High-Sensitive MAPPS Analysis for High-Confident Immunogenicity Risk Assessment

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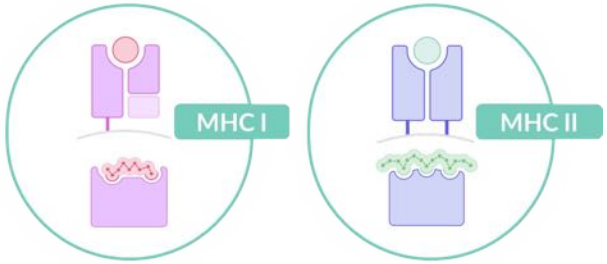
IMMUNESPEC Advanced immunopeptidomics platform



Identification of MHC presented peptides by affinity purification & MS based identification

- Identification of neoantigens/tumor antigens for development of immunotherapy/ precision medicine
- Identification of pathogen derived antigens for prophylactic vaccine development
- Immunogenicity assessment of biotherapeutics (MAPPS)

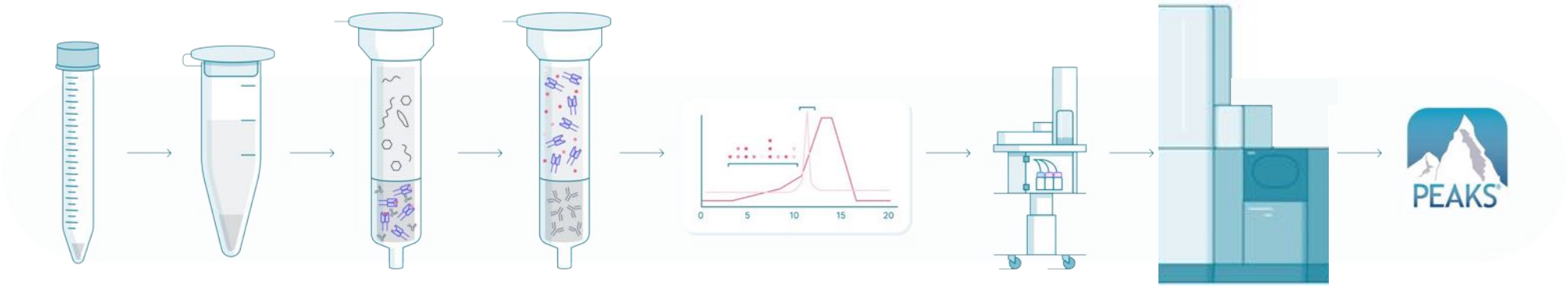
→ High-sensitivity immunopeptidomics analysis: maximize number of identified peptides

→ Minimal sample input

→ Semi-automated platform with high throughput capacity

→ Larger screening panels

IMMUNESPEC Advanced immunopeptidomics platform



1 High-outcome purification

State-of-the-art protocol for optimized solubilization • purification • extraction

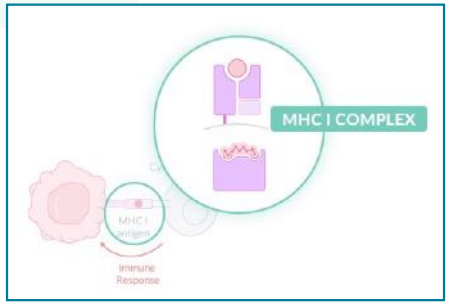
Semi-automated workflow • HT

2 Sensitive mass spectrometry

Ultraprecise mass spectrometry analysis using a TimsTOF-SCP

3 Extensive data analysis

Optimized data processing for complete immunogenic reports



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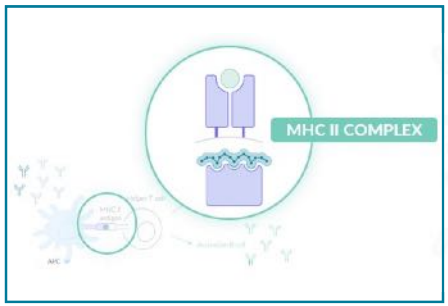
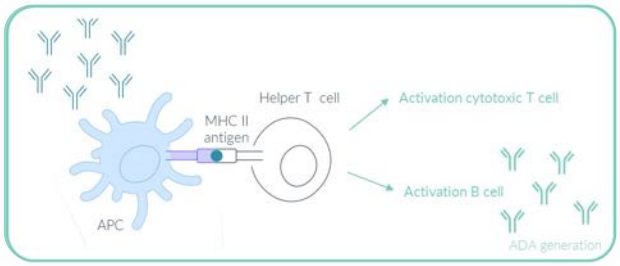
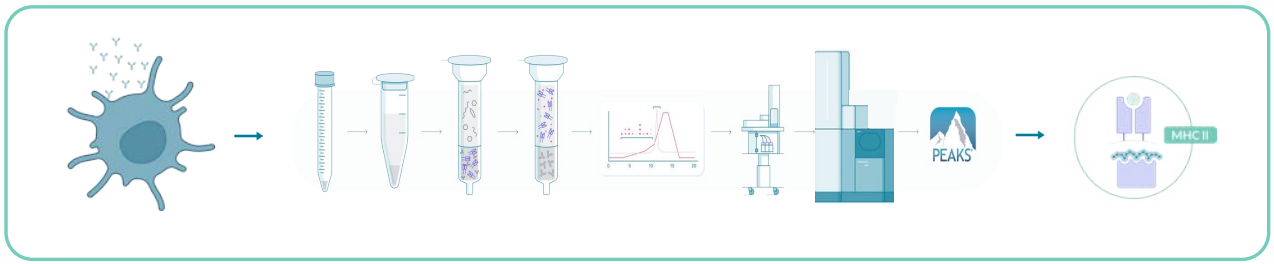


MAPPS assay. Risk assessment of your biotherapeutic agent.

