NGS typing results using Oxford Nanopore Sequencing. Can MinION data be reliably used for HLA typing?

Aim
The common practice for HLA typing at the moment is to use Sanger sequencing or NGS. With the development of new sequencing technologies, the applicability of these techniques for HLA typing should be investigated.

Nanopore sequencing is one of the latest DNA sequencing technologies, in which single molecules are sequenced directly. The technique has the potential of read lengths of tens of kilobases. However, the error rate in the reads is high. Especially the length of homopolymers and repetitive sequences cannot be determined accurately. Here we present the typing results for HLA samples using Oxford Nanopore Technologies (ONT).

Materials & Methods
We have developed NGSengine (GenDx), a software package to perform HLA typing based on NGS data. NGSengine uses an integrated HLA typing approach which includes determining the HLA locus, applying a sequence reference, aligning reads, phasing and typing into a single method. Since HLA is highly polymorphic, each of these steps contains HLA-specific features.

At DKMS Life Science Lab, the MinION sequencer from Oxford Nanopore Technologies has been used for full-length HLA sequencing. We have used a benchmarking set of 30 samples, 10 each for HLA-A, HLA-B and HLA-C to explore 3 successive Nanopore chemistries R7.3, R9 and R9.4. Additionally, a larger benchmarking set has been used to test the latest chemistry R9.5.

Results
Using successive iterations of ONT chemistries (R7.3, R9, and R9.4) we have mapped the template-complement (2D) reads to the known reference alleles using Burrows-Wheeler Aligner (BWA). The different ONT chemistries are indicated in boxes. p-values are calculated using the wilcoxon rank test.

Conclusions
HLA typing with MinION data is challenging. With the current status of the MinION data quality (obtained with R9.5 chemistry), the results of our preliminary analysis look promising. However, the base calling algorithm has not been optimized for amplicon analysis. Furthermore, the noise is still too high, and the homopolymer issues have not (completely) been solved. In our benchmarking set of 30 samples, not a single typing result matched completely with the pretyping. Further improvements are necessary before MinION sequencing can be applied for reliable HLA typing.