Changes to reporting HLA typing post NGS

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Pre NGS typing

- Apart from commonly known ambiguous results which required additional typing of other exons
- HLA genes & alleles were identified by sequencing across the exons encoding the peptide binding domains; exon 2 and 3 for HLA class I and exon 2 only for HLA class II genes
- designated by an upper case ‘G’ which follows the first 3 fields of the allele designation of the lowest numbered allele in the group.
HLA-Class I molecules and genes
HLA-Class II molecules and genes

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Saving and transforming lives by connecting the right donors and patients
Molecular Methods-NGS

Kit Components

PCR Reagent
Nine tubes of “All-in-One” preamplified master mix
to cover 11 genes: HLA- A, B, C, DRB1, DPB1,
DQA1, DQB1, DRB1, DRB3/4/5

Library Reagent
Reagents for fragmentation, and repair A-tailing, adapter
ligation, and library amplification

Adapter Index Plate
Pre-attached Adapter
index in 96 well plate

Magnetic bead Kit (not shown)
Filed washer Agencourt
AMPure XP Bead

The Highest Overall Coverage

Whole Gene Coverage

Exon 2 to Exon 4
Exon 1 to Exon 2

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NGS HLA read out screen

Displays the selected genotypes for the sample.
Post NGS HLA typing

- In the first instance, From our NGS results, we will be reporting 2 fields
- Our results will be A*01:01 or A*01:04N or A*01:32 or A*01:03 etc

<table>
<thead>
<tr>
<th>Group Designation</th>
<th>Alleles Within Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*01:01:01G</td>
<td>01:01:01/01:01:02/N01:01:03/01:01:04/01:01:05/01:01:06/01:01:07/01:01:08/01:01:09/01:01:10/01:01:11/01:01:12/01:01:13/01:01:32/01:01:51/01:01:53/01:01:64/01:01:64N/01:01:22/N01:01:32/01:01:37/01:01:45/01:01:55/01:01:67/01:01:87/01:01:103/01:01:107/01:01:109/01:132/01:141/01:142/01:155/01:177/01:212/01:217/01:234/01:237/01:246/01:248/01:249/01:251/01:252/01:253</td>
</tr>
<tr>
<td>A*01:03:01G</td>
<td>01:03/01:01/01:03/01:02</td>
</tr>
<tr>
<td>A*01:02:01G</td>
<td>01:02/01:01/01:09/01:02</td>
</tr>
</tbody>
</table>

- These are all different alleles of the HLA-A*01 antigen
- If Recipient is A*01:01,02:01 and the potential donor typed as A*01:32 and 02:01
- Previously both would be reported as A*01:01G and 02:01 (2/2)
- They are however, different alleles so really are (1/2)
- If the potential donor typed as A*01:03,02:01 again (1/2)
- Previously would be reported as A*01:03G and 02:01 (1/2)
Next generation DNA sequencing has facilitated the routine characterization of complete HLA gene sequences. These data complement structural and functional studies of HLA elements encoded outside of the exons specifying the antigen recognition domain. This commentary is focused on evaluating whether the interpretation of HLA clinical typing results should expand the region of the HLA gene considered in the assignment from the exon(s) encoding the antigen recognition domain to the full gene sequence. Our recommendation is that, at present, there is insufficient data to support considering variation in the regions outside of the antigen recognition domain in clinical decision making.
Reporting moving forward

- Our current report are generated on an ailing DOS system so no major changes are possible
- In our notes sections how would the clinical units like to see the results interpreted
  - Assessment at allele level, matched at these loci and mismatched at these with no comment re xx/xx
  - Assessment at allele level, matched at these loci and mismatched at these with xx/xx @ allelic not including HLA-DP which must be assessed for permissibility OR
  - Assessment at allele level, matched at these loci and mismatched at these with xx/xx @peptide binding group not including HLA-DP which must be assessed for permissibility
- If the XX/XX option is selected what is the optimum XX? 10 or 12?
- What about DRB3,4,5 ? Sometimes there will be no result, sometimes 1 and other times 2
  - For DRB1*01,08,10 there are no DRB3,4 or 5 results
  - So are we up to 15 possibles?
- Would any one like the class II Alpha domains included in the equation?
I would love to hear from you

Regarding any issue with HLA typing or Reporting

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