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Maximizing Immunopeptide Identification and Reproducibility from Minute Pediatric Solid Tumor Biopsy Samples

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Introduction

- Immunopeptidomics, a rapidly growing field of proteomics, plays a vital role in identifying and quantifying peptides presented by the major histocompatibility complex (MHC) to the immune system.
- Peptides presented by MHC molecules are typically present at very low levels especially in pediatric patient samples where MHC expression is often low.
- Maximizing peptide yield, sensitivity, and intra- and inter-sample reproducibility with mass spectrometry is essential to discovering peptide-MHC (pMHC) targets for modern cancer immunotherapies.
- Here we compare sample preparation methodologies incorporating sepharose bead columns and streptavidin magnetic beads combined with the ultra-high sensitivity mass spectrometry for the analysis of immunopeptides from pediatric solid malignancies.

Methods

- HLA class-I peptide samples were prepared using two immunoprecipitation(IP) methods: CNBr-activated Sepharose 4B column-based capture and streptavidin-biotin based capture. Briefly, frozen tissues and frozen cell pellets were pulverized by liquid nitrogen cooled cryomilling and then resuspended in lysis buffer to perform IP with using pan-HLA class I antibody W6/32. Peptides were eluted from MHC molecules and then purified with a 5 kDa MWCO filter and C18 resin.
- Peptides were separated by nano-HPLC (nanoElute 2, Bruker Daltonics) on a 250 mm x 75 µm, 1.7 µm column. Sixty minute gradients were analyzed in parallel on a timsTOF Pro 2 and timsTOF Ultra (Bruker Daltonics) in PASEF (Parallel Accumulation and Serial Fragmentation) mode.
- Data were processed using PEAKS software

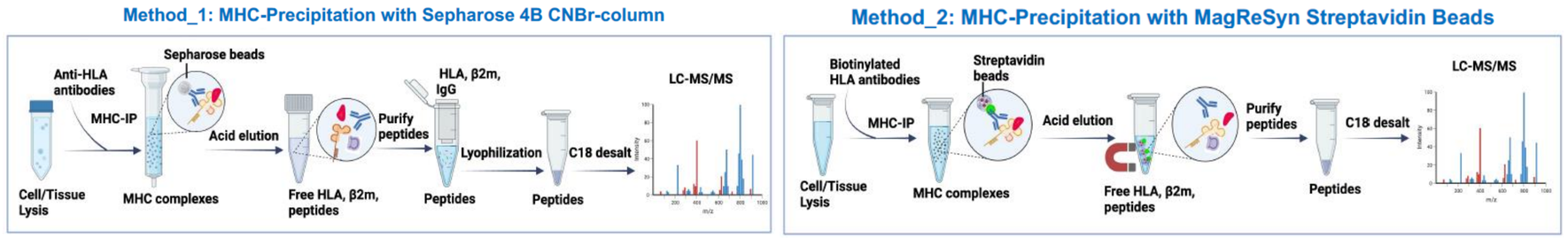

Figure 1. Immunopeptidomics experimental pipeline. Two methods for purification of MHC peptides.

