SCP on Orbitrap MS

Sensitive and Deep Immunopeptidome Analysis with Orbitrap Astral Mass Spectrometer allowing to analyze smaller sample sizes

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Abstract

Purpose: To identify HLA Class I peptides (immunopeptidome) from cell extracts spanning a wide dynamic range with high sensitivity and accuracy, allowing for smaller sample sizes (biopsy) maintaining a robust immunopeptidome.

Methods: HLA Class I peptides extracted from IM-9 human B-lymphocyte cells were analyzed on a Thermo Scientific™ Vanquish™ Neo UHPLC System coupled to a Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer equipped with a Thermo Scientific™ FAIMS Pro Duo interface.

Results: Optimized Immunopeptidomics workflow allows for deeper annotation of the immunopeptidome at high loads or equivalent sensitivity to prior analytical technologies with lower amounts of starting material (5e6 cells). Now able to obtain a robust Immunopeptidome from biopsy size samples.

Introduction

Mass spectrometry (MS) allows for direct immunopeptidomics analysis, enabling simultaneous identification and quantification of thousands of MHC peptides in a single run. The recently developed Orbitrap Astral mass spectrometer has enabled new levels of sensitivity and selectivity to provide deeper insights into the immunopeptidome. In this study, we utilized the Orbitrap Astral mass spectrometer to characterize the immunopeptidome of IM-9 cells to support the detection and annotation of potential neoantigens.

Materials and methods

Sample Preparation

Class I HLA-peptide complexes were enriched from IM-9 human B-lymphocyte cells using a pan-specific HLA class I antibody (W6/32 clone) from a starting cell culture of 5e8 cells. HLA-associated peptides, also known as immunopeptides (IMP), were enriched using the Assaymap Bravo automated system after lysis with NP-40 lysis buffer. HLA-peptide complexes were captured using a pan HLA Class I antibody, w6/32. Glycine eluted complexes were further fractioned on a C18 sep pak, using 30% acetonitrile to harvest peptides, leaving proteins captured to the C18. Samples were diluted to represent the equivalent of 5e5 to 1e7 cells of extracted IMP.

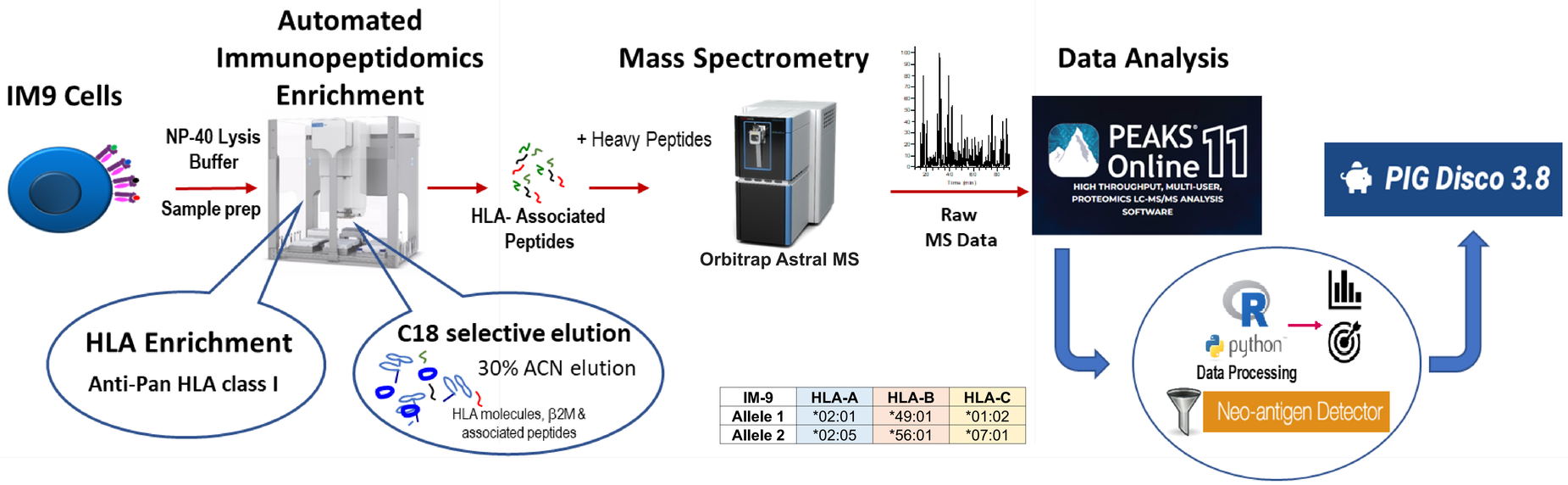
Test Method(s)

IMP extracts were analyzed using data-dependent acquisition (DDA) LC-MS/MS analysis on an Orbitrap Astral mass spectrometer interfaced with a Vanquish Neo UHPLC system and FAIMS Pro Duo interface. IMP were separated on a 25 cm x 75 µm C18 column packed with 1.7 µm particles via reverse phase chromatography with variable gradient separations (60 min, 120 min) at 200 nL/min (Fig. 1). DDA acquisition methods were used with ion cloud separations enabled by FAIMS at compensation voltages (CVs) of -25, -50 and -70 V to select for +1 and +2 - +4 charge states, respectively. Full MS scans were acquired with Orbitrap detection at 240,000 resolving power. MS/MS scans were acquired with Astral detection and 1.2 amu quadrupolar isolation of selected MS precursors.

