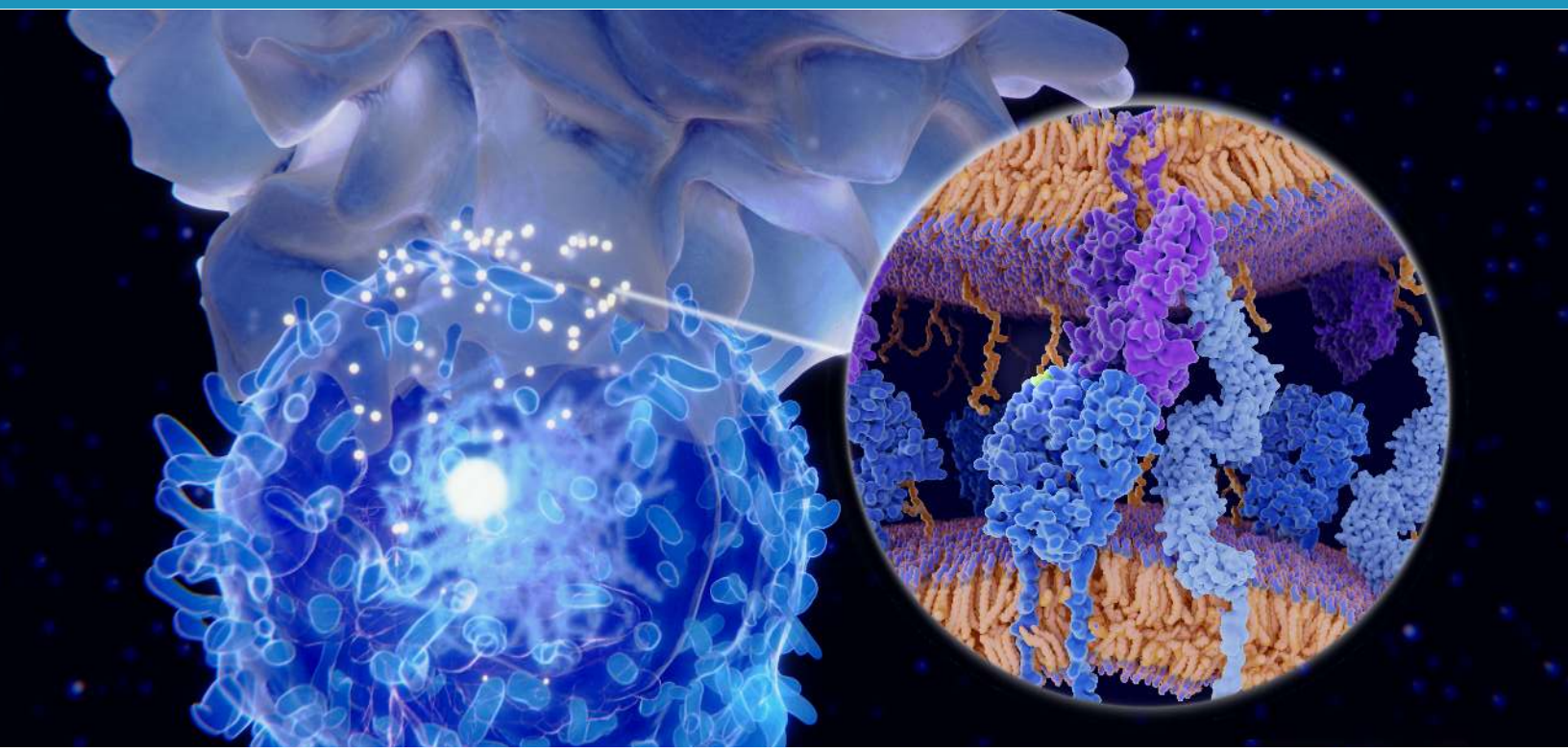

Investigation of the immunopeptidomic antigen-presented landscape of malignant pleural mesothelioma in human cell lines

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Abstract:

Malignant pleural mesothelioma (MPM) is a difficult-to-treat cancer with limited success in current therapies. Peptide-based cancer vaccines offer a potential avenue for intervention. In this investigation, a publicly available mass spectrometry dataset [1] was utilized to explore the immunopeptidomic landscape of MPM across four distinct cell lines, each with two replicates. The analysis revealed both canonical and non-canonical peptides using our PEAKS 11 DeepNovo peptidome workflow. Subsequently, the length distribution, binding prediction, allele-specific binding of identified peptides were analysed and compared across cell lines.

