DEVELOPMENT OF LARGE-SCALE METHODOLOGIES FOR MRD MONITORING IN PEDIATRIC B-ALL

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common malignant hematologic malignancy in childhood, and despite significant advances in therapy and risk stratification of patients, disease recurrence remains as a relevant clinical issue – mainly considering some specific subgroups of patients. The evaluation of therapeutic response by MRD monitoring has been used to re-stratify the risk obtained at diagnosis and, in turn, it may anticipate the hematological relapse allowing then an earlier therapeutic reorientation. In this way, the development of more sensitive, cheaper and less laborious tools for MRD detection characterizes a major challenge. In B-derived ALL, detection of MRD by allele-specific PCR (ASO-PCR) is considered the gold standard method for DRM monitoring and is based on the detection of clone-specific rearrangements in genes encoding the light (IgK, IGL) and the heavy chains (IgH) of immunoglobulins. However, this type of approach is laborious, time-consuming and it requires the development of unique conditions and specific primers for each patient. In addition, this methodology is not able to monitor the clonal evolution throughout the treatment and might generate false-negative results. Therefore, the use of new generation sequencing techniques (NGS) to monitor MRD through the V(D)J rearrangements point to very promising achievements which would certainly contribute to more efficient therapeutic strategies, as well as to increase the

OBJECTIVE

To establish and validate the value of MRD by NGS and to evaluate the value of this parameter for the re-stratification of groups with similar genetic characteristics in B-derived pediatric ALL.

MATERIAL AND METHODS

This study is a multicenter retrospective (2010-2015) and prospective approach. Bone marrow (MO) samples sent to the CEMO Molecular Biology Laboratory and / or IPPMG-UFRJ from pediatric patients (0 - 18 years) diagnosed with derived B-ALL are being analyzed. In order to standardize the SNG technique, a pilot study with DNA of bone marrow samples from 10 patients is underway, a study consisting of the identification of clonal V(D)J clonal rearrangement by allele-specific PCR, heteroduplex or fragment analysis followed by direct sequencing for definition of the clonal rearrangement and thus, this enables the rearrangement detection by SNG. The MRD monitoring by SNG will be done through the Ion Torrent® methodology with preparation of a library, preparation of the template through clonal enrichment, purification and sequencing. The results obtained will be correlated with the MRD data collected by MFC performed in the Flow Cytometry Laboratory of the IPPMG-UFRJ according to the protocols of the Euroflow Consortium. The specific subgroups are defined by the analysis of the molecular equivalents of the translocations t (4; 11), t (9; 22) and t (12; 21) by RT-PCR.

RESULTS

❖ Partial analysis of clinical, cytogenetic and molecular data of patients already included in the study

Table 1 - Clinical and cyto-molecular features

Features	Number of p atients (%)
Gender	
Female	12 (43)
Male	16 (57)
Age (years)	
Median	7,08
Range	1,92-14
Risk Group	
Standard Risk	1 (4)
Intermediate Risk	7 (25)
High Risk	13 (46)
NK	7 (25)
Cytogenetic abnormalities	
Hyperdiploidy	
t(12;21)	1 (10)
t(9;22)	1 (10)
iAMP21	7 (70)
	1 (10)

Table 2 – Distribution of samples according to the therapy phase

Therapeutic Protocol Phase	Number of samples
Diagnosis	28
D15	9
D33	10
D78	5
Pre-Protocol II	11
Total	63

❖Technical qualification and analysis of 3 cell lines of B-cell precursor leukemia (Nalm-6, SUP-B15 and SEM) and 10 bone marrow samples for the diagnosis of pediatric patients with B-ALL derived with identification and selection of V(D)J rearrangements for use in serial samples at the subsequent analysis of MRD by RQ-PCR V(D)J and simultaneously by SNG as part of the pilot study.