Data Analysis

The data analysis was performed using PEAKS Studio software (ver. 11.5) with the DeepNovo Peptidome workflow for database search and de novo peptides identification. Spectra were searched against the UniProt human database (20,607 sequences) with the no-enzyme option. Data were searched in parallel for both DB search and De Novo search. The sequence motif and binding properties of 9-mer peptides were analyzed using MHCMotifDecon 1.0 and NetMHCpan 4.0.

Figure 1. Overview of Optimized Immunopeptidome workflow for Smaller Sample size



IM-9	HLA-A	HLA-B	HLA-C
Allele 1	*02:01	*49:01	*01:02
Allele 2	*02:05	*56:01	*07:01

Results

MHC class I peptides eluted from IM-9 cells were analyzed at different cell line equivalent dilutions to produce identification of up to 40,551 peptides with 8-12 amino acid (AA) and 26,745 9 AA length from 1e7cells equivalent input. The number of 9-mer peptides constituted ~65% of total class I peptides identified (Fig. 2-4). Orbitrap Astral technology with FAIMS interface enabled detection of HLA peptides of different charge states (Fig. 5). The resulting data were analyzed for sequence motifs, and the results were consistent with known HLA alleles (Fig. 6). The depth and accuracy of these results were enabled by Orbitrap Astral technology. Specifically, the high dynamic range MS1 survey scan enhances acquisition sensitivity (Fig. 7). Confident sequence assignment was achieved because the Orbitrap Astral acquires high quality MS/MS spectra across the complete ion series (Figure 7) with very high mass accuracy (Fig. 8). Approximately ~70% of 9-mers from the 5e6 cell sample input data were predicted binders to at least one of the representative HLA supertypes with the largest proportion predicted to bind HLA-A02.

Figure 2. Full MS Base Peak chromatograms demonstrating chromatographic reproducibility across variable cell line equivalent dilutions

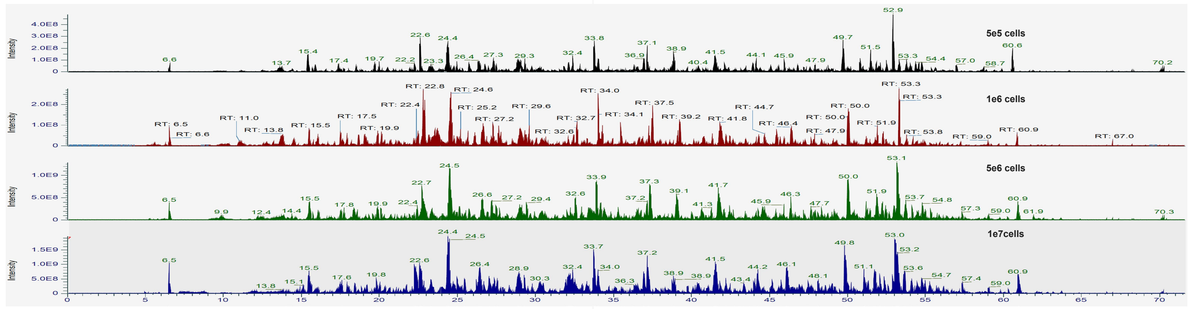


Figure 3. IM-9 HLA Class I peptides annotated from 5e5 to 1e7 cell line equivalent loads separated over 60-minute gradient

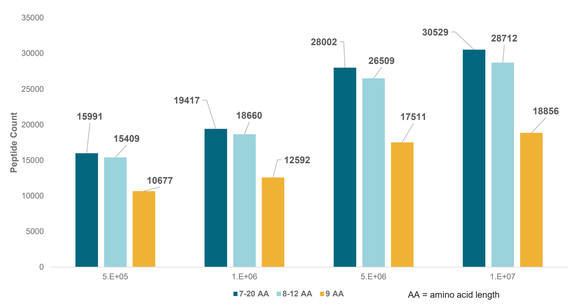


Figure 4. IM-9 HLA Class I peptides annotated from 1e6 to 1e7 cell line equivalent loads separated over 120-minute gradient

