CHARACTERIZATION OF THE MOLECULAR HETEROGENEITY AND IDENTIFICATION OF CELLS MARKERS ASSOCIATED WITH *IKZF1* PLUS IN B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKAEMIA

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INTRODUCTION

B-cell precursor acute lymphoblastic leukaemias (B-ALL) are characterized by different genomic alterations that are associated with prognosis assisting patients' risk stratification. B-ALL main cytogenetic-molecular subgroups are: high hyperdiploidy and *ETV6-RUNX1*, which are associated with good prognosis; *TCF3-PBX1*, associated with intermediate prognosis; and *BCR-ABL1* and *KMT2A* rearrangements (*MLL-r or KMT2A-r*) associated with unfavourable prognosis. Copy number alterations (CNAs) can also be important prognostic markers in B-ALL, for example IKZF1 deletions (*IKZF1*^{del}) have been associated with an increased relapse rate and an unfavourable prognosis. Recently, a new molecular subgroup called *IKZF1*^{plus} was described. This novel group is defined by the co-occurrence of *IKZF1*^{del} with deletions affecting *CDKN2A*, *CDKN2B* (necessarily in homozygosis), *PAX5* or *PAR1* region in the absence of *ERG* deletions. *IKZF1*^{plus} is associated with a worse prognosis and a significantly higher risk of relapse when compared to the presence of *IKZF1*^{del} only.

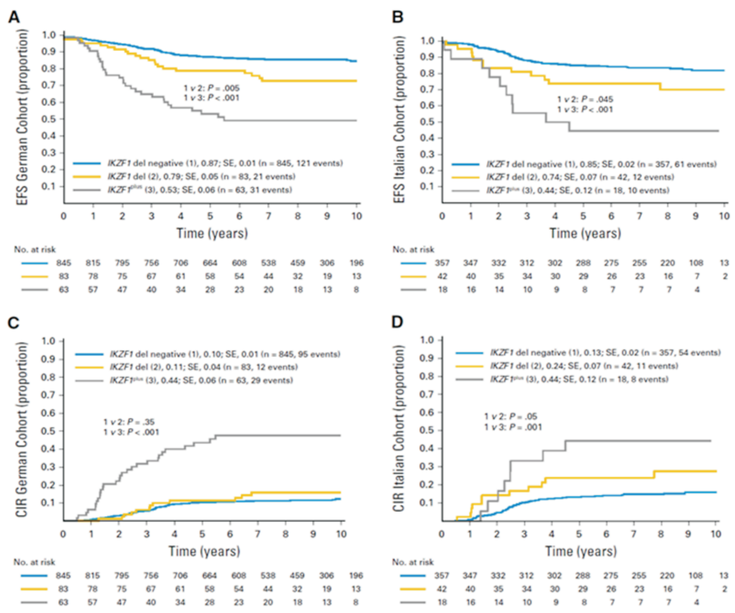

Figure 1. Event-free survival (EFS) and cumulative incidence of relapse (CIR) at 5 years according to *IKZF1* status (no *IKZF1* deletion [*IKZF1del*], *IKZF1*^{del}, *IKZF1*^{plus}) inpatients with B-cell precursor acute lymphoblastic leukaemia (ALL) treated in trial AIEOP-BFM (*Associazione Italiana Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Muenster) ALL 2000 (Stanulla M, 2018).*

AIM

To delineate the molecular profile of the *IKZF1*^{plus} subgroup and to identify specific markers to enable early detection of these cases.

