Advancing Transplantation Monitoring and Disease Insight: A Next-Generation Sequencing Assay for detecting HLA loss

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Introduction

Haploidentical stem cell transplantation (haplo-SCT) has emerged as a vital therapeutic option for patients lacking matched donors, offering hope for those with hematologic malignancies and other disorders. However, the phenomenon of human leukocyte antigen (HLA)-loss poses significant clinical challenges, with an impact on clinical outcome post transplantation. The clinical relevance of addressing HLA-loss in haplo-SCT cannot be overstated. It directly influences patient survival, overall treatment efficacy and selection of relevant treatment. Failure to effectively manage HLA-loss can result in increased morbidity and mortality which underscores the critical need for innovative and accurate diagnostic methods.

Aim

The aim of this study is to present a novel NGS assay that can detect the loss of heterozygosity as well as semi quantify HLA loss.

Method

Twenty-six markers (insertion/deletion) located within the intron boundaries in the HLA genes that have a Major Allele Frequency of >0.35 and six outside of the HLA region (chromosome 6) were selected to be used in the NGS assay (see figure 1). Specific primers were designed to amplify both the insertion and deletion to compensate for PCR efficiency and the maximum size of the amplicons were 79 bp to facilitate a 2x76bp sequencing on Illumina instruments as well as to be used in combination with One Lambda Devyser Chimerism.

Sixty-four haplo-identical HLA matched pairs were screened with the novel NGS assay to identify informative markers and assess the informativity of the assay. In addition, three chimeric dilution series with pre-defined recipient percentage (Mixed Chimerism (MC) 0.05 - 50%) were tested in replicates to assess the Limit of Detection where the One Lambda Devyser Chimerism was used as a reference. As a proof of concept, three cell separated samples (CD3, CD33 and CD34) from a patient with Acute myeloid leukemia (AML) relapse was tested.

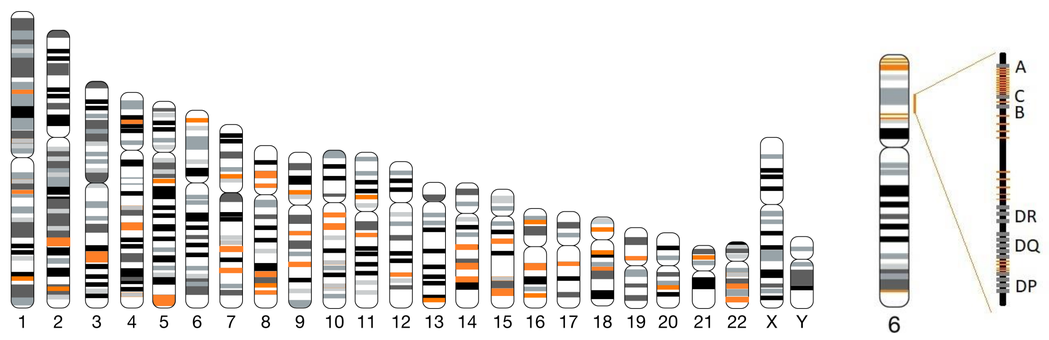

Figure 1. Schematic of Indel markers (orange) used in One Lambda Devyser Chimerism (left) and the HLA loss assay (right)

Results

The correlation (R^2) between One Lambda Devyser Chimerism and the HLA loss assay was 0.98 in the samples ≤1% MC and 0.99 in the samples with ≥1% Mixed Chimerism (MC) (see figure 2).

The informativity of the assay was evaluated using 64 haplo-identical and Matched unrelated donor (MUD) pairs. The average number of informative markers were 4.4, one pair had 0 informative markers and one had 9 (see figure 3).