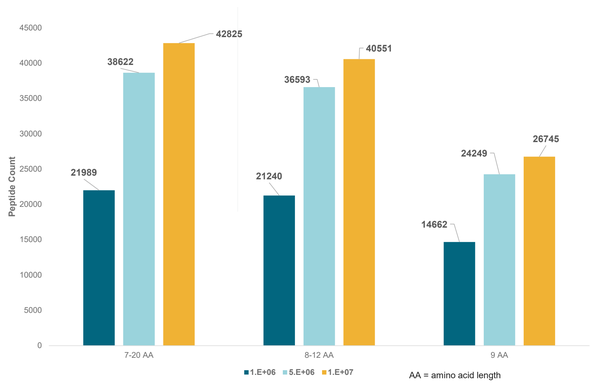


Figure 5. Relative charge distribution of annotated IM-9 Class I HLA peptides from 5e6 and 1e7 cell line equivalent loads separated over 60-minute gradient

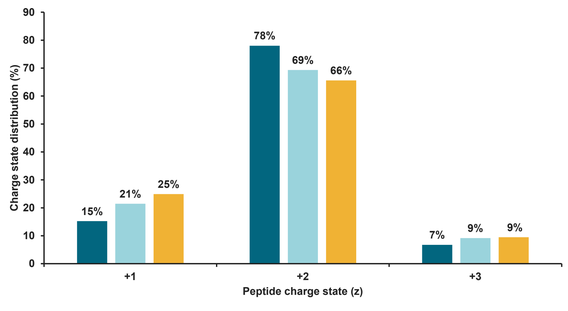


Figure 6. Sequence logo motif detected for 9-mer (9 amino acid length) peptides

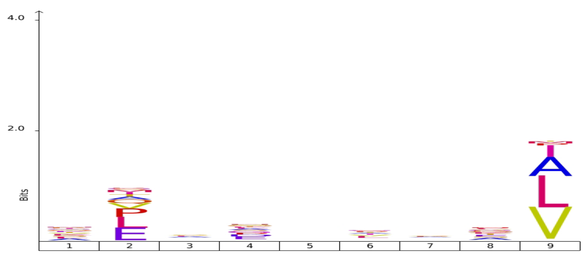


Figure 7. Example of spectral quality of the Orbitrap Astral MS/MS scans to demonstrate low abundance precursor isolation from high dynamic range Orbitrap Full scans from HLA Class I peptide annotations

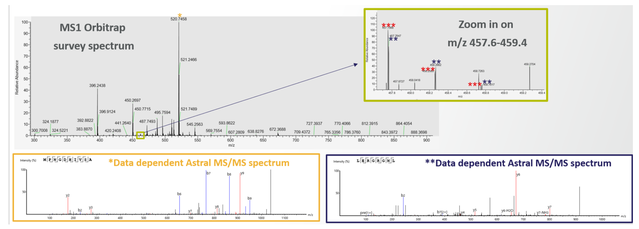


Figure 8. Annotated MS/MS spectrum of HLA Class I peptide, demonstrating full mass range of detection to capture diagnostic immonium ions, b1 and y1 ions, valuable for de novo sequencing annotation.

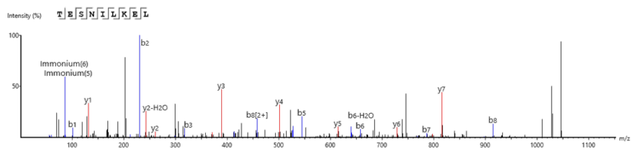
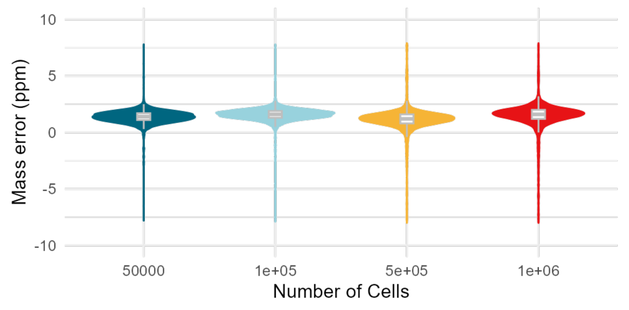


Figure 9. Plot of accurate mass measurement for precursors of all identified HLA Class I peptides from each cell line equivalent dilution



Conclusions

- Improved sensitivity of and dynamic range of detection for immunopeptide analysis with the Vanquish Neo UHPLC system coupled to an Orbitrap Astral mass spectrometer equipped with FAIMS Pro Duo interface selectivity enables deeper depth of coverage with higher throughput of analysis
- Improved sensitivity allows for equivalent IMP coverage with 5e5 cell equivalent dilution as previously obtained with 1e8 cell equivalent (data not shown)
- Increased sensitivity allows for compatibility with low levels of material equivalent to samples extracted from tissue biopsy samples

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