STUDY DESIGN, MATERIAL AND METHODS

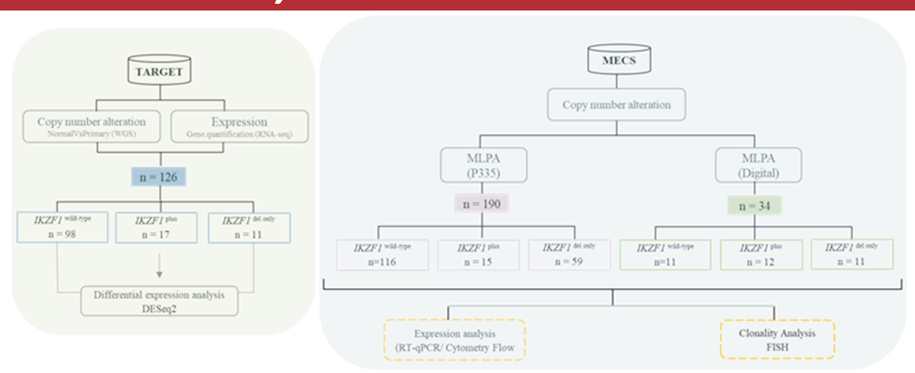

Figure 2. Study Design Flow Chart. The first flow chart shows the shows the analyzes performed on the TARGET cohort through DEseq2. The second flow chart shows the analyzes performed on the MECS cohort. Orange-dotted boxes represent prospective analyzes that will still be performed.

RESULTS

Table 1. Clinical and laboratory characteristics of the patients.

Variables	TARGET *IKZF1*^{wt} n (%)	TARGET *IKZF1*^{plus} n (%)	TARGET *IKZF1*$^{del\ only}$ n (%)	TARGET *P*-valor	MECS *IKZF1*^{wt} n (%)	MECS *IKZF1*^{plus} n (%)	MECS *IKZF1*$^{del\ only}$ n (%)	MECS *P*-valor
Age (months)								
0-12	0 (0)	0 (0)	0 (0)		12 (10,3)	0 (0)	0 (0)	
13-120	67 (68,3)	06 (35,2)	05 (45,4)	0.0879	54 (46,5)	04 (26,6)	20 (33,8)	0.004
>120	31 (31,6)	11 (64,7)	06 (54,5)		50 (43,1)	11 (73,3)	39 (66,1)	
Gender								
Female	49 (50,0)	09 (52,9)	05 (45,5)		51 (43,9)	06 (40,0)	32 (54,2)	
Male	49 (50,0)	08 (47,1)	06 (54,5)	0.927	65 (53,1)	09 (60,0)	27 (45,7)	0.374
Risk Group								
Good	33 (33,6)	0 (0)	01 (9,11)		40 (34,4)	03 (20,0)	12 (20,3)	
Intermediate	54 (55,1)	12 (70,5)	05 (45,5)	0.007	60 (51,7)	02 (13,3)	05 (8,47)	0.001
High	08 (8,10)	02 (11,7)	03 (27,2)		06 (5,17)	06 (40,0)	25 (42,3)	
No information	03 (3,06)	02 (11,7)	02 (18,1)		10 (8,62)	04 (26,6)	17 (28,8)	
WBC (cells/mm³)								
<50.000	65 (66,3)	07 (41,1)	06 (54,5)		70 (60,3)	12 (80,0)	31 (52,5)	
≥50.000	33 (33,6)	10 (5,88)	05 (45,5)	0.124	46 (39,6)	03 (20,0)	28 (47,4)	0.147
TOTAL	98	17	11		116	15	59	

Differential Expression Analysis (DESeq2)

