## Warmup: Structures and alignments

* Go to the **IPD/IMGT HLA repository** and open de MICB protein sequences  
  <https://github.com/ANHIG/IMGTHLA/blob/Latest/fasta/MICB_prot.fasta>
* Pick the full sequence of one of the alleles and copy it.
* Mark the allele.
* Go to the online **NetSurf server** and paste the sequence  
  <https://services.healthtech.dtu.dk/services/NetSurfP-2.0/>
* While waiting, select another MICB sequence of choice.
* Mark the allele.
* Go to [**https://www.ebi.ac.uk/jdispatcher/psa/lalign**](https://www.ebi.ac.uk/jdispatcher/psa/lalign)and use the two protein sequences to align OR
* **Use** [**https://www.ebi.ac.uk/ipd/imgt/hla/alignment/**](https://www.ebi.ac.uk/ipd/imgt/hla/alignment/)
* Check the NetSurf output and mark the polymorphic amino acids
* How many accessible amino acids did you find for your combination?

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Allele 1 MICB** | **Allele 2 MICB** | **Amino acid mismatches (positions)** | **Accessible mismatches (positions)** | **Accessible mismatch score (number)** |
|  |  |  |  |  |

## Case 1: Multiple living related donors for a patient without antibodies

* **PIRCHE → SOT → Single patient**
* Recipient

OSKAR,HLA-A\*01:01:01+HLA-A\*33:01:01^HLA-B\*08:01:01+HLA-B\*14:02:01^HLA-C\*07:01:01+HLA-C\*08:02:01^HLA-DRB1\*03:01:01+HLA-DRB1\*13:01:01^HLA-DQB1\*02:01:01+HLA-DQB1\*06:03:01

* Four candidate donors
  1. one father
  2. one mother
  3. one sister
  4. one brother

MAE ,HLA-A\*01:01:01+HLA-A\*02:01:01^HLA-B\*08:01:01+HLA-B\*38:01:01^HLA-C\*07:01:01+HLA-C\*12:03:01^HLA-DRB1\*03:01:01+HLA-DRB1\*07:01:01^HLA-DQB1\*02:01:01+HLA-DQB1\*03:03:02

PAI ,HLA-A\*30:01:01+HLA-A\*33:01:01^HLA-B\*13:02:01+HLA-B\*14:02:01^HLA-C\*06:02:01+HLA-C\*08:02:01^HLA-DRB1\*13:01:01+HLA-DRB1\*11:04:01^HLA-DQB1\*06:03:01+HLA-DQB1\*03:01:01

JOAO,HLA-A\*01:01:01+HLA-A\*30:01:01^HLA-B\*08:01:01+HLA-B\*13:02:01^HLA-C\*07:01:01+HLA-C\*06:02:01^HLA-DRB1\*03:01:01+HLA-DRB1\*11:04:01^HLA-DQB1\*02:01:01+HLA-DQB1\*03:01:01

LUISA ,HLA-A\*02:01:01+HLA-A\*33:01:01^HLA-B\*38:01:01+HLA-B\*14:02:01^HLA-C\*12:03:01+HLA-C\*08:02:01^HLA-DRB1\*07:01:01+HLA-DRB1\*13:01:01^HLA-DQB1\*03:03:02+HLA-DQB1\*06:03:01

* Enter them into the SOT system and calculate the PIRCHE. Compare the outcomes
* Add an uncle and evaluate again

Uncle TIO ,A\*11:01:01+HLA-A\*33:01:01^HLA-B\*07:02:01+HLA-B\*14:02:01^HLA-C\*07:02:01+HLA-C\*08:02:01^HLA-DRB1\*12:02:01+HLA-DRB1\*13:01:01^HLA-DQB1\*03:01:01+HLA-DQB1\*06:03:01

* PM: show the heath plots and hover over the items

## Case 2: One DCD donor with multiple candidate recipients (no antibodie)

* **PIRCHE → SOT → Donor Allocation**
* DCD donor

DON\_1, HLA-A\*02:01:01+HLA-A\*24:02:01^HLA-B\*18:01:01+HLA-B\*44:05:01^HLA-C\*02:02:02+HLA-C\*07:01:01^HLA-DRB1\*01:01:01+HLA-DRB1\*16:01:01^HLA-DQB1\*05:01:01+HLA-DQB1\*05:02:01^HLA-DQA1\*01:01:01+HLA-DQA1\*01:02:02

