Rode Kruis
Vlaanderen

HLA typing with 11-loci-NGS: benefits in donor selection

V. Van Sandt, A. Senev, M. Vanden Driessche, A. Torfs, J. Kerkhofs, M. Hidajat, L. Daniëls, M. Emonds

Background: After 1 year HLA genotyping using the (near to) whole gene next generation sequencing of the 11 most relevant HLA loci (MIA FORA FLEX, Immucor) we evaluated the benefit of using this technology in the context of stem cell transplantation.

Aims: We analyzed the added value of genotyping more HLA loci (DRB345, DQA1 and DPA1) and increasing the sequencing target region to nearly all coding and most of the non-coding regions.

Methods: Next generation sequencing

Results: We typed 173 potential donors with a minimal match of 9/10 for a cohort of 129 recipients in need of a MUD. 24% of these recipients had more than 1 donor with an equal number of 2nd field locus MM for HLA-A, B, C, DRB1 ,DQB1 and DPB1. For 23% of these recipients one of the donors was a better match for DRB345, despite being identical at 3rd field for DRB1, 6% could be better matched for DQA1 despite being 3rd field identical for DQB1. In 6% a better donor was available for DPA1, in all of these cases however the least matched donor had also a DPB1 mismatch.

In our total HSCT cohort (unrelated and related TX, n= 672) sequencing near to all exons allowed us to identify 93 coding variants with SNPs outside the key exons and 8 new 2nd field alleles, including a DQA1*05:XXN (mutation of the ATG start codon to ACG) and an A*03 with a mutation in the signal peptide disrupting the central hydrophobic region. All these alleles, (0,85% of total alleles sequenced) result in a different, new or even no protein on the cell surface and would have been unnoticed using Sanger sequencing of the key exons.

Sequencing the non-coding regions allowed us to confidently identify splice site variants such as DRB4*01:03:01:02N and predict 4th resolution. Homopolymers, short tandem repeats and systematical algorithm artefacts, however, prevent us from using NGS data at 4th field resolution.

Summary/conclusions: NGS allows 3rd field HLA genotyping and that both the addition of extra loci as well as an increase of the target region has an added value during the final selection of a stem cell donor.