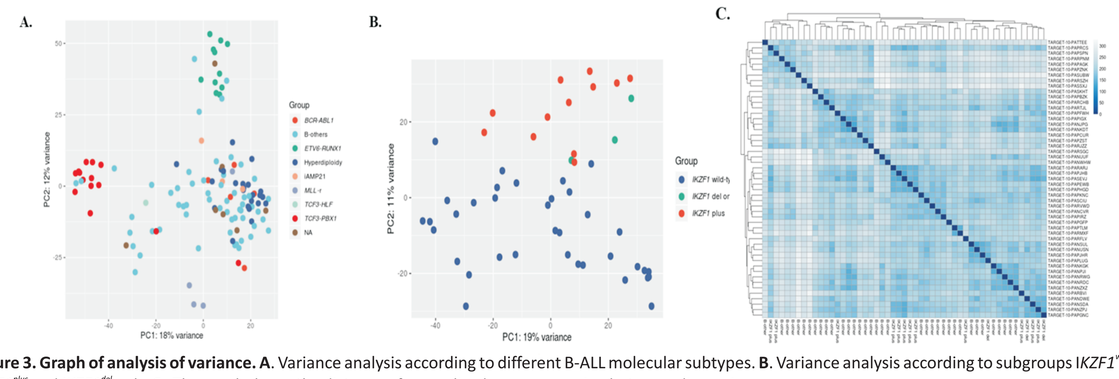

Figure 3. Graph of analysis of variance. A. Variance analysis according to different B-ALL molecular subtypes. **B**. Variance analysis according to subgroups *IKZF1*^{wt}, *IKZF1*^{plus} and *IKZF1*^{del} only. **C**. The graph shows the distance of normalized gene expression between dimensions.

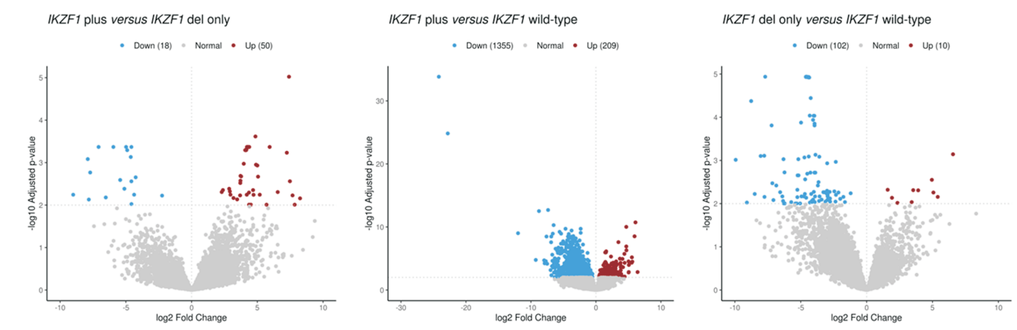

Figure 4. Distribution of up-regulated and down-regulated genes between groups. In all graphs the blue and red dots represent down-regulated and up-regulated genes, respectively. The first graph shows the distribution of genes in the *IKZF1*^{plus} group versus the *IKZF1*^{del} only group. The second shows the distribution of genes in the *IKZF1*^{plus} group when compared to the *IKZF1*$^{wild\text{-}type}$. And the last graph shows genes distribution comparing *IKZF1*^{del} only with *IKZF1*$^{wild\text{-}type.}$

Venn Diagram

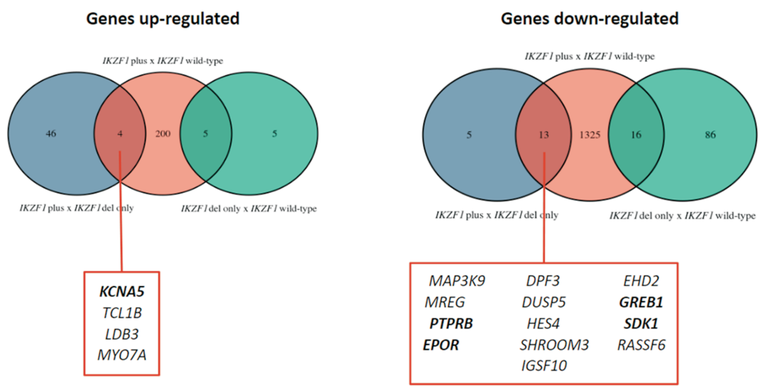

Figure 5. Venn Diagram. On the left, up-regulated genes and on the right, genes down-regulated in *IKZF1*^{plus} when compared to both the *IKZF1*$^{del\ only}$ group and the *IKZF1*$^{wild\text{-}type}$ group. Gene names in bold represent the ones found in membrane protein list. This list was assembled from data available online (https://www.proteinatlas.org/search/protein_class:Predicted+membrane+proteins).

