Next-Generation Sequencing as a Reliable Method of Quantifying Bone Marrow Engraftment in Bone Marrow Transplant Patients

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Introduction Bone marrow transplantation (BMT) is an effective curative therapy performed on patients with hematological malignancies. Patients undergo conditioning regimens that deplete their existing hematopoietic stem cells, allowing for engraftment of the donor bone marrow. After patients undergo BMT, percent engraftment is periodically measured, determining the success of the therapy. Current molecular testing for bone marrow engraftment (BME) measures short tandem repeats (STR) in the genome to identify differing alleles between the patient and the donor. However, we hypothesize that next generation sequencing (NGS) provides an efficient method of BME measurement that is more accurate and sensitive than current STR analyses. **Methods** We performed targeted NGS with an existing panel used for unrelated pharmacogenetic testing on DNA from unrelated patients mixed to create a range of varying concentrations of each patient, which was used to assess linearity. We also utilized patient blood samples from BMT samples from the donor, recipient pre-transplant, and recipient post-transplant. STR analysis was performed on samples as well, which was used to provide baseline comparative results. **Results** The targeted NGS panel utilized for these assays targets over 430 single nucleotide variants (SNVs). This was narrowed down to 40 benign SNVs that commonly occur in the human population. The STR panel, however, targets 16 loci. SNVs were considered informative if the genotypes were different between donor and recipient. The most informative loci were those that the pairs were homozygous for different alleles. The number of informative loci obtained via NGS differs from pair to pair, ranging from 15 to around 25 informative SNVs. Linear standard curves generated from NGS showed comparable R^2 values to that generated from STR analysis. Comparison of variant allelic frequencies with NGS provided an easily quantifiable measurement for percent engraftment when comparing donor samples with post-transplant samples with BMT pairs. **Conclusions** NGS offers a method for easily quantifying BME in patients who have undergone a BMT. Compared to STR analysis, NGS is comparable in accuracy of results, while including more targeted loci. The benefit of NGS includes decreased wet bench time, as well as decreased analysis time, when compared to STR analysis. This suggests that NGS could provide a more efficient method of measuring BME in the clinical setting.