Results

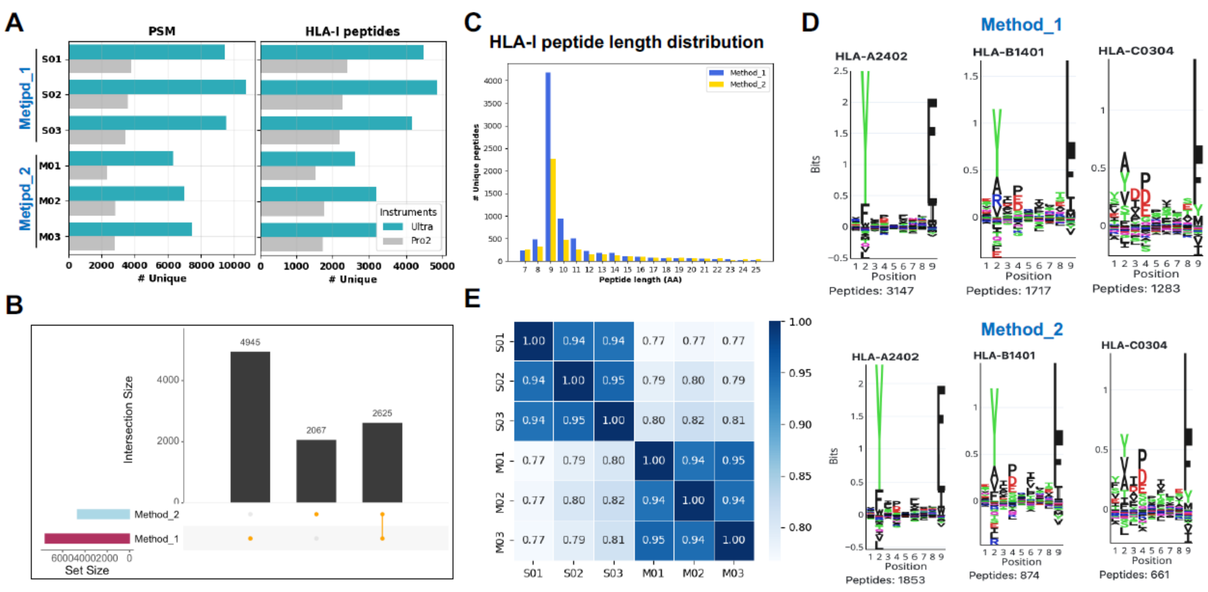

Figure 2. In-depth and sensitive analysis of HLA-I peptides at 1% FDR for peptide identifications.
A. Number of unique peptide spectrum match (PSM) and peptides identified for each sample (2e7 of SKNAS neuroblastoma cells, technical triplicates in each method) across the different MS instruments. **B.** UpSet diagrams show overlap between all merged peptides identified over the technical triplicates of each method using timsTOF Ultra. **C.** Length distributions of HLA-I peptides identified in each method using timsTOF Ultra. **D.** Peptides were assigned to the different HLA allotypes based on SKNAS HLA-I allotrope and predicted binding affinity and their binding motifs. **E.** Intra- and inter-sample reproducibility calculated by Pearson correlations of log10 transformed intensities of HLA-I peptides identified using timsTOF Ultra.

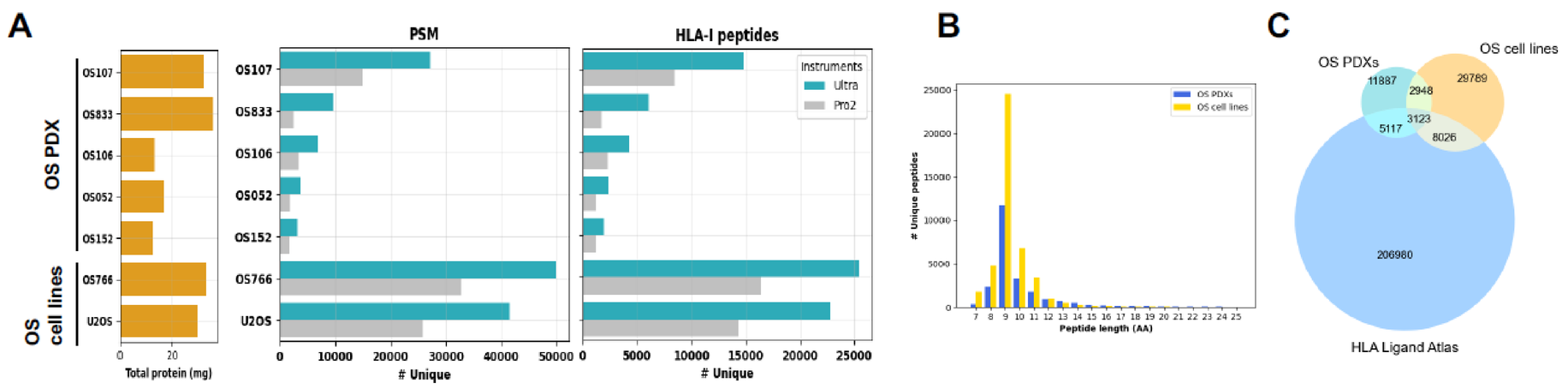

Figure 3. MS-based immunopeptidomics data of osteosarcoma. A. Number of unique PSM and peptides identified for each osteosarcoma patient-derived xenografts (OS PDX, n = 5) and 2 cell lines (1e8 cells per sample) across the different MS instruments. **B.** Length distributions of HLA-I peptides identified in OS PDXs and 2 cell lines using timsTOF Ultra. **C.** Venn diagram of all merged peptides identified in OS PDXs, OS cell lines, and normal tissues from the HLA Ligand Atlas.

Summary

- The Bruker timsTOF Ultra demonstrated a significant improvement in peptide identification capabilities, detecting over 40% more peptides compared to the timsTOF Pro2. This indicates that timsTOF Ultra offers superior sensitivity and efficiency in peptide identification, which could be highly beneficial for proteomics studies requiring high-depth peptide coverage.
- In the comparison of immunoprecipitation methods, the CNBr-activated sepharose 4B method significantly outperformed the streptavidin magnetic bead method.
- The analysis of osteosarcoma PDX samples revealed an average yield of 5904 unique peptides, with a wide range from 1967 to 14,823 peptides. This variability is linked to the tumor input mass, indicating that the amount of starting material significantly

Future Directions

- Optimize the streptavidin magnetic bead method to improve peptide recovery in small-scale IP for tumor biopsy samples.
- Identify numerous peptide-MHC targets for the development of peptide-centric chimeric antigen receptors (PC-CARs) for pediatric solid tumors

Acknowledgements

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Conflict of interest: JM and VT are employees of Bruker Daltonics GmbH & Co KG. MW and DA are employees of Bruker Scientific, Inc.

References

Yarmarkovich M, Marshall QF, Warrington JM, *et al*. Targeting of intracellular oncoproteins with peptidecentric CARs. *Nature*. 2023;623(7988):820-827.
Marcu A, Bichmann L, Kuchenbecker L, *et al*. HLA Ligand Atlas: a benign reference of HLA-presented peptides to improve T-cell-based cancer immunotherapy. *Journal for ImmunoTherapy of Cancer*. 2021;**9:**e002071.

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