Immunogenicity evoked by a biotherapeutic



MAPPS assay: identification MHC-II presented peptides from APCs loaded with a biotherapeutic



All protein therapeutics: potential to elicit unwanted immunogenicity (effect on safety, efficacy, PK, PD)

Health authorities: IND submission – requirement thorough immunogenicity risk assessment

→ Preclinical assays for evaluation and mitigation of immunogenicity risk

MAPPS: Measurement of truly presented T cell epitopes

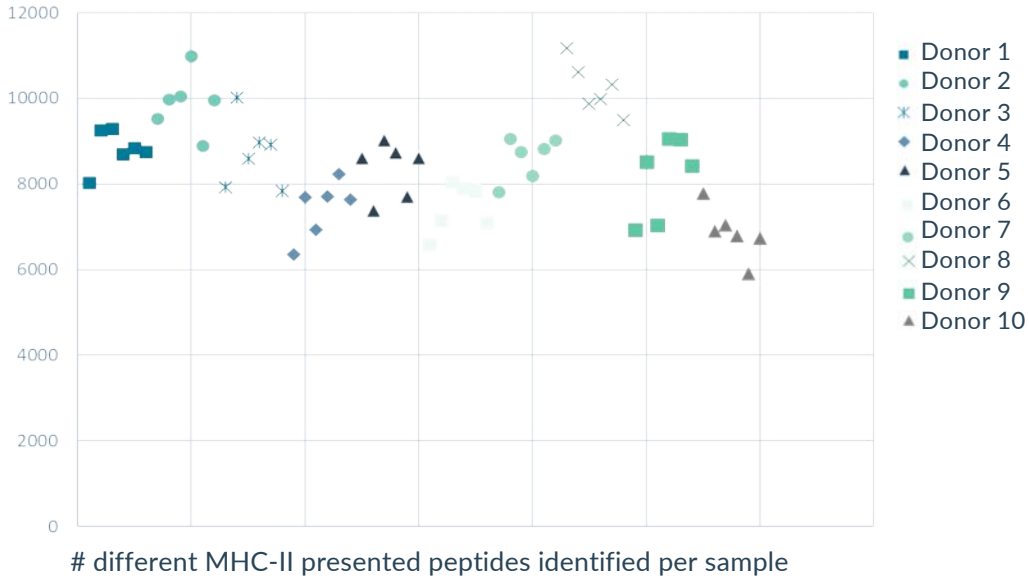
- Immunopeptidomics analysis of antigen presenting cells loaded *in vitro* with the target biotherapeutic
- Pinpointing all the T cell epitopes of the target biotherapeutic (uptake + lysosomal processing + MHC presentation)
- Overview of putative T-cell immunogenic clusters: immunogenic profile of the biotherapeutic
- Vast majority identified peptides self-peptides: high-sensitivity needed not to miss T cell epitopes
- Correlation immunogenicity risk and # presented peptides & # presented clusters

Case Study 1: High-throughput MAPPS analysis of biotherapeutic loaded moDCs

in collaboration with ImmunXperts

60 samples of moDCs (1 M cells)

10 different donors – 6 conditions:
4 biotherapeutics (test articles)
1 Positive loading control: BetV1
1 unloaded control



different MHC-II presented peptides identified per sample

- Average 8.5K different MHC peptide identifications/sample
- In total 91.029 different MHC peptides identified

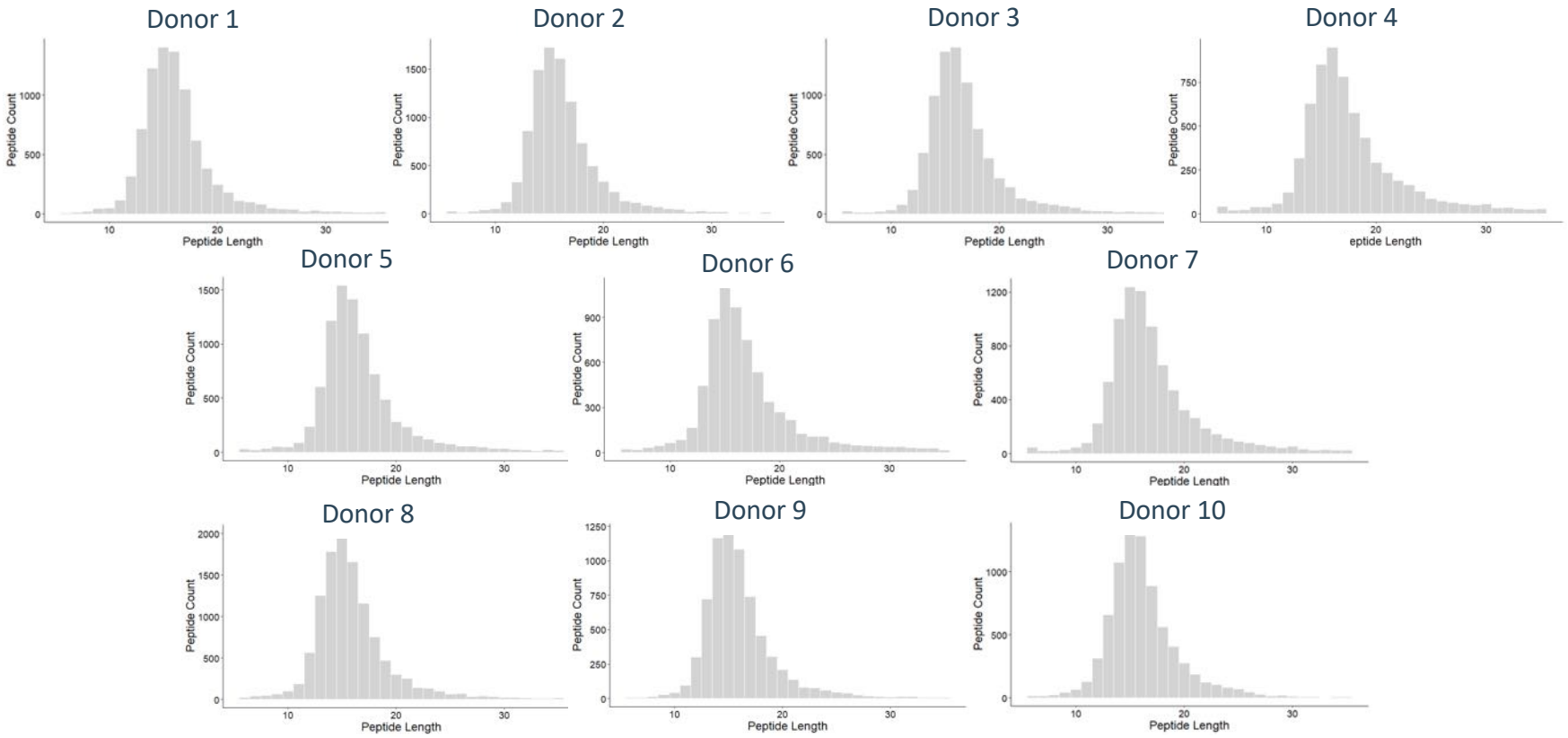
- **High numbers of presented MHC peptides are identified using only 1M moDCs per sample**
- **Per donor: reproducible # MHC peptides**