Inroduction:

Immunopeptidomics plays a critical role in advancing the development of peptide-based cancer vaccines, with a specific emphasis on identifying neoantigens vital for instigating immune responses against cancer cells. A standout strategy involves the direct immunopurification of the MHC-antigen complex, proving to be the most efficacious approach for precisely pinpointing potential CD8+ T cell targets.

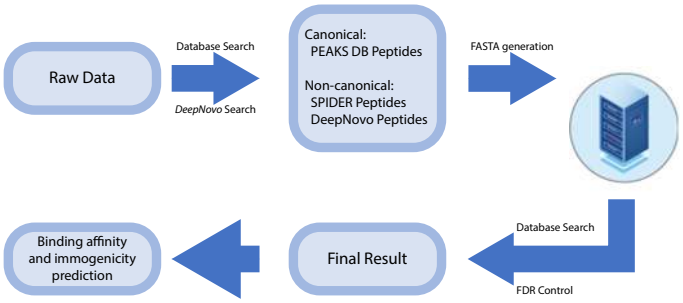

Fig 1. Schematic workflow for DeepNovo peptidome workflow.

Central to this methodology is the utilization of mass spectrometry as the principal tool for data acquisition. PEAKS 11 Deepnovo workflow analysis raw mass spectrometry data, and identifies peptides by database search, homolog and DeepNovo search. Furthermore, the platform integrates an essential component for immunogenicity assessment— a personalized model centered on T cell immunogenicity. In this study, we use our platform to investigate the immunopeptidomic landscape of MPM across four distinct cell lines.

Methods:

A published dataset was used [1]. Mass spectrometry data from four different cell lines with two duplicates are analyzed using PEAKS 11 Deepnovo workflow (Figure 1). Precursor and peptide fragment ion mass errors were set to 20.0 and 0.05 Da, respectively. Oxidation (M) and N-terminal acetylation were set as variable PTMs. Peptide identification was performed using a 1% peptide FDR with the human Uniprot reviewed database. Peptide length restriction was set to 7-20, and only unique peptide sequences were included in the final list of identified peptides. Length distribution, binding affinity prediction to specified alleles and sequence motif generation were done using MVP workflow [2][3] using a combined peptide list from all search results for each sample.

Results and Discussion:

The identified peptide numbers are listed in Table 1. The number of identified peptide between the replicates is reproducible, except for replicate 2 of H28 and H211, suggesting some degree of sample loss during the purification/preparation process (Table 1).

Cell Lines	Replicates	DB Peptides	Homolog Peptides	DeepNovo Peptides
H2452	Rep 1	2870	46	417
	Rep 2	2978	64	408
JL1	Rep 1	3635	70	602
	Rep 2	3083	34	512
H28	Rep 1	4345	110	731
	Rep 2	2242	30	555
H211	Rep 1	4256	48	735
	Rep 2	1424	16	429

Table 1. Identified immunopeptide numbers in samples. DB peptides represent peptides identified from database. Homolog peptides represent peptides identified from database with mutations. DeepNovo peptides represent peptides that are not in the database. All peptides were filtered to 1% peptide FDR, length of 7-20

Results and Discussion Cont'd:

The Venn diagram showing the common versus unique peptides across the cell lines revealed that the majority of identified immunopeptides were unique to each cell line, with less than 1% common peptides across all four cell lines, indicating the complexity and heterogeneity of MPM (Figure 2). However, it is possible to further investigate this 1% peptide subset to gain valuable insights from it.

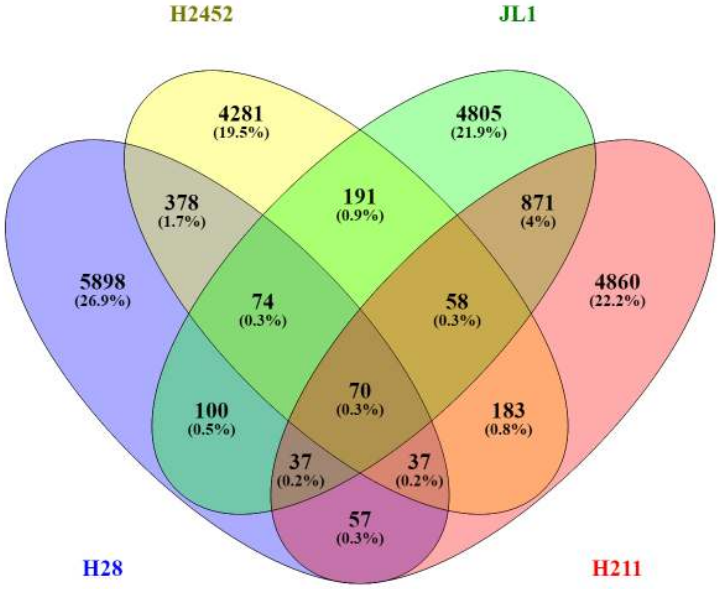

Fig 2. Venn Diagram comparing identified peptides from all four cell lines.

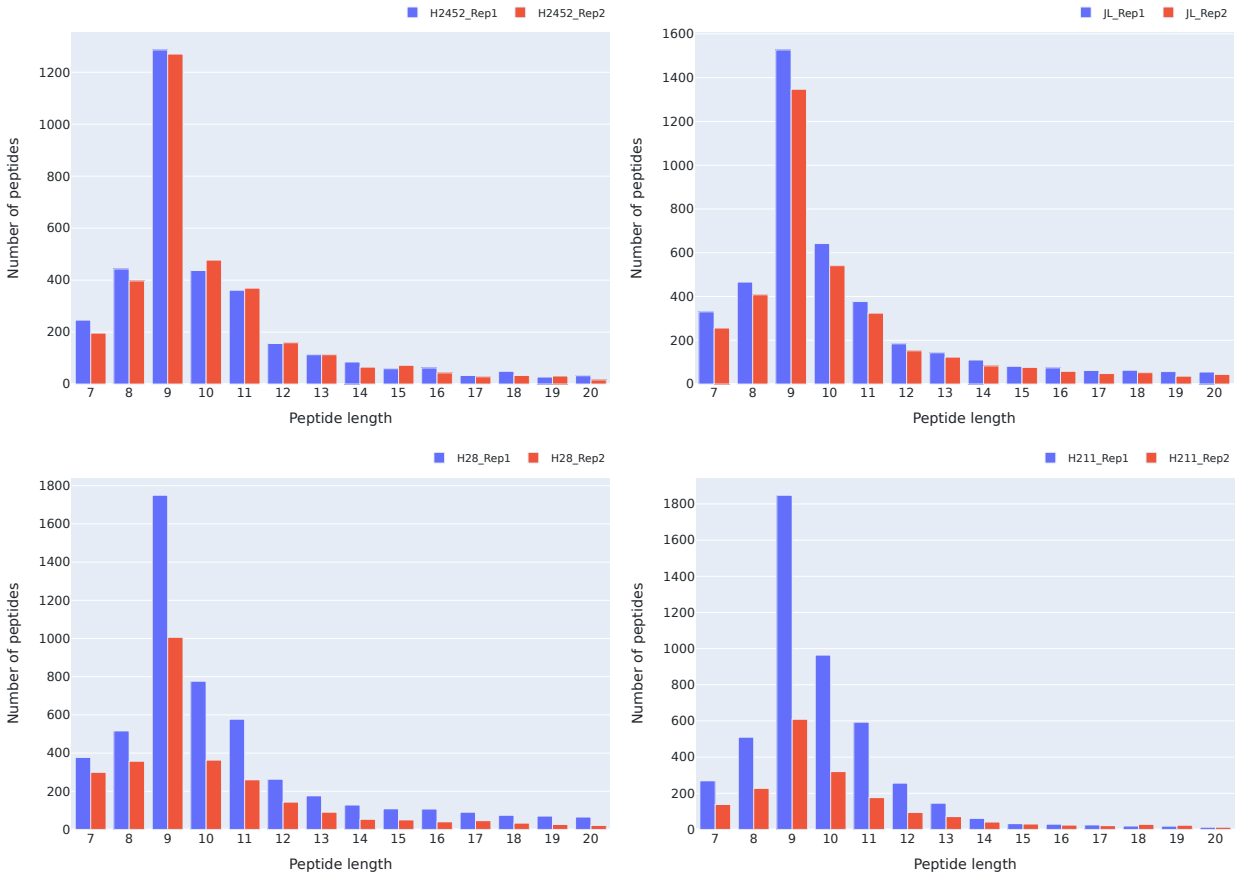

Fig 3. The length distribution of all identified peptides. Four distinct cell lines with two replicates each. Length distributions for each cell line are shown. Blue and red bars represent the different replicates in each cell line.

Results and Discussion Cont'd:

In the length distribution analysis, peptides ranging from 8 to 12 mers constituted the majority of eluted peptides. Notably, within this range, 9 mers emerged as the predominant population. The distribution of peptide lengths exhibited a characteristic pattern typical of the length distribution observed for HLA-I peptides (Figure 3). Furthermore, the peptide-MHC binding affinity prediction indicated that most peptides were predicted to bind MHC-I complex (Figure 4). However, in the case of replicate 2 of H28 and H211 we observed a lower percentage of predicted binding peptides compared to other samples, possible due to sample preparation/purification problems.

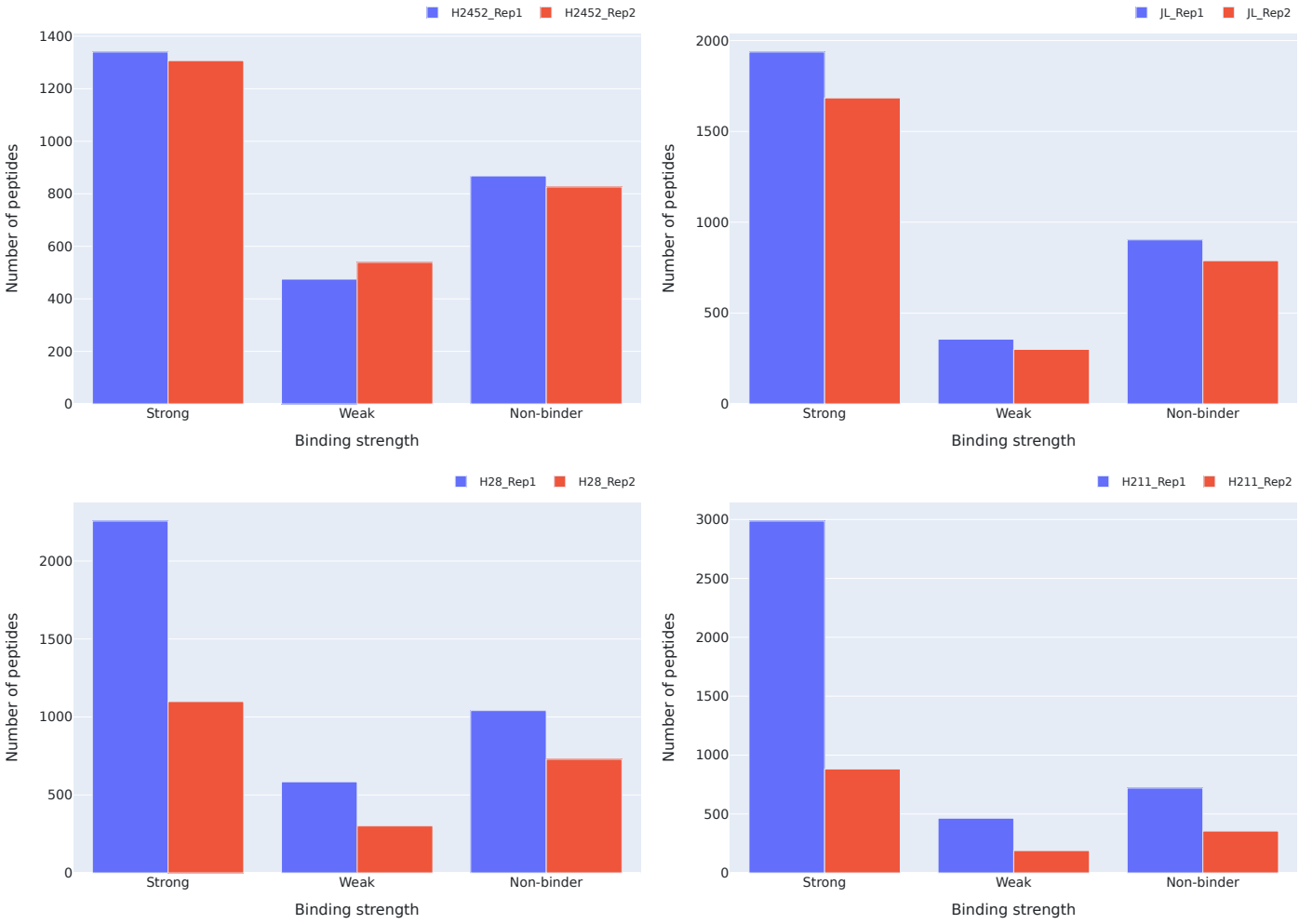

Fig 4. Comparison of the peptide predicted to be strong/weak and non-binders. Prediction performed on peptides between 8 and 12mers inclusive using NetMHCPan 4.1. Peptides were classified as "strong binders" when their predicted "affinity percentile" was lower than 0.5 and "weak binders" when their predicted "affinity percentile" lays between 0.5 – 2.

The binding specificity of eluted peptides suggested an unequal distribution of peptides across MHC alleles (Figure 5). This inequality may arise from variations in the surface expression levels of different MHC alleles, caused by epigenetic regulation or degradation of the MHC molecules [4][5], leading to differential presentation of peptides to T cells. Such differences may influence the strength and specificity of immune responses, impacting an individual's susceptibility to diseases and their response to immunotherapies [4-6]. Motif deconvolution identified the expected binding motifs for all MHC alleles, confirming the high quality of the immunopeptidomics dataset (Figure 6).

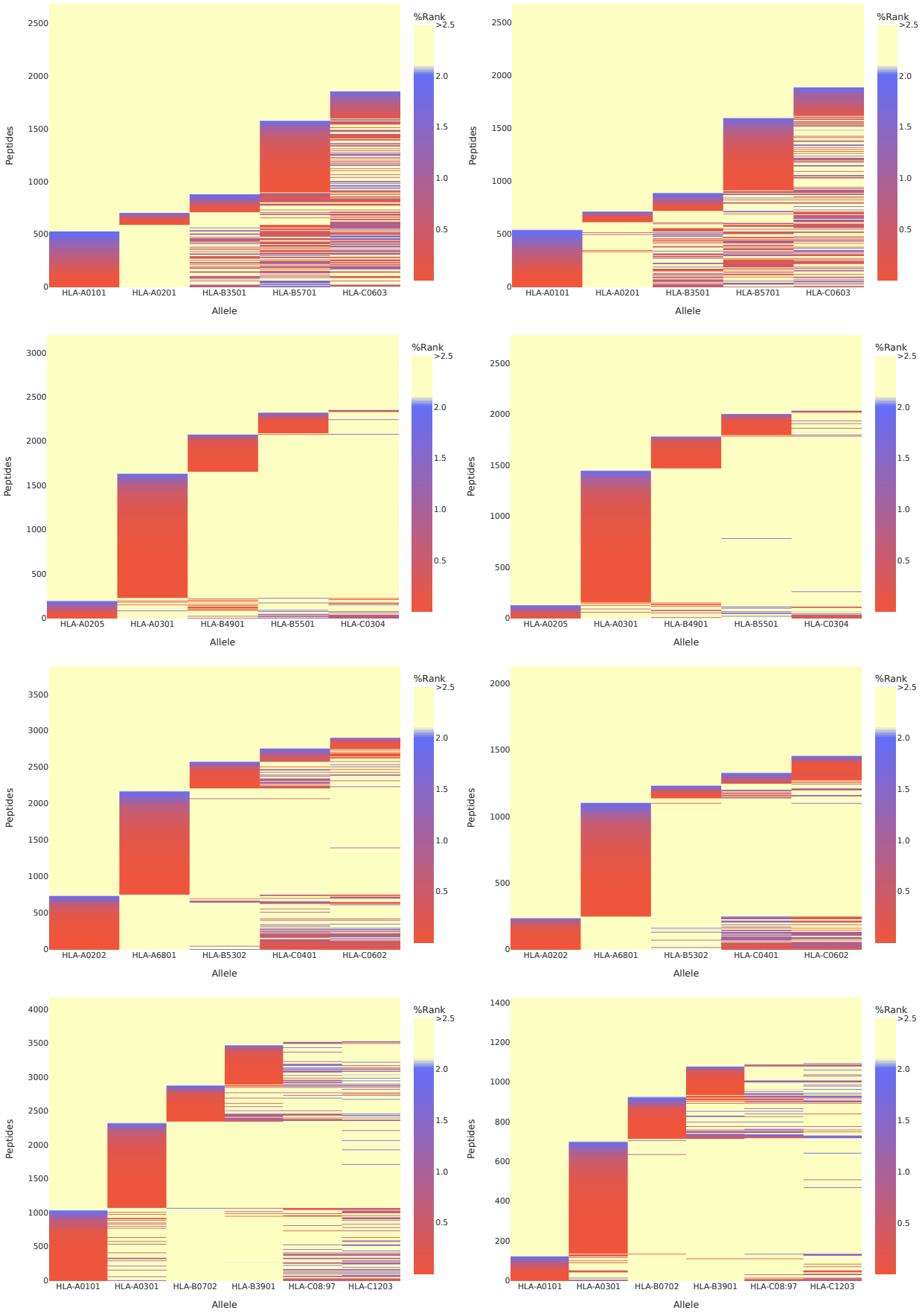

Fig 5. Eluted peptides between 8 and 12mers inclusive binding specificity deconvolution. Heatmap illustrating the outcome of peptide-specificity deconvolution based on prediction performed using NetMHCPan 4.1. Red represents predicted strong binders, blue represents week binders.