Dispersion of expression among B-other patients (DESeq2 - B-other)

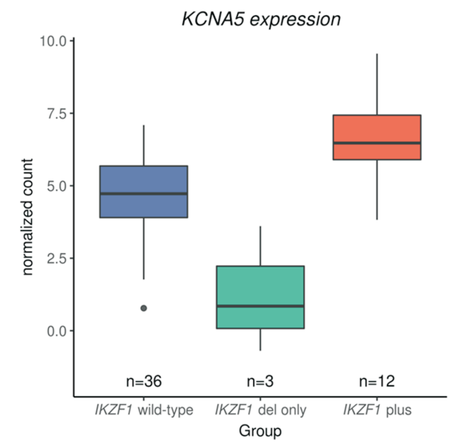

Figure 5. Analysis of KCNA5 gene expression between groups. The blue, green and red boxplot shows the expression of *KCNA5* in group *IKZF1*$^{wild\text{-}type}$, *IKZF1*$^{del\ only}$ and *IKZF1*^{plus}, respectively.

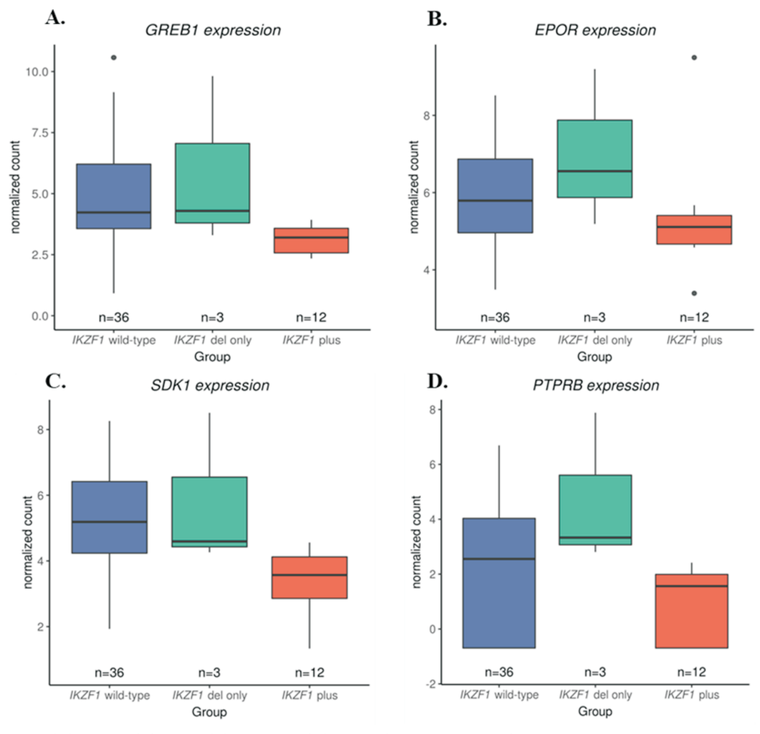

Figure 6. Gene expression analysis of (A) *GREB1*, (B) *EPOR*, (C) *SDK1* and (D) *PTPRB* (down-regulated) according to different *IKZF1* groups. The blue, green and red boxplot shows the expression of the genes referred to in the groups expression of in group *IKZF1*$^{wild\text{-}type}$, *IKZF1*$^{del\ only}$ and *IKZF1*^{plus}, respectively.

DISCUSSION

We show that *IKZF1*^{plus} has a characteristic expression profile when compared to the other subgroups. Although the frequency and clinical relevance of the CNAs that define *IKZF1*^{plus} have been established, it is still essential to define the molecular heterogeneity underlying this subgroup, since it may contribute to elucidate the sombre outcome observed in those *IKZF1*^{plus} patients. We understand that the characterization of this heterogeneity will enable the identification of cell markers that may be implemented in the diagnostic routine using a more accessible technology.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA