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EXPLORING DUX4-REARRANGED B-ALL IN INDIA: INCIDENCE, MOLECULAR PATHWAYS, AND POTENTIAL BIOMARKERS

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INTRODUCTION

- DUX4-rearranged B-ALL (DUX4-r), characterized by cryptic DUX4-rearrangements, has not been investigated in Indian patients. Gaining an understanding of the clinical and molecular characteristics is crucial for targeted therapy development.

AIM

- **We aimed to determine the incidence, clinical characteristics, gene fusions, single nucleotide variants (SNVs), and copy number alterations (CNAs) in DUX-r subtype.**
- **Gene set enrichment analysis (GSEA) and Qiagen Ingenuity Pathway Analysis (IPA) were employed, with external validation using St.Jude Children Hospital publicly available count files.**

METHOD

- We analyzed bone marrow/peripheral blood samples from 394 de-novo B-ALL patients (67.1% males, 32.9 % females, median age, 15.2 years) at AIIMS, India (2018-2022).
- Sentinel aberrations were identified using multiplex reverse transcription polymerase chain reaction and fluorescence in situ hybridization.Total RNA sequencing identified DUX4-r subtype using the Rtsne and FusionCatcher/Cicero.
- SNVs were identified using GATK HaplotypeCaller, and CNAs were assessed using MLPA. After differential gene expression analysis, GSEA was performed and pathways with P<0.05 and false discovery rate (FDR)<0.05 were considered significant. Core analysis and biomarker filtering were conducted using IPA.

RESULTS

- Twenty-nine cases (7.36%) were classified as DUX4-r ALL, with fusions including IGH::DUX4 (n=24, 82.75%),ERG::LINC01423 (n=3, 10.34%), and a novel fusion, DUX4::EML4 (n=1, 3.44%).
- ERG and IKZF1 alterations were observed in 25% and 20.83% of patients, respectively using MLPA. Mutations were found in KRAS, FLT3, JAK3, PAX5, PTPN11 (n=8, 27.58%), and ZEB2:H1038R (n=2, 6.89%) in DUX4-r samples.
- The **MYC V2 pathway** (NES=1.77) and **Unfolded protein response** (NES=) was enriched while the **P53 pathway** (NES=-2.18) was downregulated.
- In addition, **IL6 JAK-STAT3, COMPLEMENT, INTERFERON GAMMA, IL2 STAT5, PI3K/AKT/MTOR** signaling were also downregulated. (p<0.0001)
- Out of 25 gene networks, 1 was involved in hematological development, and 3 in cell death/survival processes. The merged network identified potential master regulators: CCR6, DLC1, ECM1, HMGA2, MMP2, and PAWR.
- **S100** signaling and **CREB** signaling were downregulated.
- Fifteen potential biomarkers (**BAK1, BAX, BCL2, MMP2, GP5, JAK3, COG2, DLC1, FAS, IFNG, IL-10, PCNA, WEE1, MCL1, H2AX**) were identified. All networks and biomarkers were significant with P < 0.01.
- Mutations observed from RNA seq data in DUX4-r subtype are: **PAX5:V26G, KRAS:G13D, PTPN11:G60R, JAK2:R657Q, KRAS:G12V , FLT3:A680V,FLT3:D835Y, ZEB2:H1038R**

Patients' characteristics	Pediatric (<18 years) (n=301)	Adults (>18 years) (n=93)
Age (years), median (range)	5 (4 months–17 years)	28 (18-76 years)
Males, n (%)	204 (67.78)	64 (68.81)
Females, n (%)	97 (32.22)	29 (31.19)
WBC at diagnosis (X10 9 /L), median (range)	15.0 (400- 673000)	26.0 (300-416000)
Hb (gm/dL), median (range)	6.95 (1.7-13.8)	7.55 (2.1-15.8)
Platelet count (X10 9 /L), median (range)	31.0 (2-460)	37.0 (1-314)
Cytogenetics Sentinel, n	140 (46.51)	56 (60.21)
Cytogenetics B-others, n	162 (53.82)	36 (38.70)
DUX4- rearranged, n	24 (7.97)	5 (5.37)

Table 1: Clinical characteristics of B-ALL patients at the baseline and frequency of DUX4-r subtype in AIIMS dataset.

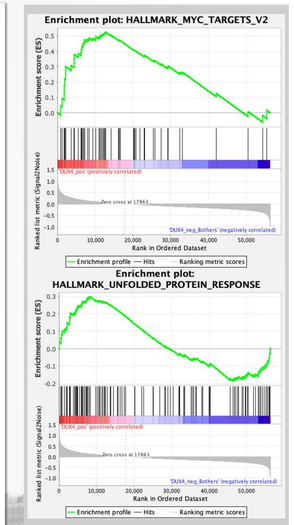


Figure 1: GSEA Enrichment plots for significantly upregulated pathways in DUX4-r AIIMS dataset (Hallmark collection).

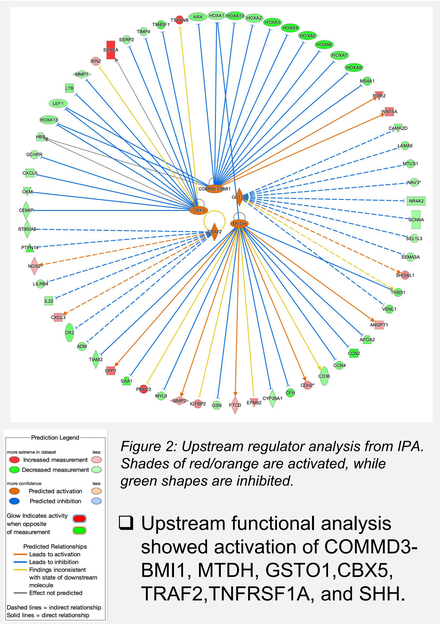


Figure 2: Upstream regulator analysis from IPA. Shades of red/orange are activated, while green shapes are inhibited.

- Upstream functional analysis showed activation of COMMD3-BMI1, MTDH, GSTO1,CBX5, TRAF2,TNFRSF1A, and SHH.

Gene	log2FoldChange	pvalue
ADAMTS15	8.86	9.48E-150
PTPRM	6.06	3.99E-82
APELA	9.07	8.21E-75
AGAP1	5.04	2.72E-60
PDGFRA	8.76	3.16E-60
SATB2	5.37	3.68E-59
CLTC	2.21	8.66E-57
CHST2	3.95	1.86E-56
GDNF	7.31	2.16E-55
GPR155	3.54	1.41E-52

Table 2: Top 10 most significantly differentially expressed genes (DEGs) obtained by DESeq2 *in DUX4-r subtype*

CONCLUSIONS

This study provides first insights into the incidence of DUX4-r B-ALL, expression profile, dysregulated pathways, and clinical features among Indian patients. Further experimental validation is warranted.

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