Assessment of hematopoietic stem cell molecular engraftment based on STR analysis

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Introduction
Donor chimerism monitoring is the main way to control the process of hematopoietic engraftment. Assessment of the engraftment course in oncohematological patients is important for the choice of treatment strategy and further management of the patient.

Patients and methods
In order to assess engraftment, 38 patients with oncohematologic diseases were analyzed who underwent 38 hematopoietic stem cell transplantations (HSCT) from 2017 to 2019, including 13 women (34%) and 25 men (66%). The median age at the time of transplantation was 33 (2–47) years. The gender distribution among donors was 19 women (50%), and 19 males (50%). The patients were divided into two groups: 26 patients (68%) after allogeneic compatible HSCT (allo-HSCT), and 12 patients (32%), after haploidentical HSCT (haplo-HSCT). The donor chimerism was determined by polymerase chain reaction of short tandem repeats (STR-PCR) in peripheral blood on days +30, +60, and +100 after HSCT. The study was conducted more frequently in cases of mixed chimerism detection. For amplification of STR markers, the commercial AmpFlSTR® Identifiler® Plus Kit (UK) kit was used; PCR products were separated by capillary electrophoresis on a 3500 Genetic Analyzer (HITACHI Applied Biosystems, Japan). The alleles were identified using the Chimer Marker v3.1.0 software

Results
Concerning ratios of donor/recipient pairs, the female donor/male recipient combinations (15 pairs), and male donor/female recipient transplants (13 pairs) were more common than male donor/female recipient and female donor/female recipient combinations. When assessing the stem cell engraftment, all markers were informative for the studied donor/recipient pairs. According to the degree of significance, the loci in the allo-HSCT pairs were distributed as follows: D13S317 / D18S51> D5S818 / D16S539 / D21S11 / D7S820> TH01 / AMEL / FGA / D8s1179 / D2S1338> CSF1PO / D3S1358 / TPOX> D19S433 / W19A. The numbers of informative genetic loci in the donor/recipient pairs varied from 4 to 13. For haplo-HSCT pairs, the distribution of loci was as follows: D13S317 / D7S820 / AMEL> D16S539 / D2S1338 / D18S51> D5S818 / FGA / D8s1179 / D2S111 / CSF1PO / D3S1358 / VWA> D19S433> TH01 / TPOX, with 1 to 8 informative loci. According to the results of our analysis, complete donor chimerism (99-100%) in haplo-HSCT was found in 2 patients (18%) on the day +30 after HSCT, according to HLA matching degree of 5/10 and 6/10; the remaining ten cases showed mixed chimerism. On day +100, 2 out of 10 reached full donor chimerism. Complete chimerism was revealed in 11 pairs with allo-HSCT, among them, the HLA matching degree was 10/10 in 9 pairs, and 5/10, in two pairs. By 100 days, 3 patients developed a transition from mixed to complete chimerism.

Conclusion
The analysis showed an association between HLA typing results, and the type of performed HSCT (allo- or haplo-HSCT). Chimerism monitoring after transplantation is an integral part of more effective prognosis for relapse and factor of improved survival for the patients after HSCT.

Keywords
Chimerism, engraftment, hematopoietic stem cell transplantation, STR loci.
Impact of additional chromosomal abnormalities on survival after allo-HSCT in CML patients

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Introduction

Widespread use of targeted therapy with 2nd and 3rd generation tyrosine kinase inhibitors (TKIs) and appropriate revision of indications for allogeneic hematopoietic stem cell transplantation (allo-HSCT) allowed to achieve optimal therapeutic responses in the majority of chronic myelogenous leukemia (CML) patients. Nevertheless, inadequate therapeutic response and relapses, which are in most cases associated with additional chromosomal aberrations (ACAs) and mutations in BCR-ABL kinase domain (BCR-ABL KD), still remain a problem leading to decreased overall survival (OS) in patients. Moreover, there is still no comprehensive concept delineating ACAs role and prognostic value on therapy responses. There are scarce data on the role of ACAs in allo-HSCT outcomes. The aim of our study was to evaluate the ACAs impact upon long-term OS in allo-HSCT recipients.

Patients and methods

This study included retrospective data on the cohort of 101 CML patients with median age of 38 years (range, 19-61) undergoing allo-HSCT from HLA-matched sibling (n=26), haploidentical donor (n=14), or unrelated donor (n=61) in the R. M. Gorbacheva Memorial Institute between 2010 and 2019. By the time of allo-HSCT, 11 of these patients (11%) had both BCR-ABL KD mutations and ACAs. A cytogenetic procedure, mutation analysis was performed by Sanger sequencing. OS were estimated by Kaplan-Meier long-rank test. A univariate analysis was carried out using the log-rank test. The following variables were considered as potential prognostic factors: donor type, donor-recipient gender, donor-recipient age, donor-recipient HLA matching, donors’ age, patients’ age, patients’ sex, the presence of ACAs, and treatment with TKIs. All the patients received 1st, 2nd or 3rd generation TKIs prior to allo-HSCT. 39 patients (39% of the total) had BCR-ABL KD mutations, whereas T315I mutation was found in 15 of them (15%). All the patients were divided into prognostic groups, depending on ACAs, according to revision of ACAs prognostic value on therapy results. 34 patients (34%) had any ACAs in Ph+ cells at any given moment starting from diagnosis, 22 (22%) of these patients had high risk group ACAs (single i(17)(q10), -7/del7q or 3q26.2 or as a component of complex ACAs and complex ACAs without these chromosomal abnormalities). Sixteen patients (16%) had both BCR-ABL KD mutations and ACAs. A cytogenetic study of bone marrow was carried out according to standard cytogenetic procedure, mutation analysis was performed by Sanger sequencing. OS were estimated by Kaplan-Meier (long-rank test).