‘Delineating the Genomic and Proteomic Landscape of *BCR-ABL1*-like Acute Lymphoblastic Leukemia Subtype: Bench-to-Bedside Translational Research’

Dikshat Gopal Gupta1*, Neelam Varma2, Ashish Kumar3, Shano Naseem2, Man Updesh Singh Sachdeva2, Sreejesh Sreedharanunni2, Jogeshwar Binota2, Parveen Bose2, Minakshi Gupta2, Preeti Sonam2, Palak Gupta2, Pankaj Malhotra2, Alka Khadwal2, Amita Trehan2, Subhash Varma2

1Ann Roberts H Lurie Children's Hospital, USA. 2Post Graduate Institute of Medical Education & Research, India. 3Nucleome Informatics Pvt Ltd, India.

Abstract

Gold standard for the identification of *BCR-ABL1*-like ALL patients is gene expression profiling (GEP). Due to its diverse nature, its identification is extremely difficult and expensive. On the genomic and proteomic landscape of *BCR-ABL1*-like ALL patients, there is the paucity of published literature from developing countries. We used digital barcoded nCounter Nano String GEP & qPCR for its detection, followed by molecular & proteomic characterization using fluorescence in situ hybridization and Liquid Chromatography-Mass Spectrometry. In a cohort of 1021 B-ALL cases, Multiplex assay revealed 18.66% *BCR-ABL1*, 3.85%, *TCF3-PBX1*, 5.27% *ETV6-RUNX1,* and 1.21% *KMT2A-AFF1*.Using transcriptome GEP, we found 32.05% *BCR-ABL1*-like ALL patients using NanoString and 26.67% using RQ-PCR, and their median age at presentation was considerably higher than *BCR-ABL1*-negative ALL cases (p=0.0306). Further, we identified 20% *CRLF2* overexpressed cases having 8.33% *CRLF2-IGH* translocation with concomitant R683S mutation and 8.33% *CRLF2-P2RY8* translocation. In 80% *CRLF2* down- regulated cases, we identified 10% having *JAK2* rearrangement. MRD- positivity was more common in *BCR-ABL1*-like ALL cases (55.55% vs. 25% in Ph-negative ALL cases). Gene enrichment analysis indicated that involvement of spliceosomal mediated mRNA splicing pathway and three miRNAs was statistically significant in *BCR-ABL1*-like ALL patients. For the first time, we have described incidence, molecular and proteomic characterization of *BCR-ABL1*-like ALL, in developing nations.

Background

Baseline detection of Ph/*BCR-ABL1*-like-ALL cases is extremely beneficial for hematologists/physicians to design personalized medicine and to treat these patients efficiently.

Objective

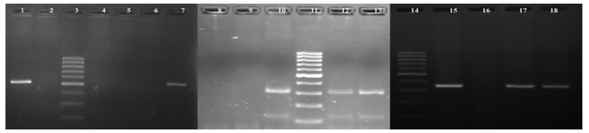
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Materials & Methods

This study was conducted at PGIMER, India (2016–2021). Flowcytometric-immunophenotyping and multiplex RT-PCR was performed to detect four recurrent fusion transcripts (n=1021). Transcriptome GEP was performed on 12 Ph-positive ALL cases. *BCR-ABL1*-like-ALL cases identified using nCounter Platform (n=96) and RQ-PCR (n=536) were characterized using FISH, DD-PCR, RQ-PCR and MRD-positivity. Nine HKGs’ expression was evaluated using 6 algorithms. Furthermore, we built and validated PHi-RACE classifier (n=629 cases) for its rapid detection. Lastly, we delineated the proteomic landscape of BCR-ABL1-like ALL cases using LC-MS/MS.

Results

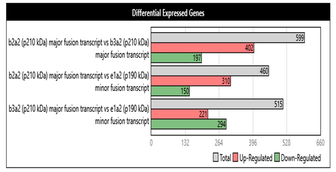
In the cohort of 1021 B-ALL cases, the incidence of *BCR-ABL1* fusion transcripts was 18.66%, *TCF3-PBX1* 3.85% , *ETV6-RUNX1* 5.27% , and *KMT2A-AFF1* 1.21% as revealed by multiplex RT-PCR assay.

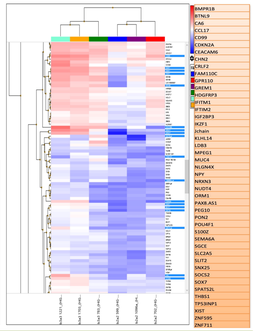


Lane 1	Positive Control	521 bp (e1a2)	Lane 10	Positive Control	310 bp (b2a2)	Lane 15	Positive Control	385 bp (b3a2)
Lane 3	Ladder	100 bp	Lane 11	Ladder	100 bp	Lane 14	Ladder	100 bp
Lane 7	Minor breakpoint (e1a2; p190kDa)	521 bp (e1a2)	Lanes 12 &13	major breakpoint (b2a2; p210kDa)	310 bp (b2a2)	Lanes 17 & 18	Major breakpoint (b3a2; p210kDa)	385 bp (b3a2)

Microarray Transcriptomic GEP profiling (n=12) *BCR-ABL1*+ve ALL cases

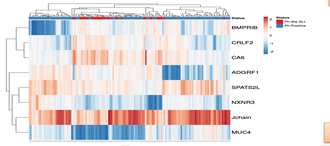
- A total of 1574 differentially expressed (DE) genes (460 genes in patients with minor fusion transcript e1a2, 515 genes in patients with b2a2 major fusion transcript and 599 genes in patients with b3a2 major fusion transcript) have been identified.
- Differentially expressed (DE) genes were identified according to the criteria: Anova p (Corr) cut-off = 0.05 and log fold change cut-off of 2.0.
- DE genes were further filtered through hierarchal clustering analysis and a total of 45 DE genes with 10- to -86-fold change were identified.



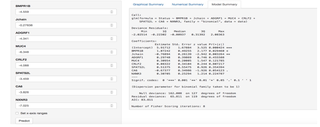


Detection of *BCR-ABL1*-like ALL cases using Nanostring & qPCR

32.05% (25/78) & 26.67% (143/536) *BCR-ABL1*-negative cases showed clustering with *BCR-ABL1*-positive ALL cases.



PHi-RACE: PGIMER In-House Rapid & Cost Effective Classifier for Ph-like ALL Detection



Sensitivity=95.20%, Specificity=83.70%, cut-off >0.28)

Penalized logistic Regression

45 genes

(Intercept)	8.645879093
BMPR1B	0.089797222
BTN3	0.217924401
C4A	-0.078598432
CCL17	-0.171371336
CD99	0.130913236
CDKN2A	0.163100508
CEACAM6	-0.074742027
CHN2	0.106110634
CRLF2	0.103790034
FAM110C	0.254601103
GPR110	0.169439599
GREM1	-0.163673151
HSP2BP3	0.156804277
IFITM1	-0.181529764
IFITM2	0.068432904
IGF2BP3	-0.038921815
JCHAIN	-0.492166892
KLHL14	-0.020338862
LDB3	0.13759933
MPEG1	-0.004099949
MUC4	0.120950094
NLGN4X	-0.047882113
NPY	-0.165369748
NRXN3	-0.193948369
NUDT4	-0.117730017
OMG	0.009974955
PAX8-AS1	0.059737324
PEG10	0.205993894
PON2	-0.020041185
POU4F1	-0.083595803
S100Z	-0.232107309
SEMA6A	0.179634085
SGCE	0.068272125
SLC2A5	-0.086831472
SLIT2	-0.213093137
SNX25	0.127402867
SOCS2	0.106604425
SOX7	-0.073702432
SPATS2L	0.159749945
THBS1	-0.216802512
TP53INP1	-0.168748193
XIST	0.914162231
ZNF595	-0.169850902
ZNF711	0.564291884
	-0.081850932

15 best genes

nCounter Nanostring

BMPR1B
CHN2
CRLF2
GPR110
Jchain
MUC4
NRXN3
NUDT4
PON2
POU4F1
S100Z
SEMA6A
SNX25
SPATS2L
TP53INP1

8 best genes

RQ-PCR

BMPR1B
CRLF2
GPR110
Jchain
MUC4
NRXN3
SPATS2L
CA6

Categories of *BCR-ABL1*-like ALL cases

***BCR-ABL1*-like ALL cases (32.05%)**

CRLF2 up regulated (5/25 20%)

8.33% *CRLF2-IGH* & 8.33% *CRLF2-P2RY8* translocation

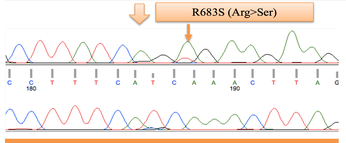
CRLF2 dowregulated (20/25 (80%)

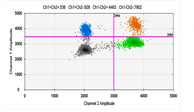
10% (1/10) *JAK2* rearrangements

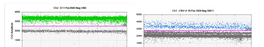
IKZF1 gene (4-7) deletion in ALL cases DDPCR

Sanger Sequencing of exon 16 of *JAK2* in 5 *CRLF2* up-regulated cases

R683S (Arg>Ser)





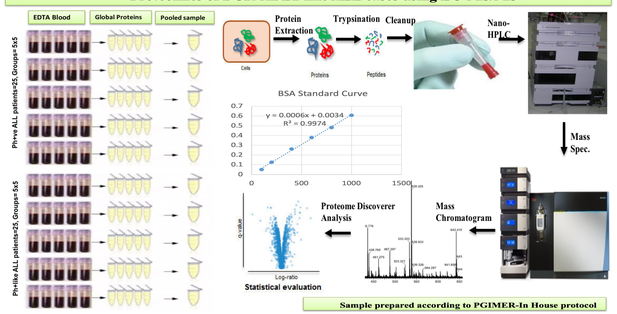


R683S (Arg>Ser) Missense Variant 1/25 = 4%

MRD- positivity was more common in Ph-like ALL cases (55.55% vs. 25% in Ph-negative ALL cases).

We found 8.33% positive *IKZF1* (4-7) deletion in B-ALL cases

Proteomics of BCR-ABL1-like ALL cases using LC-MS/MS



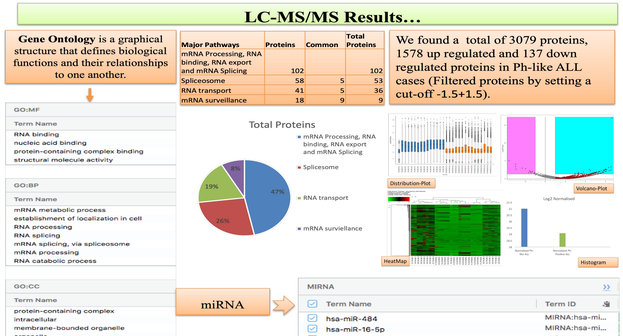
Sample prepared according to PGIMER-In House protocol

LC-MS/MS Results…

Gene Ontology is a graphical structure that defines biological functions and their relationships to one another.

Major Pathways	Proteins	Common	Total Proteins
mRNA Processing, RNA binding, RNA export and mRNA Splicing	102		102
Spliceosome	58	5	53
RNA transport	41	5	36
mRNA surveillance	18	9	9

We found a total of 3079 proteins, 1578 up regulated and 137 down regulated proteins in Ph-like ALL cases (Filtered proteins by setting a cut-off -1.5+1.5).



“g:profiler software” “DAVID Software”

Summary

We have created and published the PHi-RACE classifier to detect *BCR-ABL1*-like ALL cases, which has direct clinical implications. Further, we have delineated the genomic and proteomic landscape of *BCR-ABL1*-like-ALL for the first time from developing countries.

Discussion

We have detected *BCR-ABL1*-like ALL cases using 2 independent platforms; nCounter NanoString (32.05%) and RQ-PCR (26.67%) using hierarchal clustering and PCA approach. Our findings are in accordance with Chiaretti et al (2021) and Jain et al (2017), who found the higher incidence of 31.8% and 33.1% *BCR-ABL1*-like ALL cases using TLDA approach. As TLDA is not available in India, we have created a PHi-RACE classifier for *BCR-ABL1*-like-ALL detection for the first time in developing countries. It is very important to detect *BCR-ABL1*-like ALL cases, so as to provide them the benefits of desirable TKI therapy. We have delineated the genomic and proteomic profile of *BCR-ABL1*-like ALL cases for the first time in Indian Patients. Our study showed aberrant mRNA splicing pathways with overexpressed splicing factors and three enriched miRNAs in *BCR-ABL1*-like ALL cases, and these might find use as potential therapeutic targets.

References

Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-405.

Roberts KG, Li Y, Payne-Turner D, Harvey RC, Yang Y-L, Pei D, et al. Targetable kinase-activating lesions in BCR-ABL1-like acute lymphoblastic leukemia. N Engl J Med. 2014; 371(11):1005–15.

Jain N, Roberts KG, Jabbour E, Patel K, Eterovic AK, Chen K, et al. BCR-ABL1-like acute lymphoblastic leukemia: a high-risk subtype in adults. Blood. 2017;129(5):572-81.

Reshmi SC, Harvey RC, Roberts KG, Stonerock E, Smith A et al. Targetable kinase gene fusions in high-risk B-ALL: a study from the Children's Oncology Group. Blood. 2017;129(25):3352-61.

Chiaretti S, Messina M, Grammatico S, Piciocchi A et al. Rapid identification of BCR/ABL1-like acute lymphoblastic leukaemia patients using a predictive statistical model based on quantitative real time-polymerase chain reaction: clinical, prognostic and therapeutic implications. Br J Haematol. 2018;181(5):642-52.

Den Boer ML, Van Slegtenhorst M, De Menezes RX, Cheok MH, Buijs-Gladdines JG, Peters ST, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol. 2009; 10(2):125–34.

Gupta DG, Varma N, Kumar A, Naseem S, Sachdeva MUS, Binota J, et al. PHi-RACE: PGIMER in-house rapid & cost effective classifier for the detection of BCR-ABL1-like acute lymphoblastic leukaemia in Indian patients. Leuk Lymphoma. 2021:1-11.

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