In the proof-of-concept samples, four markers were identified from the patient and four from the donor as informative. In the T-cell fraction (CD3) no MC or HLA loss was detected. In the myeloid cell fraction (CD33), a MC of 4% recipient was identified and the HLA loss assay identified a MC of 1.5% recipient indicating a loss of heterozygosity in the recipient's fraction. The hematopoietic cell fraction (CD34) had a MC 85% recipient. The HLA loss assay showed a MC of 3.6% recipient indicating that 81.4% of the recipient's fraction cells had a loss of the HLA loci (see figures 4 and 5).

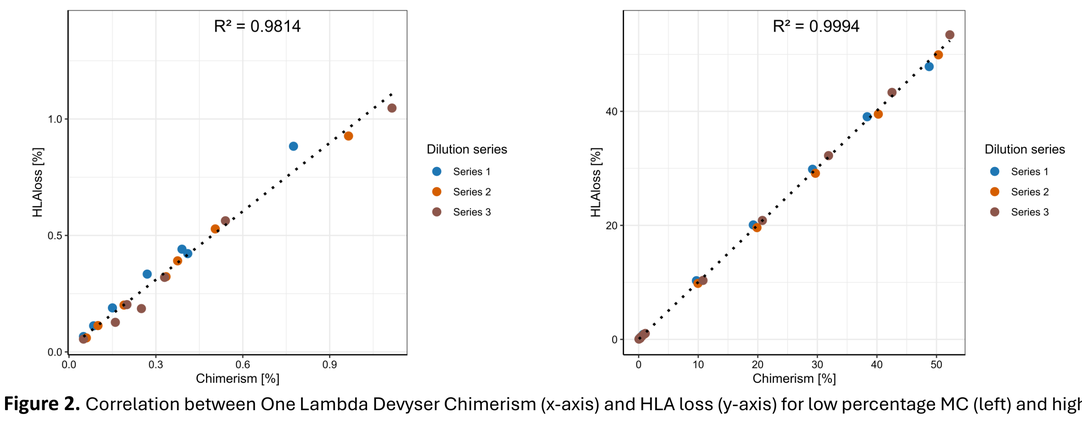

Figure 2. Correlation between One Lambda Devyser Chimerism (x-axis) and HLA loss (y-axis) for low percentage MC (left) and high (right).

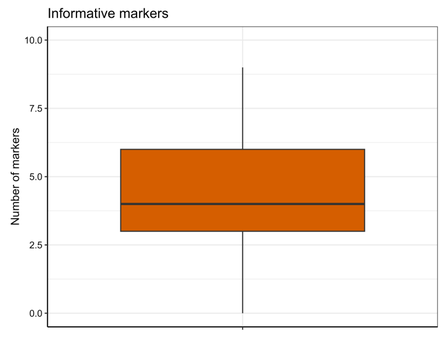

Figure 3. Number of patient informative markers in haplo and MUD matched pairs.

Figure 4. Recipient levels using chimerism (blue) or HLA loss assay (orange) in the three cell fractions.

Markers that usually are deemed non-informative in chimerism analysis (both donor and recipient are heterozygous) can also be used in the loss of heterozygosity analysis due to the skewed allele ratio that was seen in the CD34 fraction. A normal ratio is between 0.5-2 but the allele ratio in the CD34 fraction was 0.01-0.05 (see figure 6).

Using the combination of the heterozygous markers as well as the informative markers, the CD34 fraction sample indicate a loss of the whole HLA complex (A to DP).

Figure 5. Cells within the CD34 fraction. 15% originated from donor, 3.6% originates from the recipient that has an intact HLA region and 81.4% originates from recipient with a loss of heterozygosity.

Figure 6. Allele ratio in heterozygous HLA-loss markers for a normal sample (blue) and in a sample with loss of heterozygosity (orange).

Conclusion

The NGS assay exhibited at least 2 informative markers in more than 90% of the haplo-identical pairs with an average of 5 informative markers. The dilution series showed that a LoD <1% is feasible. The assay was also able to identify and quantify loss of heterozygosity in a relapse sample from an AML patient. In conclusion, this novel NGS assay is powerful and sensitive enough for detecting HLA-loss after haplo-transplantation.

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