CYTO-MOLECULAR STUDIES AND NOVEL *KMT2A-AFDN* SEQUENCING DATA IN A PRO-B-ALL INFANT: DISCUSSING THE DIFFERENCE BETWEEN ACUTE LEUKEMIAS TREATMENT PROTOCOLS

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Objectives

Chromosomal translocations involving the *KMT2A* gene (aka MLL) are reported in 18% of pediatric patients with acute myeloid leukemia (AML) and 6-8% of children with acute lymphoblastic leukemia (ALL). However, in infants (<1 year of age), 70% of patients with ALL and 35-50% of patients with AML present *KMT2A* rearrangements (KMT2A-r). *KMT2A-r* are genetically heterogeneous, and the presence of distinct *KMT2A-r* is an independent dismal prognostic factor, while very few *KMT2A* rearrangements display either a good or an intermediate outcome. The translocation t(6;11)(q27;q23) represent about 5% of acute leukemia with 11q23/*KMT2A-r* and is more frequent in AML than in ALL (7.8% of *KMT2A-r* AML vs 1.8% of *KMT2A-r* ALL). It has been mainly found in AML-M5 and M4 and is associated with a poor prognosis. This abnormality is rare in infants (0,3%) and more frequent in children (6,6%) and adults (6%). Balgobind and coworkers (2009), in a study with 35 pediatric patients with *KMT2A-r* AML, reported a frequency of 8% infant cases. The t(6;11) can be very elusive, escaping recognition with conventional banding techniques, and thus may have been overlooked and mistakenly described as deletion on 11q23. Martineau and colleagues (1998), in a series of 30 AL cases with t(6;11)(q27;q23), (27 AMLs and 3 ALLs), reported additional chromosome abnormalities in 10% of the studied cases. Molecularly, the t(6;11)(q27;q23) is characterized by the fusion between the KMT2A gene located on 11q23, and the *AFDN* gene (aka *AF6* and *MLLT4*) on 6q27. The *AFDN* protein is a scaffolding protein with a role in cell-cell junctional complexes. It plays an essential role in regulating apical-basal polarity and adherens junction integrity. Loss of expression or lower expression of AFDN is found correlated with poor prognosis in different types of cancer. In this sense, systematic cyto-molecular and cytogenomic monitoring have proved necessary, not only for demonstrating the t(6;11)(q27;q23) / *KMT2A-AFDN* true frequency but also to ascertain the *KMT2A* partner gene and its genomic mutational profile.

Herein we report the case of an infant with Pro-B-ALL, which presented a novel breakpoint in the fusion *KMT2A-AFDN* involving intron 23 of the *KMT2A* gene.

**This project was approved by the research ethics committee of INCA (#077/08).*

Material and Methods

At the diagnosis, bone marrow sample from a 1 year and 1-month-old boy was referred to the Laboratory of Cytogenetics – INCA. Immunophenotyping showed 95% of blast Cells, and a profile compatible with Pro-B-ALL diagnosis, being treated under the ALL-BFM-2009 protocol. Currently, the baby is alive.

Cytogenetic analysis was performed at diagnosis, in bone marrow samples cultured for 24 hours, according to the standard protocol. FISH experiments were conducted using homemade and commercial probes, according to the manufacturers' instructions.

LDI-PCR assays were used to identify the *KMT2A* partner genes and their corresponding breakpoints.

Resultados

Conventional cytogenetics by G-Banding technique revealed the karyotype 50,XY,+6,t(6;11)(q27;q23),+12,+19,+22 **(Figure 1).**

FISH assay with the MLL break apart, dual color probe was positive for *KMT2A* gene rearrangement **(Figure 2).**

The LDI-PCR sequencing LDI-PCR sequencing showed the fusion MLL (Intron 23) - *AFDN* (Intron 1). **(Figure 3).**

