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TECHNICAL ADVANCE

Challenges in Determining Genotypes for Pharmacogenetics in Allogeneic Hematopoietic Cell Transplant Recipients

Loralie J. Langman,* Lior Nesher,† Dimpy P. Shah,‡ Jacques M. Azzi,‡ Elizabeth J. Shpall,§ Katy Rezvani,§ John L. Black, III,* and Roy F. Chemaly‡

From the Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota; the Infectious Disease Institute,† Ben-Gurion University, Beer-Sheba, Israel; and the Departments of Infectious Diseases, Infection Control, and Employee Health‡ and Stem Cell Transplantation and Cellular Therapy,§ Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas*

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Address correspondence to Loralie J. Langman, Ph.D., Clinical and Forensic Toxicology Laboratory, H-730, Mayo Clinic, 200 First St. SW, Rochester, MN 55905. E-mail: langman.loralie@mayo.edu.

As part of a pharmacogenetic study, paired blood and oral fluid samples were tested for the *IL28B* polymorphism (rs12979860) before and after hematopoietic cell transplantation (HCT) to evaluate changes in the genotype and investigate the utility of genotyping in oral fluid in HCT recipients. In 54 patients with leukemia >18 years of age, samples were collected approximately 7 days before HCT and 60 days after HCT. *IL28B* polymorphism testing was performed using real-time PCR with allele-specific probes. Twenty-four patients had the same genotype as their donors. In 30 patients, the genotype was different from that of the donor. In the oral fluid samples, 4 retained the recipient's genotype, and 18 had a genotype that matched that of the donor. In the remaining 8 patients, the results could not be characterized and appeared to be a combination of both, suggesting mixed proportions of donor and recipient cells. The assumption was that the sloughed epithelial cells of the mouth are of recipient origin. However, oral fluid is a mixture that contains varying numbers of cells of the recipient and immunomodulatory cells from the donor. Therefore, the use of oral fluid after HCT for clinical pharmacogenetics purposes needs further investigation. *(J Mol Diagn 2016, 18: 638–642; http://dx.doi.org/10.1016/j.jmoldx.2016.03.007)*

Pharmacogenetics are increasingly being studied in medicine. In particular, the effect of pharmacogenetics on hematopoietic cell transplantation (HCT) is being studied, especially with regard to dosing of medications.[1,2]

IL28B is an innate cytokine in the interferon (IFN)-γ family that is expressed at low levels by a broad variety of cells in response to viral infections.[3] A single-nucleotide polymorphism called rs12979860 CT, located 3 kb upstream from the *IL28B* gene locus on human chromosome 19q13 (Online Mendelian Inheritance in Man no. 607402), is associated with the response to pegylated interferon and ribavirin combination therapy in individuals with hepatitis C virus infection. Patients with the rs12979860CC genotype, compared with either the CT or TT genotypes, have approximately twofold to threefold greater rates of sustained viral response to combined pegylated interferon and ribavirin therapy.[4] The CC genotype has also been associated with a threefold increase in rate of spontaneous clearance of hepatitis C virus.[5] In addition, IFN-y is a common pathway for other viral infections, such as cytomegalovirus (CMV). Therefore, the rs12979860 polymorphism, which is presumed to affect the production of IFN-y, may also play an important role in CMV infection and disease. A recent study of 151 allogeneic HCT recipients found that the risk of

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CMV infection could be lower when donors have the rs12979860 TT genotype compared with donors with the CT and CC genotypes. In addition, recipients from donors with the TT genotype had a significantly shorter duration of first episodes of CMV compared with those with the CT and CC genotypes, thus indicating that the T allele (recessive genetic model) may be a protective factor against CMV infection in allogeneic HCT recipients.[6]

Blood is traditionally the specimen of choice for pharmacogenetic analysis; however, blood from HCT recipients would reflect the genotype of the donor not the recipient. Traditionally, oral fluid or buccal swabs, which contain the recipient's sloughed buccal cells, have been used to determine an HCT recipient's germline genotype. In this study, we used paired blood and oral fluid samples obtained from HCT recipients before and after HCT to describe changes in the *IL28B* genotype in these matrices.