Case Study 1: High-throughput MAPPS analysis of biotherapeutic loaded moDCs

in collaboration with ImmunXperts

QC: Length distribution of identified peptides

Collaboration ImmunXperts

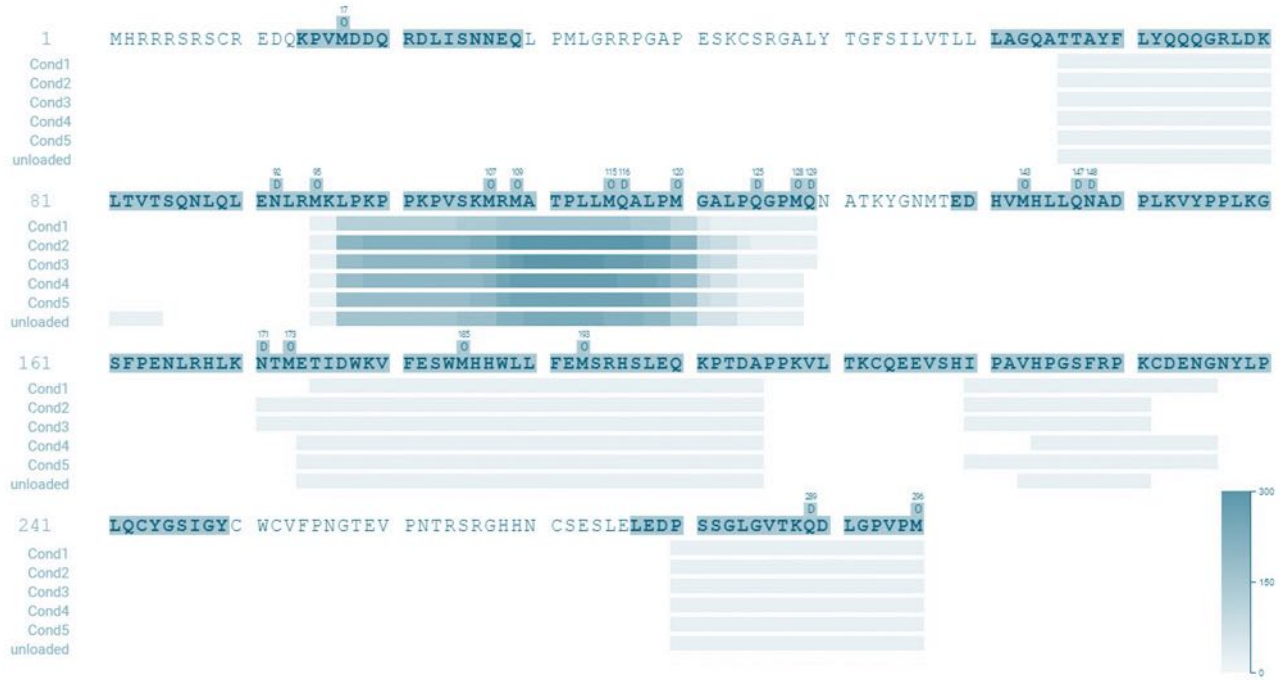


- **Size distribution of identified peptides is conform for MHC-II presented peptides**

Case Study 1: High-throughput MAPPS analysis of biotherapeutic loaded moDCs

in collaboration with ImmunXperts

QC: MHC-II presented self-peptides in different samples from the same donor



Heat map HG2A – distribution of identified immune peptides per sample (different conditions – same donor)

- **MAPPS analysis of samples from same donor: same presentation pattern (self-peptides): reproducibility**

Case Study 1: High-throughput MAPPS analysis of biotherapeutic loaded moDCs

in collaboration with ImmunXperts

Positive loading control: BetV1

Distribution of identified immune peptides from BetV1

- 11 to 78 different BetV1 derived peptides/sample
- Total of 218 different BetV1 derived peptides
- Identified peptides in putative immunogenic clusters.

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
#Total different peptides	8980	**8986**	8846	7864	9097	7976	9465	10546	9047	6455
#Different BetV1 peptides	74	**33**	27	38	69	31	36	78	21	11
% BetV1 peptides	0,8%	**0,4%**	0,3%	0,5%	0,8%	0,4%	0,4%	0,7%	0,2%	0,2%

- **Numerous overlapping MHC-presented peptides are identified, a crucial factor for pinpointing putative immunogenic clusters with high confidence**

Case Study 1: High-throughput MAPPS analysis of biotherapeutic loaded moDCs

in collaboration with ImmunXperts

Positive loading control: BetV1

Heat map Betv1 – distribution of identified immune peptides per donor

- Identified peptides in putative immunogenic clusters.
- Different HLA genotype: different clusters

⇨ **High numbers of presented MHC peptides are identified using only 1M moDCs per sample**

⇨ **Reproducibility between samples**

⇨ **Putative immunogenic regions are identified by multiple peptides: high-confidence immunogenic profile of test article**

Case Study 2: Identification of MHC-II presented peptides from marketed biologics

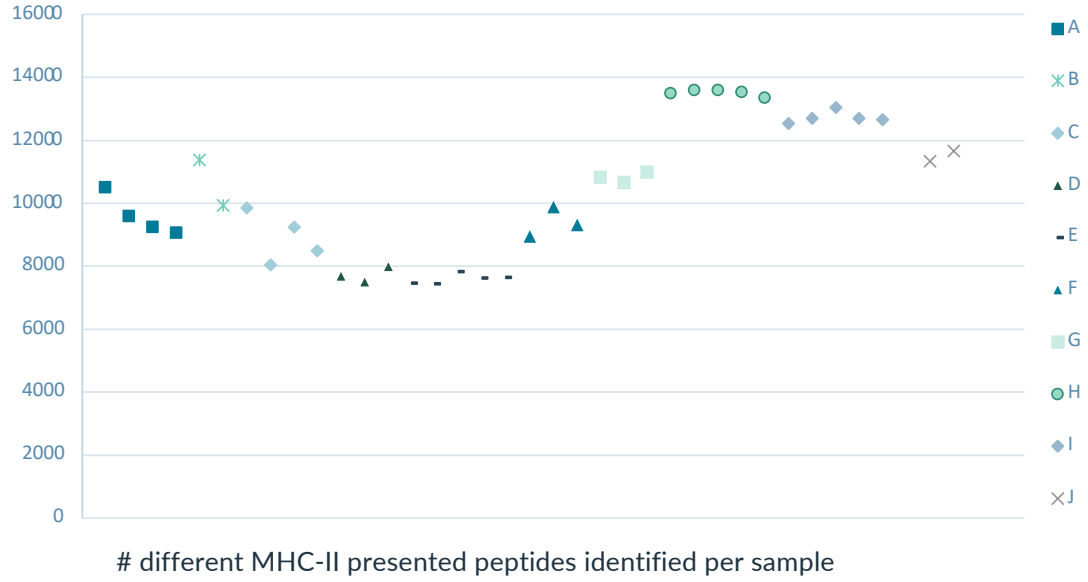
in collaboration with ImmunXperts

Explorative study to compare immunogenic profile from different biologics

moDCs (<1 M cells)

10 different donors
5 different biologics

- #Total different MHC peptides: 7437 - 13595



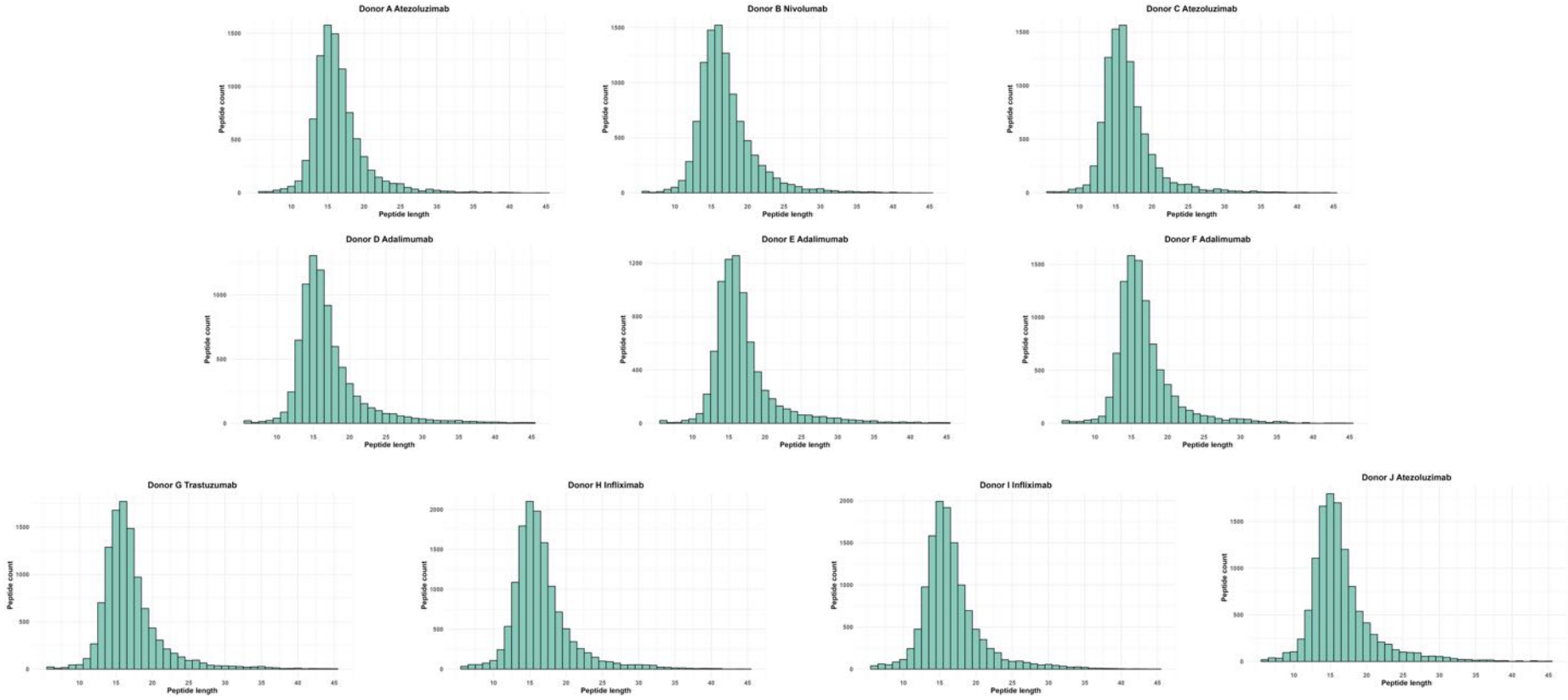
different MHC-II presented peptides identified per sample