RESULTS

Scheme 1 - *Identification of Ig/TCR rearrangements*

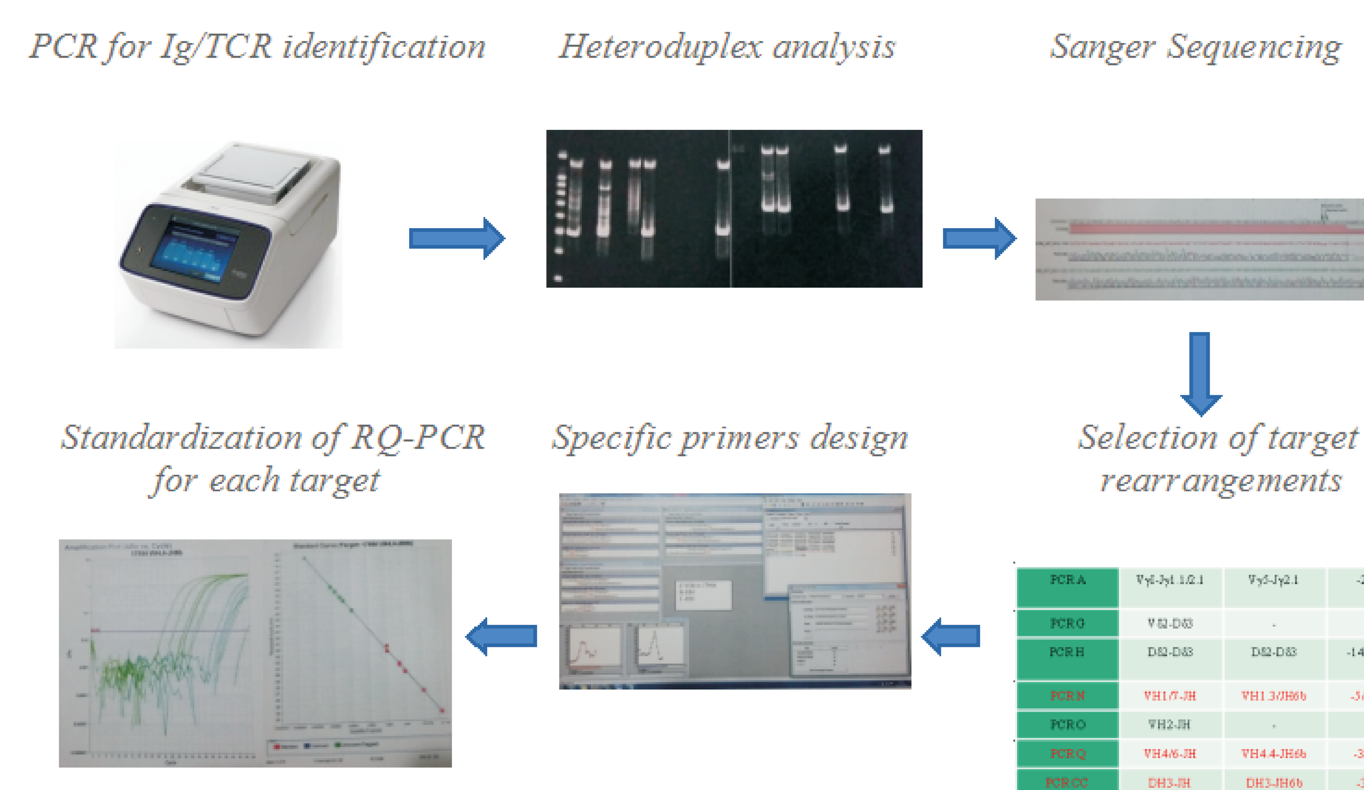

Table 3 - *Rearrangements V(D)J identified and selected in **patient Id1***

PCR H	Dδ2-Dδ3	Dδ2-Dδ3	-2/+9/-2
PCR O	VH2-JH	VH2.26-DH6.6-JH6b	0/+8/-5 -4/+9/-4
PCR P	VH3-JH	VH3.9-DH2.2-DH3.10-JH6b	-3/+9/-4 -6/+26/-1 -9/+5/-5

Table 4 - *Rearrangements V(D)J identified and selected in **patient Id2***

PCR G	Vδ2-Dδ3	Dδ3	-3/+6/0 ou -3/+8/-3
PCR H	Dδ2-Dδ3	-	m[vw]
PCR AA	DH1-JH	-	m[vw]
PCR BB	DH2-JH	DH2.15-JH6b	-6/+6/-6
PCR CC	DH3-JH	DH3.9	-

Table 5 - *Rearrangements V(D)J identified and selected in **patient Id3***

PCR B	VγI – Jγ1.3/2.3	Vγ3 – Jγ1.3	0/+8/-5
PCR G	Vδ2-Dδ3	Dδ2-Dδ3	-24/+15/-25
PCR Q	VH4/6-JH	VH6.1-JH5b	-14/+34/-13
PCR U	TCRB-Tube3	Dβ2-Jβ2.2	-5/+2/-2
PCR AA	DH1-JH	DH1.7-JH5b	-10/28/-13

Table 6 - *Rearrangements V(D)J identified and selected in **patient Id4***

PCR G	Vδ2-Dδ3	Vδ2-Dδ3	0/+2/0
PCR J	VkII-Kde	Vk2.30-Kde	-5/+5/-1
PCR M	Intron-Kde	Intron-Kde	-2/0/-9
PCR N	VH1/7-JH	VH1.1-DH4.11-JH5b	-13/+9/-8 0/+1/-3
PCR T	TCRB-Tube2	Vβ27.1-Dβ2-Jβ2.3	-4/+9/0 0/+6/-6
PCR U	TCRB-Tube3	Dβ2-Jβ2.3	0/+6/-6
PCR W	Vδ2-Jα Tube2	-	m[vw]
PCR UU	Vl-Jl	Vl2.5-Jl1	-6/+3/-4

CONCLUSION

NGS can be an advantageous methodology for the detection of DRM in patients with B-ALL, allowing a better stratification of risk of these patients, besides providing a greater understanding of the biological aspects of ALL.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

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