B28

MS-Based HLA Peptide Discovery: Tumor Neoantigens and Biotherapeutic T-Cell Epitopes

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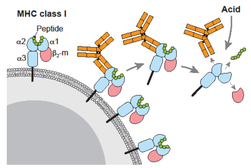
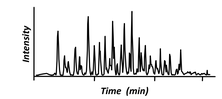
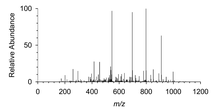
MS Bioworks
Protein Mass Spectrometry Services

Workflow

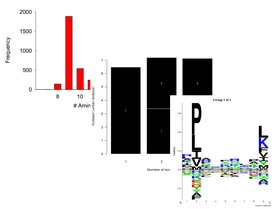


- Cells are lysed and resulting preparations are subjected to immunoaffinity capture. Following multiple washes peptides are eluted from the MHC cleft with acid.





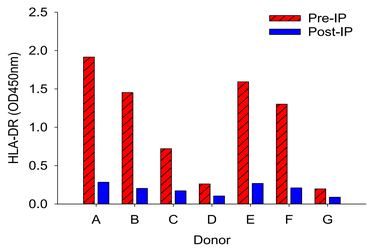
- Purified peptides are analyzed by LC-MS/MS using nano-scale chromatography combined with a Fusion Lumos mass spectrometer using electron-transfer/higher-energy collision dissociation (EThcD) fragmentation. MaxQuant is used for peptide identification and quantitation.



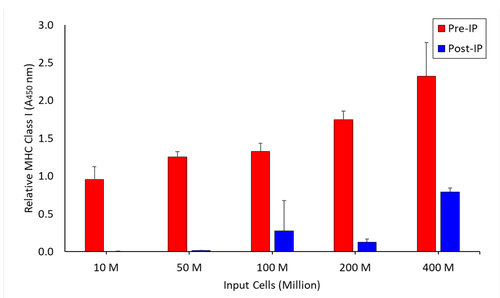
- Processed data can be further interrogated using bioinformatics tools. For example NetMHC or GibbsCluster allow the elucidation of binding strengths and motifs.

MHC Recovery

- Custom ELISAs detect intact MHC Class I and MHC Class II complexes to monitor recovery of the MHC molecules.
- This is used for both tissue/cell specific optimization and as a quality control check during the studies.

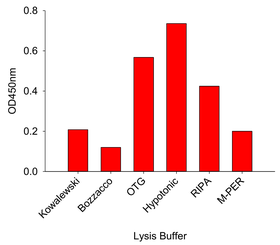


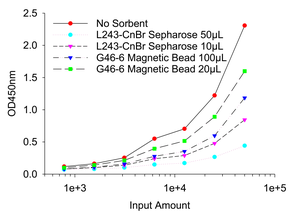
- Seven dendritic cell samples (A-G) had widely divergent levels of MHC Class II expression, but all the MHC Class II was captured in the IP.



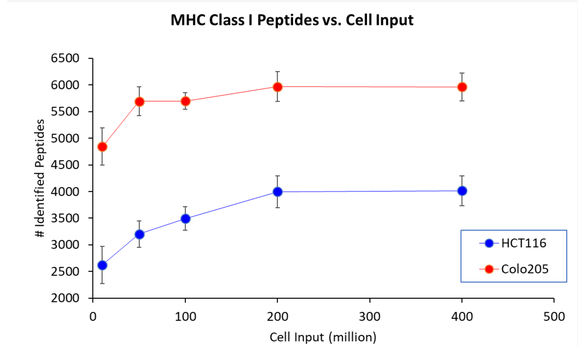
- Quantity of input Colo205 cells was varied (10 – 400 M, +IFNγ) showing the relative amount of MHC Class I complexes captured for each IP experiment.

Optimization

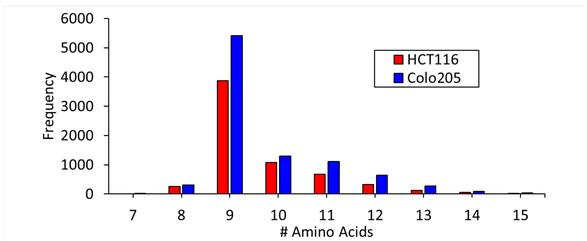




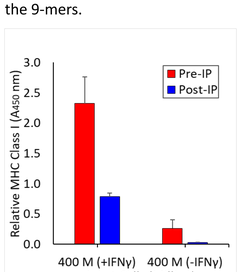
- Using an ELISA that detects the intact Class II heterodimer, we optimized lysis buffer formulations, IP antibodies, and IP sorbents to maximize Class II immunoaffinity capture.



- To better understand the MHC Class I peptide yield and to improve practical application of this assay we varied the input amount while holding the antibody concentration constant (20 mg/mL).



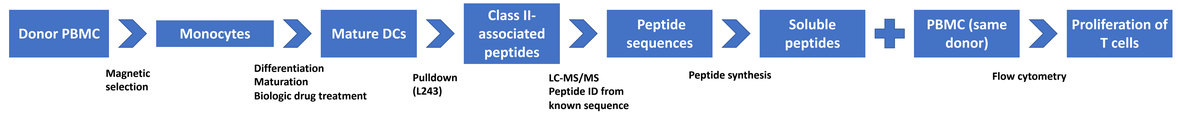
- The histogram above shows the distribution of peptide lengths in the combined data. The scatter plot includes data for all the observed peptides and exclusively the 9-mers.



# Peptides	+IFNγ	-IFNγ
mean	4015.3	3761.0
Std.Dev.	277.74	166.62
CV	7%	4%

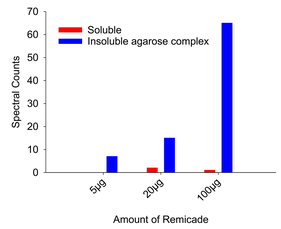
- Effect of IFNγ on MHC Class I expression in Colo205 cells as detected by W6/32 ELISA (left) and number of observed peptides by MS (table, right).

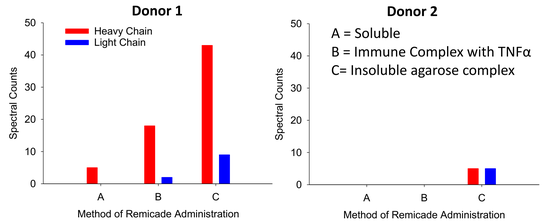
Biotherapeutic T-Cell Epitopes



DC Pulsing With Biotherapeutic

- Human dendritic cells are adept at ingesting apoptotic whole cells, immune complexes, and insoluble aggregates, and then processing those materials for presentation on Class II.
- We used the commercial biologic Remicade to assess treatment conditions with samples from two donors.





- Dendritic cells prefer immune complexes or insoluble aggregates over soluble proteins for ingestion, processing, and display on Class II.
- Soluble protein antigen (Remicade) is taken up and processed far less efficiently than aggregated antigen.
- The ability to present peptides derived from Remicade is donor-specific. Increasing the concentration of soluble protein antigen does little to improve the outcome.

Peptide ID

- The sequence coverage maps for the heavy chains of the target protein in the donor 1 samples is below. Identified peptides are highlighted in yellow.

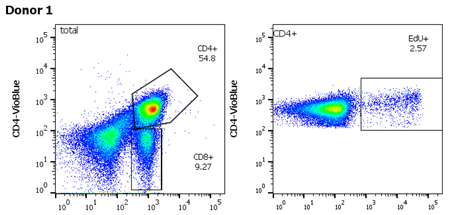
Remicade_HC (100%), 49,515.8 Da
Remicade_HC
19 exclusive unique peptides, 34 exclusive unique spectra, 43 total spectra, 54/450 amino acids (12% coverage)

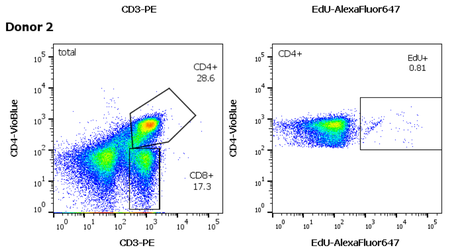
EVKLEESGGG LVQPGGSMKL SCVASGFIFS NHWMNWVRQS PEKGLEWVAE
IRSKSINSAT HYAESVKGRF TISRDDSKSA VYLQMTDLRT EDTGVYYCSR
NYYGSIYDYW GQGTTLTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK
DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT
YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSLSP GK

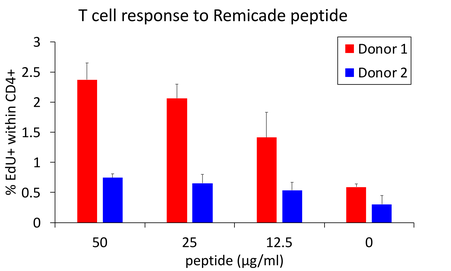
- Note the highest number of peptides were identified in the AA402 – AA 421 region of the sequence.

T-Cell Activation

- Peptides presented by MHC class II are capable of stimulating T cell proliferation. One of the peptides discovered by LC-MS/MS (red box, “Peptide ID”) was synthesized and fed to PBMCs from the same donor from the discovery experiment (Donor 1) and another donor not known to present Remicade peptides (Donor 2). After 5 days in culture, T cell proliferation in the presence of this peptide was assessed by EdU incorporation analyzed by flow cytometry.







Summary

- Presented optimized methods for the identification of neoantigens (MHC class I) and biotherapeutic neoepitopes (MHC class II).
- These assays have utility to improve prediction of neoantigen identification and mitigate risk of biotherapeutic immunogenicity.