- **High numbers of presented MHC peptides are identified using < 1M moDCs per sample**
- **Per donor: reproducible # MHC peptides**

Case Study 2: Identification of MHC-II presented peptides from marketed biologics

in collaboration with ImmunXperts

QC: Length distribution of identified peptides



- **Size distribution of identified peptides is conform for MHC-II presented peptides**

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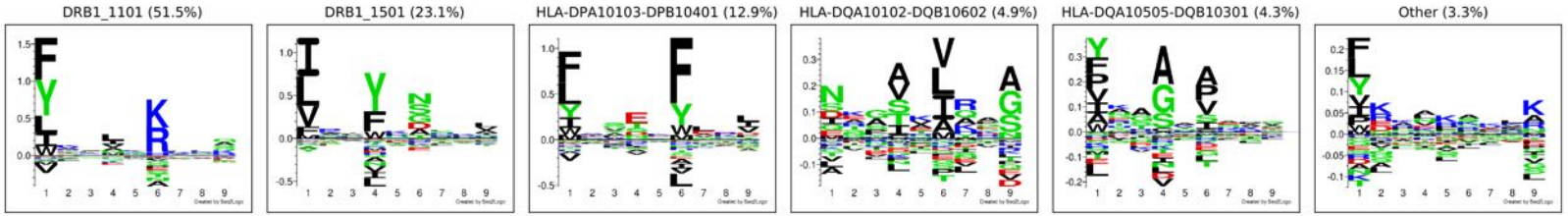
Case Study 2: Identification of MHC-II presented peptides from marketed biologics

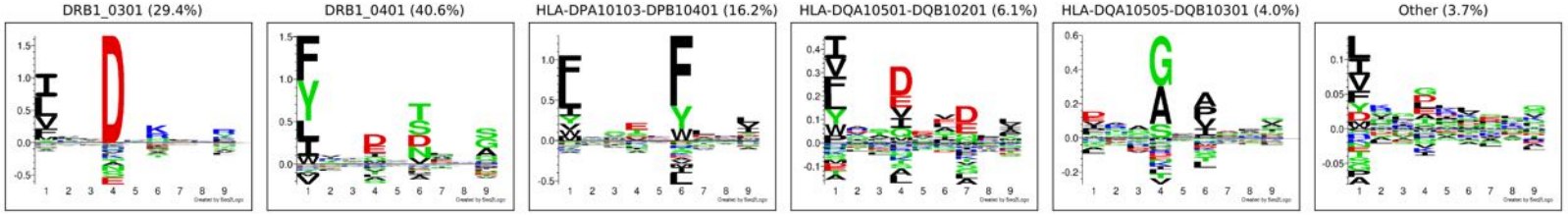
in collaboration with ImmunXperts

QC: MHC Motif Decon Tool: Attribution identified peptides to HLA-DR, HLA-DP and HLA-DQ

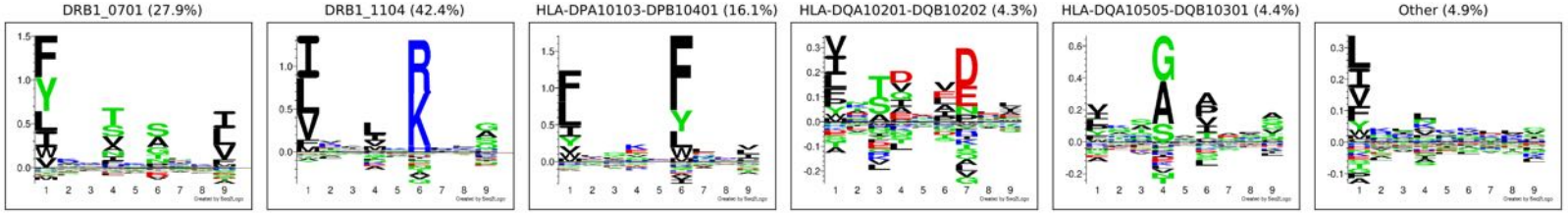
	Donor B		
DRB1	11:01:01	15:01:01	74.6%
DPB1	04:01:01	04:01:01	12.9%
DQB1	06:02:01	03:01:01	9.2%



	Donor E		
DRB1	03:01:01G	04:01:01G	69.7%
DPB1	04:01:01G	04:01:01G	16.2%
DQB1	03:01:01G	02:01:01G	10.1%



	Donor I		
DRB1	11:04:01	07:01:01	70.3%
DPB1	02:01:02	04:01:01	16.1%
DQB1	02:02:01	03:01:01	8.7%



- **HLA-DR presented peptides: dominant part of all identified MHC-II peptides**
- **HLA-DP & HLA-DQ presented peptides: subsidiary part of all identified MHC-II peptides**

