Mutation screening in the genes *ASXL1*, *TP53*, and *KRASNRAS* of patients with myelodysplastic syndrome

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INTRODUCTION AND OBJECTIVE

Myelodisplastic Syndrome (MDS) is a heterogeneous group of clonal bone marrow disorders characterized by ineffective hematopoiesis, different degrees of cellular dysplasia, and increased risk of progression to acute myeloid leukemia (AML). IPSS is the gold standard for MDS classification, but patients with different clinical behaviors often coexist in the same group, suggesting that the currently available scores are insufficient.

The several genes that have been identified recently as mutated in MDS, including *ASXL1, TP53,* and *KRAS/NRAS*, could contribute to a deeper classification, as well as participate in the prognosis and the disease progression. In this study, we studied the mutations in A*SXL1, TP53*, and *NRAS/KRAS* genes in 50 patients with MDS and AML secondary to MDS, revealed by sequencing genomic bone marrow DNA.

MATERIAL AND METHODS

We studied 50 patients with diagnosis of MDS and AML secondary to MDS, treated at the Pedro Ernesto University Hospital (HUPE), Rio de Janeiro, Brazil, from 2012 to 2014. The scientific analysis of the samples was approved by the Ethics Committee of the Pedro Ernesto University Hospital, under the code CAAE 08084712.4.0000.5259. To validate possible mutations and polymorphisms (germline changes) of genes evaluated, peripheral blood of 129 eligible healthy controls (men and women) were collected in Rio de Janeiro. About 100 ng of genomic DNA was used for the amplification of the coding region of *ASXL1* (exon 1 to 6), *TP53* (exons 4 to 9) and *KRAS/NRAS* genes (exon 1 to 2). The purified products were sequenced in 3130 sequencer Genetic Analyzer from Applied Biosystems.

RESULTS AND CONCLUSION

Of the 50 patients studied, 9⁄50 (18%) had mutation in of the target genes (Table 1). Mutation in the *TP53* gene was the most frequent in 6⁄50 patients (12%), followed by *ASXL1* in 2⁄50 patients (4%), and NRAS in 1⁄50 patient (2%). Regarding treatment, 9⁄50 (18%) of the patients with AML received chemotherapy. Of these patients, 5⁄9 (55%) died and one is in disease remission after conventional chemotherapy. The three remaining patients underwent allogeneic bone marrow transplantation; two of them are alive and in disease remission, and the last died due to complications of the transplant.

We observed no statistical relationships among hematological, cytogenetic, and survival variables or cases mutated and those not mutated.

Table 1 – Summary of the mutations

Gene	Case number	Classification (WHO 2008)	Nucleotide Change⁄Amino Acid Change	Gender	Age	Survival (months)	Tratment
ASXL1	5	AML	c.1846G>A, N615N	F	44	16 (dead)	Decitabine
	29	RCUD (RT)	c.1923C>T, I641I	F	56	110 (alive)	No specific therapy with conservative approach
TP53	20	RCMD	c.384T>A, P128P	F	65	Missed	No specific therapy with conservative approach
	24	RAEB2	c.204InsT, Q68V	F	NI	40 (alive)	Azacitidine + Exjade® + allogeneic bone marrow transplantation
	28	RCUD (RT)	c.216InsA, A72T	M	70	44 (alive)	No specific therapy with conservative approach
	37	NI	c.669C>T, P 223P c.711T>A, M237K	M	NI	0 (dead)	No specific therapy with conservative approach
	40	AML	c.669C>T, P223P	M	31	4 (dead)	7 +3 + FLAG-IDA +MEC
	49	RCMD	c.384T>A, P128P	M	67	32 (alive)	No specific therapy with conservative approach
NRAS	38	RCUD (RT)	c.Ins279C, R97R	F	68	46 (alive)	No specific therapy with conservative approach

Keywords: *ASXL1, TP53, KRAS/NRAS*, bone marrow, myelodisplastic syndrome

Acknowledgements
This study was supported by Programa de Oncobiologia, Rio de Janeiro, Brazil.

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