Serology: DON\_1, A2, A24(9), B18, B44(12), Cw2, Cw7, DR1, DR16(2), DQ5(1)

* Four candidate recipient, create four first

REC\_1, HLA-A\*02:01:01+HLA-A\*23:01:01:01^HLA-B\*18:01:01+HLA-B\*27:03^HLA-C\*02:02:02:01+HLA-C\*07:04:01^HLA-DRB1\*13:02:01+HLA-DRB1\*13:03:01^HLA-DQB1\*03:01:01+HLA-DQB1\*06:04^HLA-DQA1\*01:02+HLA-DQA1\*05:05

REC\_2, HLA-A\*02:01:01+HLA-A\*23:01:01^HLA-B\*08:01:01+HLA-B\*18:01:01^HLA-C\*07:01:01+HLA-C\*07:01:01^HLA-DRB1\*11:04:01+HLA-DRB1\*13:02:01^HLA-DQB1\*03:01:01+HLA-DQB1\*06:04:01^HLA-DQA1\*01:02:01+HLA-DQA1\*05:05:01

REC\_3, HLA-A\*02:01:01+HLA-A\*25:01:01^HLA-B\*18:01:01+HLA-B\*51:01:01^HLA-C\*01:02:01+HLA-C\*12:03:01^HLA-DRB1\*15:01:01+HLA-DRB1\*11:01:01^HLA-DQB1\*03:01:01+HLA-DQB1\*06:02:01^HLA-DQA1\*01:02:01+HLA-DQA1\*05:05:01

REC\_4, HLA-A\*01:01:01+HLA-A\*02:01:01^HLA-B\*08:01:01+HLA-B\*07:02:01^HLA-C\*07:02:01+HLA-C\*07:01:01^HLA-DRB1\*03:01:01+HLA-DRB1\*01:01:01^HLA-DQB1\*02:01:01+HLA-DQB1\*05:01:02^HLA-DQA1\*02:01:01+HLA-DQA1\*01:02:01

* Enter them into the SOT system and calculate the PIRCHE. Compare the outcomes and store them

## Case 3: RAMP evaluation

* **PIRCHE → SOT → RAMP**
* Use the data from recipient 1, 2, 3, and 4 from case 2 and plot a RAMP
* Evaluate whether this donor is good or bad donor in a relative way. Do this by checking the actual PIRCHE scores from case 2 with the RAMP histograms

## Case 4: Adding unacceptables to the RAMP for a patient without antibodies

* **PIRCHE → SOT → RAMP**
* Enter the typing (recipient 4)
* REC\_4, HLA-A\*01:01:01+HLA-A\*02:01:01^HLA-B\*08:01:01+HLA-B\*07:02:01^HLA-C\*07:02:01+HLA-C\*07:01:01^HLA-DRB1\*03:01:01+HLA-DRB1\*01:01:01^HLA-DQB1\*02:01:01+HLA-DQB1\*05:01:02^HLA-DQA1\*02:01:01+HLA-DQA1\*01:02:01
* Create a RAMP
* Select unacceptables to get into a vPRA of ~ 50%
* Check the distribution of the risk classifications

## Case 5: Adding unacceptables to the RAMP for a patient with antibodies

* **PIRCHE → SOT → RAMP**
* Enter the typing
* REC\_5, HLA-A\*02:01:01+HLA-A\*03:01:01^HLA-B\*35:01:01+HLA-B\*51:01:01^HLA-C\*04:01:01+HLA-C\*14:02:01^HLA-DRB1\*01:01:01+HLA-DRB1\*07:01:01^HLA-DQB1\*02:02:01+HLA-DQB1\*05:01:01^HLA-DQA1\*01:01:01+HLA-DQA1\*02:01:01
* Add the MFI data → **get from file *epitopes\_class\_I.csv***
* Select from which run you want to evaluate the data
* Select unacceptables to create a pattern that suits you
* Evaluate the RAMP