MHC Motif Decon Tool: Morten Nielsen (Kaabinejadian et al, 2022)

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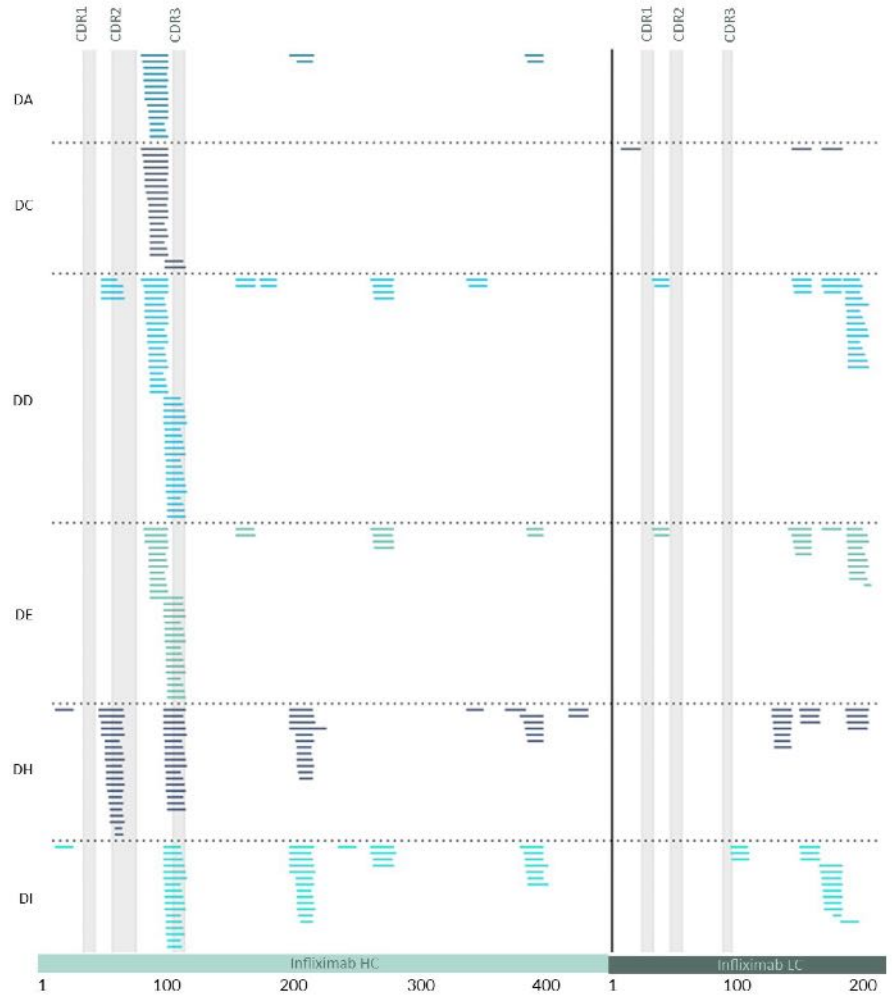
Case Study 2: Identification of MHC-II presented peptides from marketed biologics

in collaboration with ImmunXperts

MHC-II Presented peptides from Infliximab

	Total peptides identified (PSM)	Total different peptides	# Heavy chain peptides	# Light chain peptides	% Infliximab peptides
A	18085	9060	18	0	0.20%
C	17155	8476	20	3	0.27%
D	16971	7512	53	23	1.01%
E	16961	7620	36	18	0.71%
H	28257	13516	60	14	0.55%
I	27225	12690	43	16	0.46%



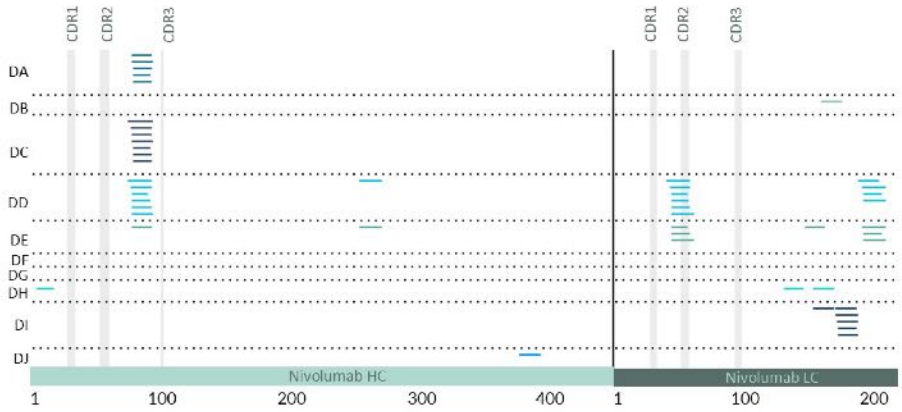
- **Infliximab: mAb with high prevalence ADAs - relatively high numbers of presented peptides**
- **Clusters: in HC CDR2 & CDR3 but most peptides match to HC & LC constant region**

Case Study 2: Identification of MHC-II presented peptides from marketed biologics

in collaboration with ImmunXperts

MHC-II Presented peptides from Nivolumab

	Total peptides identified (PSM)	Total different peptides	# Heavy chain peptides	# Light chain peptides	% Nivolumab peptides
A	18978	9572	5	0	0.05%
B	20068	9931	0	1	0.01%
C	15910	8041	6	0	0.07%
D	17678	7846	7	10	0.22%
E	16747	7437	2	7	0.12%
F	21004	9912	0	0	0.00%
G	21940	10649	0	0	0.00%
H	28305	13581	1	2	0.02%
I	27190	12694	0	6	0.05%
J	24382	11333	1	0	0.01%



