LETTER TO THE EDITOR

Does early chimerism testing predict outcomes after allogeneic hematopoietic stem cell transplantation?

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Allogeneic stem cell transplantation (HCT) is a potentially curative treatment for patients with various hematologic malignancies. Following HCT it is common practice to assess peripheral blood sorted T cell and myeloid chimerism to monitor genotypic origins of post-HCT hematopoiesis and monitor for relapse [1]. Multiple methods of assessing chimerism after HCT have been used, including assessing short tandem repeats, fluorescent polymerase chain reaction (PCR), quantitative real-time PCR of single nucleotide polymorphisms, and fluorescence in situ hybridization in gender-mismatched transplants [2]. Hematopoiesis that is fully genetically derived from donor is referred to as a complete donor chimerism (CC), whereas hematopoiesis with genetic origins from both donor and patient is referred to as a mixed chimerism (MC) [3]. It has been shown that MC in the setting of malignant hematologic conditions is associated with inferior relapse-free survival (RFS) and overall survival (OS) [4–6]. Identifying the development of MC carries therapeutic implications such as the use of donor lymphocyte infusions (DLIs) or reduction in immunosuppression in effort to eliminate host cells and achieve CC [7–9]. However, studies have also shown that early MC can improve to CC by day +90 without intervention and early chimerism testing does not appear to provide any prognostic information [10–12]. The American Society for Transplantation and Cellular Therapy have formally recommended against testing chimerism in patients who receiving myeloablative (MA) conditioning and consideration of chimerism testing in reduced intensity or non-myeloablative (RIC/NMA) conditioning [13]. While chimerism testing remains controversial, the cost of testing is substantial. The Clinical Laboratory Fee Schedule issued by the Centers for Medicare and Medicaid Services (CMS) estimates cost of each sorted chimerism test to range between $750 and $800 [14]. Hence, the utility of this repeated testing needs to be better understood to further define the role of this additional test and added cost to the process of HCT.

At our institution, sorted chimerism for T cells by CD3+ and myeloid cells by CD33+ are assessed from peripheral blood via quantitative PCR on days +30, +60, and +90 with additional monitoring per provider discretion. This is done in patients who have received RIC/NMA or MA conditioning regimens. We performed an Institutional Review Board approved retrospective study to evaluate the correlation of early donor T cell and myeloid chimerism (day +30 and +60) with clinical outcomes in patients undergoing HCT for hematologic malignancies.

Patients who underwent HCT for any hematologic malignancy between January 2007 and December 2016 at our institution were identified for review. To allow for a homogenous patient population, we only included patients who underwent a peripheral blood stem cell transplant with at least an 8/8 human leukocyte antigen matched unrelated donor. Mismatched unrelated and matched related donors were excluded. Donor chimerism and clinical data were obtained for these patients by retrospective chart review. Patients with CD3+ or CD33+ PCR of 100% donor were considered CC, whereas CD3+ or CD33+ PCR of <100% donor were considered MC. Patients who were undergoing a second HCT or HCT for nonmalignant conditions were excluded from this analysis.

The clinical outcomes of interest were OS and RFS. Patients were censored at the time of last follow up or at the time of death. Univariate Cox proportional hazard regression models were conducted using variables of interest to predict OS and RFS. Multivariate Cox
In a multivariate analysis, adjusted for aforementioned variables, neither CD3+ or CD33+ chimerism at day +30 or day +60 had statistically significant impact on OS (HR for CD3+ 0.72, 95% CI (0.37, 1.41), p=.34 and .61, 95% CI (0.27, 1.4), p=.25, for day +30 and day +60, respectively; (HR for CD33 + 1.81, 95% CI (0.38, 6.8), p=.52 and HR = 0.65, 95% CI (0.09, 4.9), p=.67, for day +30 and day +60, respectively) for OS and RFS, respectively. Of the patients included, 133 had myeloid malignancies. In a secondary multivariate analysis for myeloid malignancies, CD33 early MC did not impact RFS (HR for day +30 2.29, 95% CI (0.67, 7.77), p=.19), (HR for day +60 2.34, 95% CI (0.55, 10.4), p=.242). Of note, a total of 58 patients (28%) had CD3+ and/or CD33+ MC at day +30 and/or day +60. Of these, four (7%) underwent reduction in their immunosuppression and none received DLI.

In our study sorted MC at day +30 and +60 were not associated with inferior OS or RFS. These findings are consistent with that of prior studies showing that MC prior to day +90 do not offer prognostic information [10]. Our findings are different than those found in a recent retrospective study of patients undergoing MA conditioning for acute myeloid leukemia and myelodysplastic syndrome in which MC at day +30 and +60 was not associated with inferior OS; however, the presence of MC at day +60 was associated with inferior RFS [15].

Monitoring for evidence of disease relapse is a critical component for post-HCT care. As technology advances more sensitive testing strategies like PCR and high sensitivity flow are being used more routinely to monitor early disease relapse; and these more advance testing strategies bring into question the utility of continued early chimerism testing. In the current era, thoughtful utilization of limited medical resources and costs of care are becoming ever more important considerations for our patients and the health care system. In the United States, the cost of peripheral blood chimerism is several hundred dollars as per CMS fees. For the 209 patients reviewed in this study, early chimerism lab testing costs alone were estimated to be more than $3,000,000 and this expense had no impact on patient outcomes. The controversial nature of early chimerism testing, the findings in

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**Table 1. Chimerism outcomes.**

| Outcome                  | Predictor                  | Hazard ratio (95%CI for HR) | p-value |
|--------------------------|----------------------------|----------------------------|---------|
| **Univariate analysis**  |                            |                            |         |
| Relapse-free survival    | CD3 chimerism at day +30 (MC vs. CC) | 1.0 (0.59, 1.72) | .99     |
|                          | CD3 chimerism at day +60 (MC vs. CC) | 0.9 (0.48, 1.71) | .76     |
|                          | CD33 chimerism at day +30 (MC vs. CC) | 3.1 (0.97, 9.79) | .06     |
|                          | CD33 chimerism at day +60 (MC vs. CC) | 2.3 (0.72, 7.36) | .16     |
| Overall survival         | CD3 chimerism at day +30 (MC vs. CC) | 0.65 (0.34, 1.22) | .18     |
|                          | CD3 chimerism at day +60 (MC vs. CC) | 0.55 (0.25, 1.23) | .15     |
|                          | CD33 chimerism at day +30 (MC vs. CC) | 2.12 (0.52, 8.68) | .30     |
|                          | CD33 chimerism at day +60 (MC vs. CC) | 0.64 (0.09, 4.64) | .66     |
| **Multivariate analysis**|                            |                            |         |
| Overall survival         | CD3 chimerism at day +30 (CC vs. MC) | 0.72 (0.37, 1.4) | .34     |
|                          | CD3 chimerism at day +60 (CC vs. MC) | 0.61 (0.27, 1.4) | .25     |
|                          | CD33 chimerism at day +30 (CC vs. MC) | 1.81 (0.38, 6.8) | .52     |
|                          | CD33 chimerism at day +60 (CC vs. MC) | 0.65 (0.09, 4.9) | .67     |

CC: complete chimerism; MC: mixed chimerism.
this study, and the probable unnecessary medical costs have led us to consider cessation of early chimerism testing at our institution. Additional large-scale studies in the era of high sensitivity relapse testing modalities are required to further define the utility continued consideration of early chimerism testing.

Disclosure statement
Mountjoy, Palmer, Kuzne, Khera, Sproat, Leis, Noel, and Slack have no conflict of interest; Jain: consulting for Takeda and CareDx.

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