

DNA methyltransferase 3a hot-spot locus is not mutated in pediatric patients affected by acute myeloid or T-cell acute lymphoblastic leukemia: an Italian study

Acute lymphoblastic and myeloid leukemia (ALL, AML) are neoplasms characterized by a clonal, abnormal and self-maintaining proliferation of hematopoietic cells. These acute leukemias can be categorized into several subtypes, based principally on phenotype and according to specific genetic abnormalities. Nowadays, the outcome of most pediatric patients with acute leukemia is favorable; 5-year event-free survival is 76-86% for ALL and 49-63% for AML.¹ However, new therapies are needed to cure all leukemia patients and to reduce the burden of treatment-related complications. To this end, the development of new drugs, as well as a better understanding of the biology of these malignancies, is warranted.

The comprehension of leukemogenesis passes through the identification of the genetic alterations causing abnormal proliferation of cancer cells. Molecular characterization of pediatric leukemias revealed a number of recurrent genetic lesions, and the advent of the large-scale DNA resequencing has allowed the identification of new somatically mutated genes.² Recently, improvements in sequencing and bioinformatic technologies have provided the means of performing analyses of whole cancer genomes from AML adult patients and somatic mutations have been found at the DNA methyltransferase 3a (*DNMT3A*) gene. Twenty-two percent of adult AML showed *DNMT3A* mutations; in particular, the substitution at the R882 codon represents 15% of all mutations identified.³ *DNMT3A* recurrent somatic mutations have been described also in a significant proportion of patients with myelodysplastic syndromes⁴ and acute monocytic leukemia.⁵

Two studies reported the frequency of *DNMT3A* mutations in childhood AML. A report from Thol *et al.*⁶ found a total of 2 patients with a mutation in codon R882 in a cohort of 195 pediatric AML patients, and the Children's Oncology Group identified no *DNMT3A* mutation in a cohort of 180 children with AML, showing that *DNMT3A* mutations are rare in childhood AML.⁷

Here, we investigated the mutational status of *DNMT3A* exon 23 in a pediatric cohort of 264 Italian patients. For this analysis, 94 pediatric patients with AML and 170 pediatric patients with T-ALL (Tables 1 and 2) were selected on the basis of availability of DNA samples. Pediatric patients with diagnosis of *de novo* AML or T-ALL were enrolled in the AIEOP (Italian Association of Pediatric Hematology and Oncology) treatment protocols.⁸ Bone marrow (BM) samples of children with AML and T-ALL were collected and stored in the national reference laboratories from 2000 to 2008 and from 2000 to 2006, respectively. Morphological evaluation, immunophenotyping and immunoglobulin and T-cell receptor (TCR) rearrangement analysis were performed at the laboratory of Pediatric Hematology of the University-Hospital in Padova and at the San Gerardo Hospital in Monza; these are both centralized national reference laboratories. In accordance with the principles of the Declaration of Helsinki, consent was obtained from parents or legal guardians of all study participants. Both cohorts, AML and T-ALL, were chosen because recent publications showed that these leukemias shared biological features, such as several translocations and somatically acquired mutations in crucial genes, e.g. *RAS*, *WT1*, *FLT3*, *NF1*, and

PHF6.⁹ Moreover, aberrant methylation events have recently been identified as being highly relevant also in T-ALL since they are associated with clinicopathological features, as is already known for AML.¹⁰ We performed a comprehensive analysis of mutations occurring in the "hot-spot" locus of *DNMT3A*, by processing and sequencing the target region of exon 23 using the Roche 454-Junior platform with the Universal Tailed Amplicon Sequencing. Its experimental design allows the same amplicon to be rapidly sequenced in a large number of samples. Since the technology of 454 amplicon sequencing is still relatively new, we also analyzed 20 adult AML patients at diagnosis as putative positive controls. We obtained 271,253 sequences aligned with *DNMT3A* exon 23. The average number of sequences per patient was 965±446 for 281 out of the 284 (98.9%) patients studied. None of the pediatric AML or T-ALL patients analyzed showed mutations at the "hot-spot" *DNMT3A* region. By contrast, 3 out of the 20 (15%) AML adult patients carried a mutation in exon 23 of *DNMT3A*, confirming a mutational rate similar to that previously described.⁵ All three mutations occurred at amino acid R882: 2 patients had the R882H mutation, while one patient carried the R882P mutation. These mutated sequences were present with mean frequencies of 46.16±2.9%, indicating the heterozygous status of the mutation.

Our results confirmed that also in our Italian pediatric AML patients, *DNMT3A* mutations at the R882 locus are rare; moreover, we highlighted the fact that *DNMT3A* is also not mutated in pediatric T-ALL. We enhanced mutational analysis of methyltransferases by sequencing *DNMT3B*, also a member of the *DNMT3* family of

Table 1. Clinical features of AML pediatric patients.

	Total (n)	(%)
Patients enrolled	94	100
median age, years (range)	8.1 (0.3-16)	
Sex		
male	52	55
female	42	45
WBC count, median, 10 ⁹ /L, (range)	66.4 (1-263)	
Percentage of BM blast count, median (range)	71.6 (20-98)	
FAB		
M0	4	4
M1	17	18
M2	29	31
M3	2	2
M4+M4Eo	16	17
M5+M5A	17	18
M6	2	2
M7	5	6
unclassified	2	2
Cytogenetic features		
t(8;21)(q22;p22)	22	23
inv 16 (p13;q22)	8	9
t(15;17)	2	2
11q23	17	18
normal karyotype	22	23
complex karyotype	9	10
unclassified	14	15
Gene mutation		
<i>FLT3</i>	4	4
<i>CEBPa</i>	2	2
<i>IDH1</i>	1	1

Table 2. Clinical features of T-ALL pediatric patients.

	Total (n)	(%)
Patient enrolled	170	100
median years (age) (range)	8.5 (1.2-17.8)	
WBC count, median, 10 ⁹ /L (range)	145 (2.9-1000)	
Immunophenotype		
early-T	65	38
mature-T	22	13
thym-T	68	40
biclinal	11	6
T not specified	4	2
DNA index		
1	148	87
≥ 1.16	7	4
> 1 and < 1.16	6	4
unclassified	9	5
Prednisone response		
good	107	63
poor	61	36
unclassified	2	1
MRD risk stratification		
standard	18	11
medium	73	43
high	28	16
unclassified	51	30

genes.^{5,11} *DNMT3B* is considered functionally complementary to *DNMT3A* with common and differential target specificities, and may be considered to play a role in the aberrant methylation observed in acute leukemia.¹² The mutational screening was performed in some of our pediatric cohort. In particular, 60 T-ALL patients and 90 AML patients were sequenced at the homolog amino acid R823 at exon 23 of *DNMT3B* and no mutations were found, suggesting that neither methylase provides information about pediatric leukemia at the focused genomic locus.

Specifically, the difference between genetic alterations observed in childhood leukemia with respect to adults confirmed that only in a few cases were genes found to be mutated from adult whole-genome sequencing relevant for childhood AML. It is to be expected that other genes play a key role in the pathogenesis of childhood leukemia, and most likely in other pediatric solid tumors. These will be identified in extended somatic whole-genome sequencing of pediatric specimens of diseased cells. This study also highlights how recent improvements in sequencing and bioinformatic technologies provide new, rapid and extremely reliable methods to provide information on gene mutation status in large cohorts of patients. This opportunity to genetically characterize patients opens a new era of study in which the discovery of specific lesions might improve our knowledge on leukemogenesis and allow specific gene lesions to be therapeutically targeted.

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