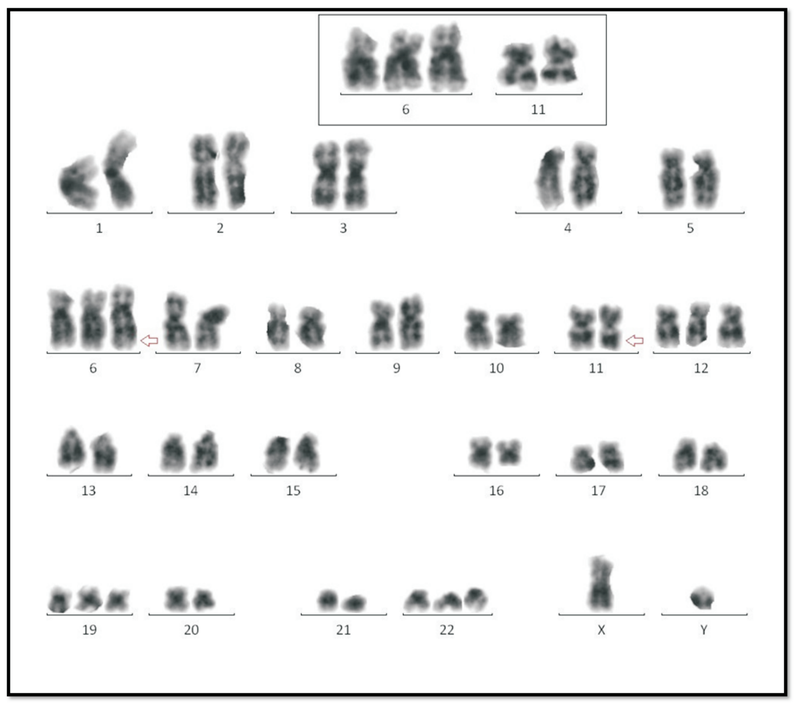

Figure 1: Cytogenetic analysis by G-banding karyotype. The red arrows show the derivative chromosomes 6 and 11. Also, a partial karyotype highlights the trisomy 6 and derivative chromosomes 6 and 11.

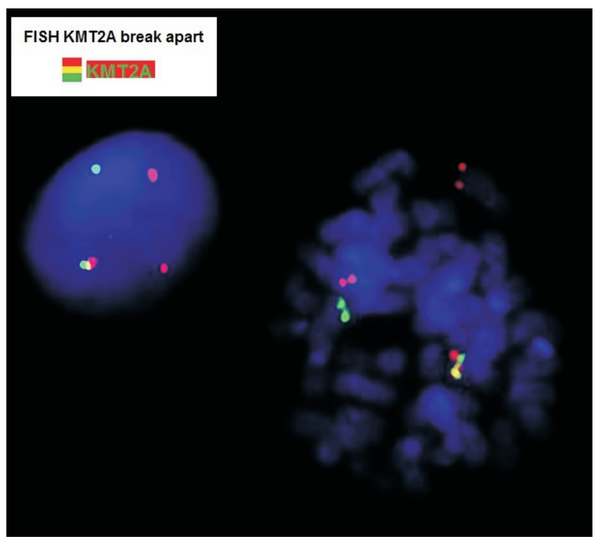

Figure 2: Molecular cytogenetics. FISH analysis in interphase nuclei showing the *KMT2A* gene rearrangement. The fused signals show the normal gene. The green and red signals broke apart confirm the rearrangement. The extra red signal corresponds to the extra chromosome 6.

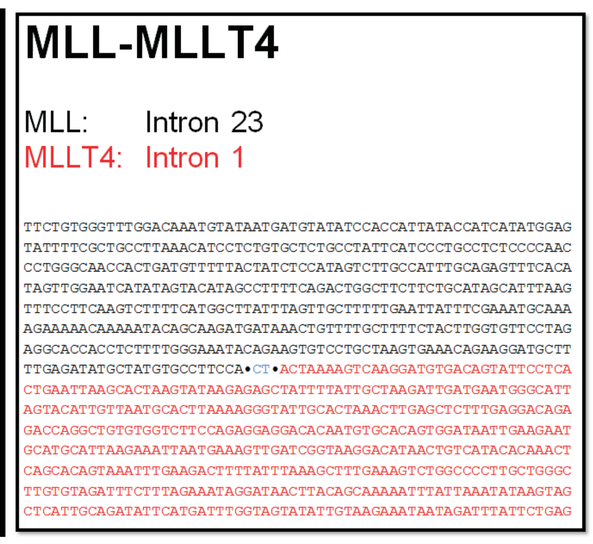

Figure 3: Results of LDI-PCR assay. The LDI-PCR sequencing identified the *KMT2A-MLLT4* fusion gene, and the corresponding breakpoints.

Discussion and Conclusion

Infants with AML harboring the translocation t(6;11)(q27;q23) are directed to the high-risk protocol. On the other hand, to be referred as high-risk, in infants with ALL it is dependent on:

(i) the response to the initial treatment;
(ii) the information from the minimal residual disease (MRD) follow-up.

The presence of the new variant observed by LDI-PCR sequencing may be related to the rare presence of this transfer in infants.

Besides, further sequencing approaches may contribute to immunological studies in the management of the child presenting this rare translocation of childhood AML.

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