- **Nivolumab: mAb with low prevalence ADAs - relatively low numbers of presented peptides**
- **Clusters: in LC CDR2 but most peptides match to HC & LC constant region**

Case Study 2: Identification of MHC-II presented peptides from marketed biologics

in collaboration with ImmunXperts

⇨ Identification of HLA-DR, -DP and –DQ peptides

⇨ HLA-DR presented peptides: dominant part of all identified MHC peptides

HLA-DP & HLA-DQ presented peptides: subsidiary (but non neglectable) part of all identified MHC-II peptides

⇨ Correlation between # identified peptides and #identified clusters in MAPPS assay and immunogenicity incidence & immunogenicity risk

Case Study 3: Identification of MHC-II presented peptides from B cells, myDCs and moDCs

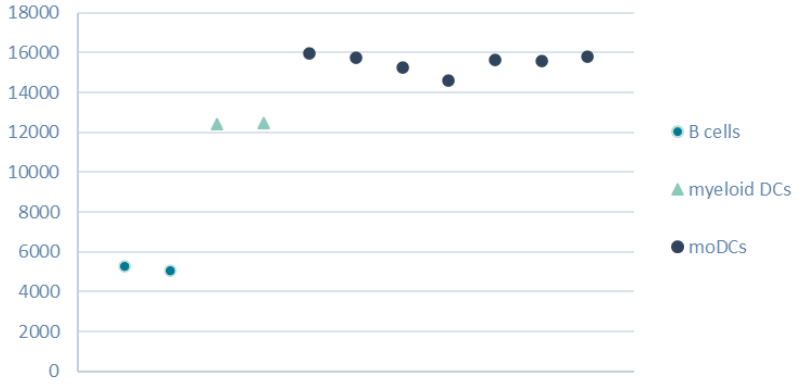
in collaboration with ImmunXperts

One donor – comparison MAPPS analysis using moDCs vs myeloid DCs & B cells

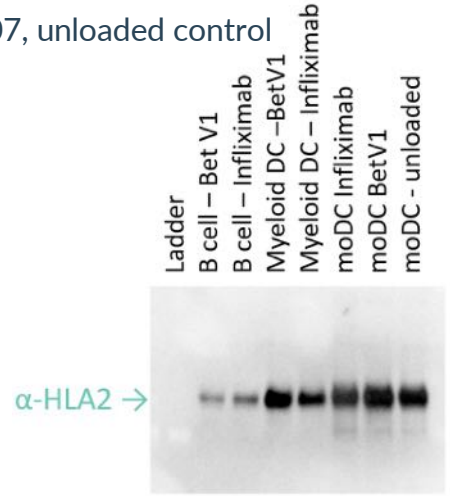
B-cells (#800 k): BetV1 & Infliximab

Myeloid DCs (#520k): BetV1 & Infliximab

moDCs (#800k) : BetV1, Infliximab, Trastuzumab, Nivolumab, Atezolizumab, ATR-107, unloaded control



different MHC-II presented peptides identified per sample



Western Blot showing MHC-II expression levels in different cell types (Equivalent of 750 cells loaded per lane)

- **High sensitivity allows MAPPS analysis of true APCs** (B cells #5k – myeloid DC #12k – moDCs #16k)
- **Per sample type: reproducible # identified MHC-II peptides**
- **#Identified peptides correlate with HLA expression levels**

Case Study 3: Identification of MHC-II presented peptides from B cells, myDCs and moDCs

in collaboration with ImmunXperts

Comparison MAPPS analysis of BetV1 loaded cells: B cells / myeloid DC / moDCs

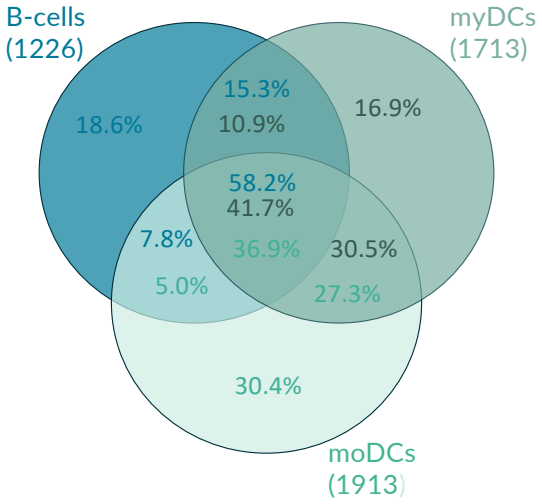
	Total peptides identified (PSM)	Total different peptides	# BetV1 peptides	% BetV1 peptides
myDC	38606	12491	34	0.27%
B cell	14158	5052	11	0.22%
moDC	34959	15781	88	0.56%

- **Same clusters identified in B cells & myDCs found in moDCs from same donor (but higher # peptides)**
- **True APCs (myDCs & B cells) can be used in high-sensitive MAPPS assay**

Case Study 3: Identification of MHC-II presented peptides from B cells, myDCs and moDCs

in collaboration with ImmunXperts

Comparison MAPPS analysis B cells / Myeloid DCs / moDCs



Overlay identified presented self-peptides at protein level in 3 cell types

Heat map HG2A – distribution of identified immune peptides per sample (different cell types– same donor)

