Donor chimerism in blood cells and free circulating plasma DNA in patients after allo-HSCT

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Introduction

Free circulating DNA (cfDNA) is present in blood plasma and is formed as a result of active secretion or cell death. In patients after transplantation of allogeneic hematopoietic stem cells (allo-HSCT), the DNA of the recipient's origin should still be present in the peripheral blood (PB) and bone marrow (BM), even in cases of complete donor chimerism (dc). However, an increase in the proportion of recipient cfDNA in patients with acute leukemia may be associated with a potential relapse of the disease or a transplant rejection.

Aims: To evaluate the association between dc measured in cfDNA and adverse events after allo-HSCT in patients with acute leukemia (AL). At the same points, to assess the minimal residual disease (MRD) by flow cytometry in BM. Additionally for cases with *FLT3-ITD* mutation to measure this specific tumor marker in the cfDNA.

Results

In the control group, the median proportion of donor cfDNA in plasma was 88% (76%-100%). In patients of the study group with complete dc in BM and PB, the median proportion of the donor cfDNA was 77% (35%-100%). In 10 patients, we observed a decrease in the proportion of donor cfDNA in several consecutive measurements (from one time point to another) while maintaining complete dc in PB and BM. In this group, we observed: 1 relapse, 2 molecular relapses, and the establishment of mixed hematopoiesis in 2 patients. In 5 AML patients with *FLT3-ITD* gene mutation it was also tested in cfDNA. (Tab.1). All 5 patients had an increase in the proportion of the recipient in the cfDNA over time. A significant decrease in the donor's contribution and the presence of a mutation have been observed in cfDNA prior to relapse (Patient 3*). In patients with stable donor levels of 65% or higher in cfDNA, we did not see the development of relapses. The results of parallel assessment of MRD in BM by flow cytometry and chimerism in cfDNA are presented in Tab.2.

Pt.#	Month after allo-HSCT	MRD status, +Positive -Negative	cfDNA		PB		BM	
			FLT3-ITD VAF(%)	Recipient's fraction (%)	FLT3-ITD VAF(%)	Recipient's fraction (%)	FLT3-ITD VAF(%)	Recipient's fraction (%)
1	2	-	**1,5**	**10**	0	0	0	0
1	3	-	**0,6**	**70**	0	0	0	0
1	6	-	**15**	**44**	2	1	0	0
2	3	-	**0,5**	**42**	0	9	0	9
2	6	-	**0**	**75**	0	0	0	0
3	1	0,0026	**1**	**55**	0	0	0	0
3*	2	0,3162	**17**	**57**	0	22	37	60
4	1	-	**0**	**15**	0	0	0	0
4	2	-	**0**	**31**	0	2	0	2
5	1	-	**9**	**30**	0	0	0	1
5	2	-	**0**	**49**	0	0	0	0

Table 1: Variant allele frequency (VAF) of the FLT3-ITD mutation and the recipient's proportion in cfDNA, MRD status

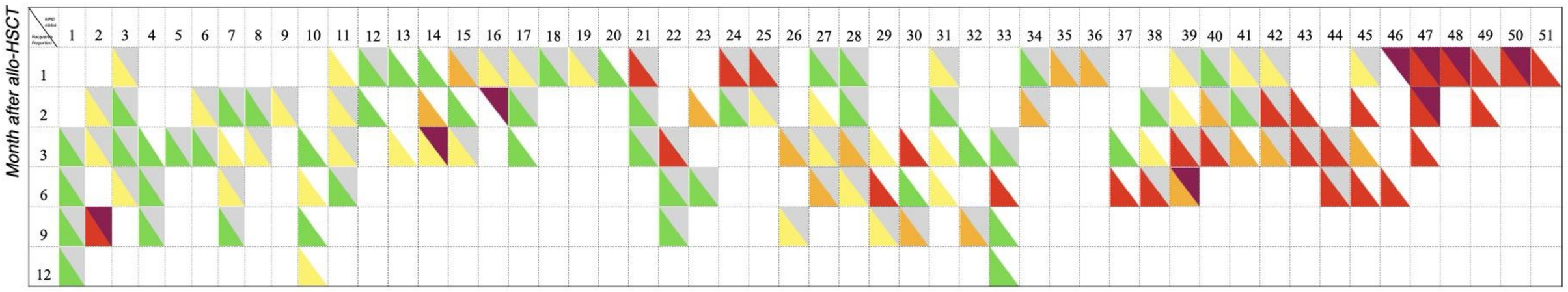

Table 2: The recipient's proportion in cfDNA (green up to 20%, yellow from 20% to 30%, orange from 30% to 40%, red over 40%) MRD status (grey— negative, burgundy— positive, white-no date), 3 patients had primary graft failure and are not included in this table.

Materials and methods

DNA samples were isolated from PB, BM and plasma of 54 AL patients who underwent allo-HSCT at the NMRC for Hematology (Moscow, Russia) between 08/14/2023 and 05/30/2024 (median age is 38 years (20-66 years); 35 are women, 19 are men). The control group consisted of 12 patients in long-term remission (more than three years). DNAs from PB, BM and plasma at total of 141 time points were taken into analysis. Cellular DNA was isolated by a modified salt extraction method, and cfDNA was isolated using the CF Extra 50 kit (Raissol, Russia). BM samples from most patients were taken at the same time as blood collection. STR DNA profiles were determined using the COrDIS Plus PCR kit (Gordiz LLC, Russia), chimerism was analyzed using GeneMapper v.4 software (Applied Biosystems, USA). MRD in the BM was measured using flow cytometry at the same time points chimerism assas the plasma cfDNA chimerism assay. For 5 AML patients from the study cohort who had F L T 3 -ITD gene mutation at the onset of the disease, cfDNA samples were also tested for this marker by PCR followed by fragment analysis.

Conclusion

In the cases with donor cfDNA fraction below 65%, the development of adverse events, especially relapses, is much more frequent, despite the fact that the study of PB and BM chimerism indicates the absence of even mixed hematopoiesis. In some cases, an increase in the recipient's fraction in cfDNA is accompanied by the appearance of FLT3-ITD (VAF 0.5-19%) tumor marker before the clinical manifestation of relapse. Longer follow-up is needed to assess the prognostic significance of these findings.

References

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