Materials and Methods

Patient Selection and Sampling

We prospectively enrolled 63 adult (>18 years old) patients diagnosed as having leukemia in complete remission before they underwent allogeneic HCT at MD Anderson Cancer Center during 2014. Peripheral blood and oral fluid samples were collected from these patients approximately 7 days before HCT or conditioning regimen and approximately 60 days after the transplant, when most patients are fully engrafted, their clinical condition stabilized, and donor cells dominate in the blood. Patients in whom we did not obtain pre- or post-HCT genotypes (ie, those who died before day 60, dropped out, or refused to give a sample) were excluded. The final cohort included 54 patients. All samples were tested for the *IL28B* polymorphism (rs12979860) at the Mayo Clinic. Approval for this study was obtained from the institutional review boards where the patients were enrolled and where the genetic assays were performed. All patients provided written informed consent for participation in this study. Their demographic data are given in Table 1.

Table 1 Characteristics of the 54 HCT Recipients

Characteristic	Value
Age at time of HCT, years	
Median (range)	56 (21–73)
Means ± SD	51 ± 14
Sex, No. (%)	
Male	34 (63)
Female	20 (37)
Time from HCT to engraftment, days	
Median (range)	12 (10–33)
Means ± SD	13 ± 4
Race/ethnicity, No. (%)	
White	41 (76)
Black	6 (11)
Non-white Hispanic	6 (11)
Asian	1 (2)
Type of malignancy, No. (%)	
Acute myelogenous leukemia	24 (44)
Myelodysplastic syndrome	12 (22)
Acute lymphocytic leukemia	11 (20)
Chronic myelogenous leukemia	5 (9)
Chronic lymphocytic leukemia	1 (2)
T-cell leukemia	1 (2)
Type of transplant, No. (%)	
Matched related donor	21 (39)
Matched unrelated donor	28 (52)
Cord blood	5 (9)
Type of conditioning regimen, No. (%)	
Myeloablative	50 (93)
Nonmyeloablative	4 (7)

HCT, hematopoietic cell transplantation.

IL28B Determination

Oral samples were collected from patients in the following manner: patients were asked to refrain from drinking or eating for 3 hours and then to fill the Oragene DNA Self-Collection Kit with saliva. The kits were then shipped to the Mayo Clinic Referral Laboratory for processing and analysis. TaqMan real-time PCR analysis with allele-specific probes (Life Technologies, Carlsbad, CA) based on a method described by Cook et al[7] was used to detect rs12979860 CT. Briefly, the assay consists of a primer pair that amplifies an upstream region in the *IL28B* gene. Two probes that are sequence specific for the 2 alleles, the C allele (wild type) and the T allele (mutant type), are labeled with 2 different reporter dyes, 1 for each allele. During PCR amplification, each probe annealed specifically to its complementary sequence between the forward and reverse primer sites. When the probe binds in the proximity of a quencher dye, the result is a quenching of the probe's fluorescence. Because the AmpliTaq-Gold DNA polymerase extends the primers, the polymerase cleaved probes hybridized to the target, separating the reporter dye from the quencher, resulting in increased fluorescence from the reporter. Thus, the fluorescence signal generated by PCR amplification indicates which alleles were present in the sample.

Results

The frequency of changes in genotype from baseline to 60 days after HCT are shown in Figure 1. Among patients with an rs12979860 CC genotype before HCT ($n = 17$), 11 had the same genotype (CC) in blood samples after HCT, indicating that the donor had the same genotype as the recipient. For the remaining 6 patients, the genotype of the donor, as indicated by recipient post-HCT blood samples, was different from that of the recipient, with 5 patients receiving a CT genotype and 1 receiving a TT