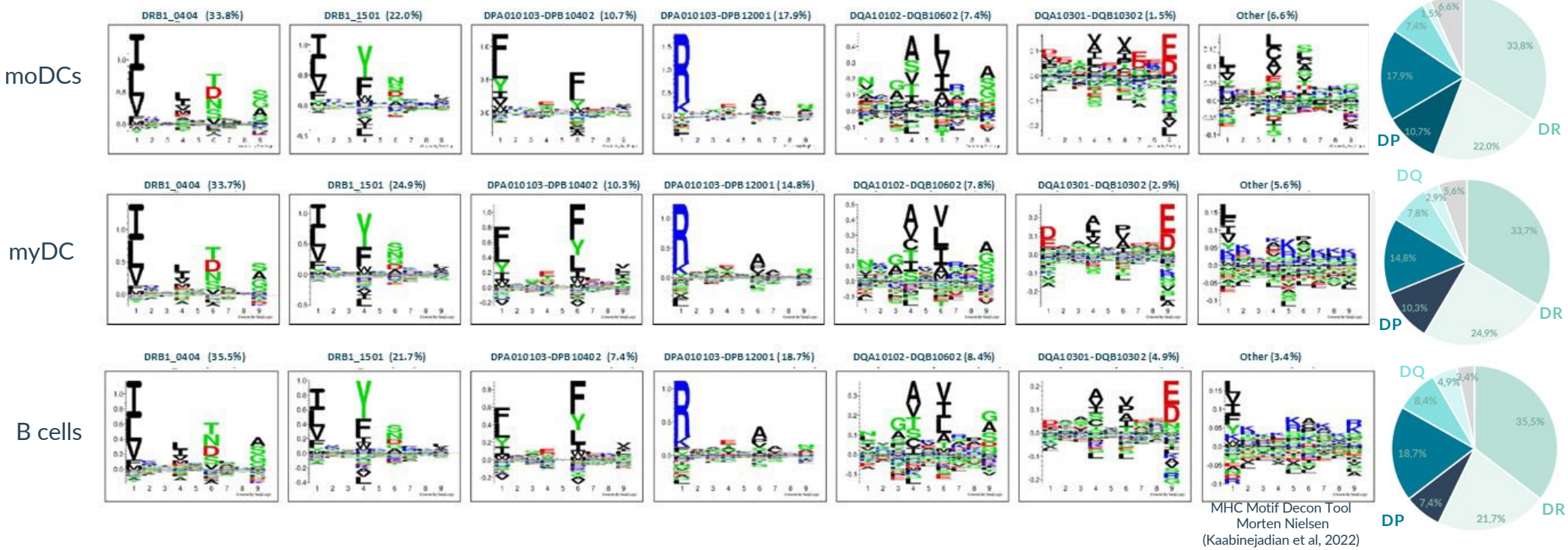
- **Differences in presented peptide repertoire at protein level**
- **For proteins from which relatively high # peptides are presented in the three cell types: presentation patterns are highly similar**

Case Study 3: Identification of MHC-II presented peptides from B cells, myDCs and moDCs

in collaboration with ImmunXperts

Comparison MAPPS analysis of BetV1 loaded cells: B cells / myeloid DC / moDCs

	Donor X	
DRB1	04:01:01	15:01:01
DPB1	20:01:01	04:02:01
DQB1	06:02:01	03:02:01



MHC Motif Decon Tool
Morten Nielsen
(Kaabinejadian et al, 2022)

- **Portion of identified DR / DP /DQ peptides highly similar between different cell types from same donor**

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Case Study 3: Identification of MHC-II presented peptides from B cells, myDCs and moDCs

in collaboration with ImmunXperts

Comparison MAPPS analysis of different biologics loaded on moDCs

	Total peptides identified (PSM)	Total different peptides	# Heavy Chain peptides	# Light Chain peptides	% Biologic peptides
Infliximab	34904	15797	25	21	0.29%
Trastuzumab	33790	15251	5	4	0.06%
Nivolumab	32250	15290	8	2	0.08%
Atezolizumab	34844	15644	16	9	0.16%
ATR107	34330	15666	20	25	0.29%
Unloaded	34846	15753	0	0	0%

- **Correlation between # identified peptides and #identified clusters in MAPPS assay and immunogenicity incidence & immunogenicity risk**
- **High-sensitivity of MAPPS assay: high-confidence immunogenic profile of test article**

Case Study 3: Identification of MHC-II presented peptides from B cells, myDCs and moDCs

in collaboration with ImmunXperts

⇨ High sensitivity allows MAPPS analysis of reduced number of moDCs and of true APCs

⇨ # Identified peptides correlate with HLA expression levels

⇨ Correlation between # identified peptides and #identified clusters in MAPPS assay and immunogenicity incidence & immunogenicity risk

⇨ Comparison B cells – myeloid DCs - moDCs:

- Portion of identified DR / DP /DQ peptides highly similar between different cell types from same donor
- Presentation patterns for same protein are highly similar

⇨ High-sensitivity of MAPPS assay: high-confidence immunogenic profile of biotherapeutic

Conclusion

- Correlation between # identified peptides and #identified clusters in MAPPS assay and immunogenicity incidence & immunogenicity risk

- MAPPS assay: majority identified peptides: self peptides
  ⇨ high-sensitivity needed for high confident immunogenicity risk assessment
  ⇨ Maximized # identified peptides: certainty about immunogenic profile of biotherapeutic

- High-sensitive, high-throughput immunopeptidomics platform for ultrasensitive MAPPS assay

  ⇨ Reduced requirement sample input
  - Use limited # cells
  - Less biotherapeutic required for loading cells
  - Large screening panels possible
  - use moDCs / myDCs / B cells / cyno moDCs

  ⇨ Pinpoint putative immunogenic clusters with great accuracy
  ⇨ Full overview including DR / DP / DQ presented peptides
  ⇨ High throughput – fast analysis

High-confident immunogenic profiling via MAPPS for reliable immunogenicity risk assessment

⇨ enhanced compound selection & modulation
⇨ higher efficacy
⇨ safety

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ImmuneSpec. Meet the team.

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