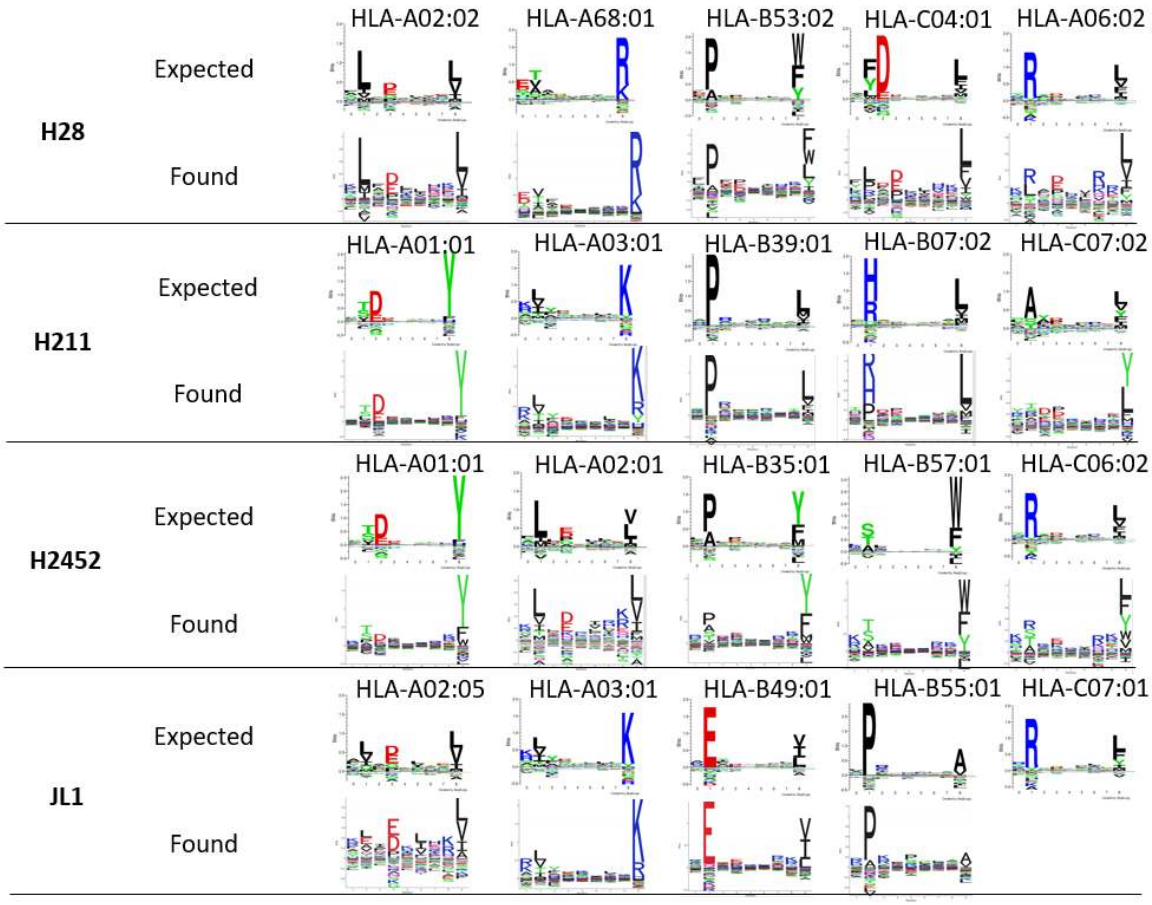

Fig 6. HLA allele-specific sequence motif from immunopeptides. Comparison between the expected motifs of specific MHC alleles obtained from NetMHCpan 4.1 Motif viewer referred as "expected", and the cluster results of the Eluted peptides referred as "Found".

Conclusion:

In conclusion, despite the intricate nature of MPM, with the right tools and analysis, identifying promising peptides for immunotherapy is feasible. By leveraging these insights, we can pursue the development of effective immunotherapies, offering hope for improved outcomes in treating this challenging disease.

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