-positive B-ALL is very well documented,[78] and 100% positivity for CD34 in *BCR::ABL1*-positive B-ALLs has been reported in published studies[52] along with high CD20 expression[79] and a low frequency of CD38 and CD117 in this subtype. However, algorithms incorporating the above CD markers as a predictive strategy to identify the molecular group are lacking. For the *BCR::ABL1*-like ALL cases, CD36 expression strongly predicts this ALL subtype, displaying the largest regression coefficient value. This is intriguing, because studies have observed patients with *BCR::ABL1*-like genetic alterations with CD36 positivity and inferior outcomes in such patients.[80] *KM2TA::AFF1*-positive B-ALLs were characterized as a distinct high-risk group with frequent CD10 negativity on immunophenotyping,[81–83] also confirmed by our own logistic regression analysis. CD20 and CD34 proved strong negative predictors for *TCF3::PBX1*-positive B-ALL, as described previously.[74,83]

Our study found lymphadenopathy in *BCR::ABL1*-like and *ETV6::RUNX1*-positive ALLs was statistically significant ($p < .05$) compared to *BCR::ABL1*-negative ALLs. Jaime-Perez et al.[84] showed that organomegaly at diagnosis was a significant predictor for relapse in ALL patients. Validation in a larger cohort of ALL patients of Indian ethnicity will be required to assess lymphadenopathy in high-risk ALLs as a significant predictor of *BCR::ABL1*-like ALL cases.

MRD at EOI is the strongest predictor of risk behavior as well as risk stratification in B-ALL patients.[85,86] MRD response differs across various genetic subtypes of ALLs, indicating that the genetics of multiple subtypes of ALLs play a significant role in the treatment outcomes.[85,86] MRD positivity translates into poor clinical outcomes and a high risk of disease relapse.[87–93] We studied the correlation between the genetic subtypes of B-ALL and disease outcome in MRD status at the EOI chemotherapy. B-ALL patients having *TCF3::PBX1* and *ETV6::RUNX1* have favorable outcomes compared to other high-risk genetic subtypes of ALLs.[94] Indeed, our study found low MRD positivity in these two subtypes. Meanwhile, we found that the flow cytometric MRD positivity at the EOI was significantly higher in the

TABLE 4 Comparison of expression of myeloid-associated markers in B-ALLs reported from India and other countries.

Publication (country of origin)	No. of patients	CD13, %	CD33, %	CD117, %
Present study 2022 (India)	1021	21.8	19.7	4.7
Santos 2022 (Brazil)[62]	277	21	11	0
Gupta 2022 (India)[38]	986	20.58	18.66	3.34
Ahuja 2022 (India)[75]	150	41.9	23.3	18.6
Hajra 2021 (India)[63]	244	10.5	52.6	5.3
Sivakumar and Basu 2021 (India)[65]	75	22.2	77.7	0
Gupta 2021 (India)[64]	100	50	2.6	5.3
Rezaei 2020 (Iran)[66]	89	5.8	2.9	1.74
Salem 2012 (Egypt)[70]	164	7.9	10.5	0
Sharma 2016 (India)[39]	303	32.9	34.5	26.2
Jalal 2016 (Iran)[68]	242	49.8	35.7	2.5
Sharma 2015 (India)[67]	100	15	10	54
Mazher 2013 (Pakistan)[69]	50	20	15	0
Seegmiller 2009 (US)[74]	200	54.5	43	1
Vitale 2007 (Italy)[71]	377	25	23	0
Suggs 2007 (US)[72]	71	53	89	5
Supriyadi 2006 (Indonesia)[73]	239	21	10	4

Abbreviations: B-ALL, B-lineage acute lymphoblastic leukemia; CD, cluster of differentiation; US, United States.

BCR::ABL1-positive subgroup ($p < .05$) and *BCR::ABL1*-like positive subgroup compared to others. A number of studies have demonstrated that the identification of *BCR::ABL1*-like and *BCR::ABL1*-fusion in B-ALL confers a high-risk status and poorer prognosis and/or overall survival compared with *BCR::ABL1*-negative B-ALLs.[1,6,12–15,31,37,95–98] The increased frequency of MRD positivity in the *BCR::ABL1*-like ALL cases reflects the aggressive clinical behavior of this subtype. This concurs with recently published works citing high rates of induction failure or MRD positivity in *BCR::ABL1*-like B-ALLs.[6,53,99] Novel therapies and personalized treatment regimens need to be incorporated to improve the treatment outcomes of newly incorporated entities.

We have characterized four RGFs and *BCR::ABL1*-like ALL signature in patients of Indian ethnicity for the first time. However, the authors from high-income countries reported >30 different genetic subtypes in the B-ALL genetic landscape. Thus, a main limitation of our research study cohort involves a lack of further subclassification of *BCR::ABL1*-negative ALL cases ($n = 592$) due to limited well-standardized multiplex RT-PCR assay. To overcome this, with ongoing B-ALL research in our center, we are actively screening the newer incorporated genetic subtypes of ALLs using RNA sequencing at baseline diagnosis. We instead employed the recently published PHi-RACE classifier to detect *BCR::ABL1*-like ALL cases at baseline. We have validated the PHi-RACE classifier in an independent B-ALL cohort ($n = 108$) that we recently published.[100]

In conclusion, our research study is a novel attempt to elucidate the clinical, hematological FCM-IP with aberrant myeloid-associated markers and MRD outcomes of prognostic significant genetic subtypes of ALLs. *BCR::ABL1*-positive and *BCR::ABL1*-like ALLs were considerably older at baseline presentation and had male preponderance and *ETV6::RUNX1*-positive ALLs were significantly younger at presentation ($p < .05$). Interestingly, we observed that myeloid-associated markers CD13 and CD33 were statistically significant in *BCR::ABL1*-positive and *ETV6::RUNX1*-positive ALLs ($p < .05$). This is the first extensive report on the characterization of prognostic significant genetic subtypes and MRD outcomes of ALLs in patients from India. Evaluating hematological, FCM-IP, and clinical symptoms at diagnosis are useful for making alternate curative decisions in various subtypes of ALLs.

AUTHOR CONTRIBUTIONS

Dikshat Gopal Gupta: Conceptualization, design, performed experiments, statistical analysis, data analyzation; and writing–original draft. **Neelam Varma:** Conceptualization, design, and writing–original draft. **Praveen Sharma:** Statistical analysis, data analyzation, and writing–original draft. **Mihai I. Truica:** Statistical analysis and data analyzation. **Sarki A. Abdulkadir:** Statistical analysis and data analyzation. **Mohammad Rizwan Siddiqui:** Statistical analysis and data analyzation. **Parveen Bose:** Performed experiments. **Jogeshwar Binota:** Performed experiments. **Pankaj Malhotra:** Provided the adult and pediatric B-ALL samples. **Alka Khadwal:** Provided the adult and pediatric B-ALL samples. **Subhash Varma:** Provided the adult and pediatric B-ALL samples. All the authors read, approved, and provided the necessary intellectual comments to the submitted manuscript.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The data will be provided on request, and all data generated during this study are included in this research article.

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Additional supporting information can be found online in the Supporting Information section at the end of this article.

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