AACR 2020
Poster #3210

Elucidation of key parameters for effective neoantigen prediction through a consortium effort: the Tumor nEoantigen SeLection Alliance

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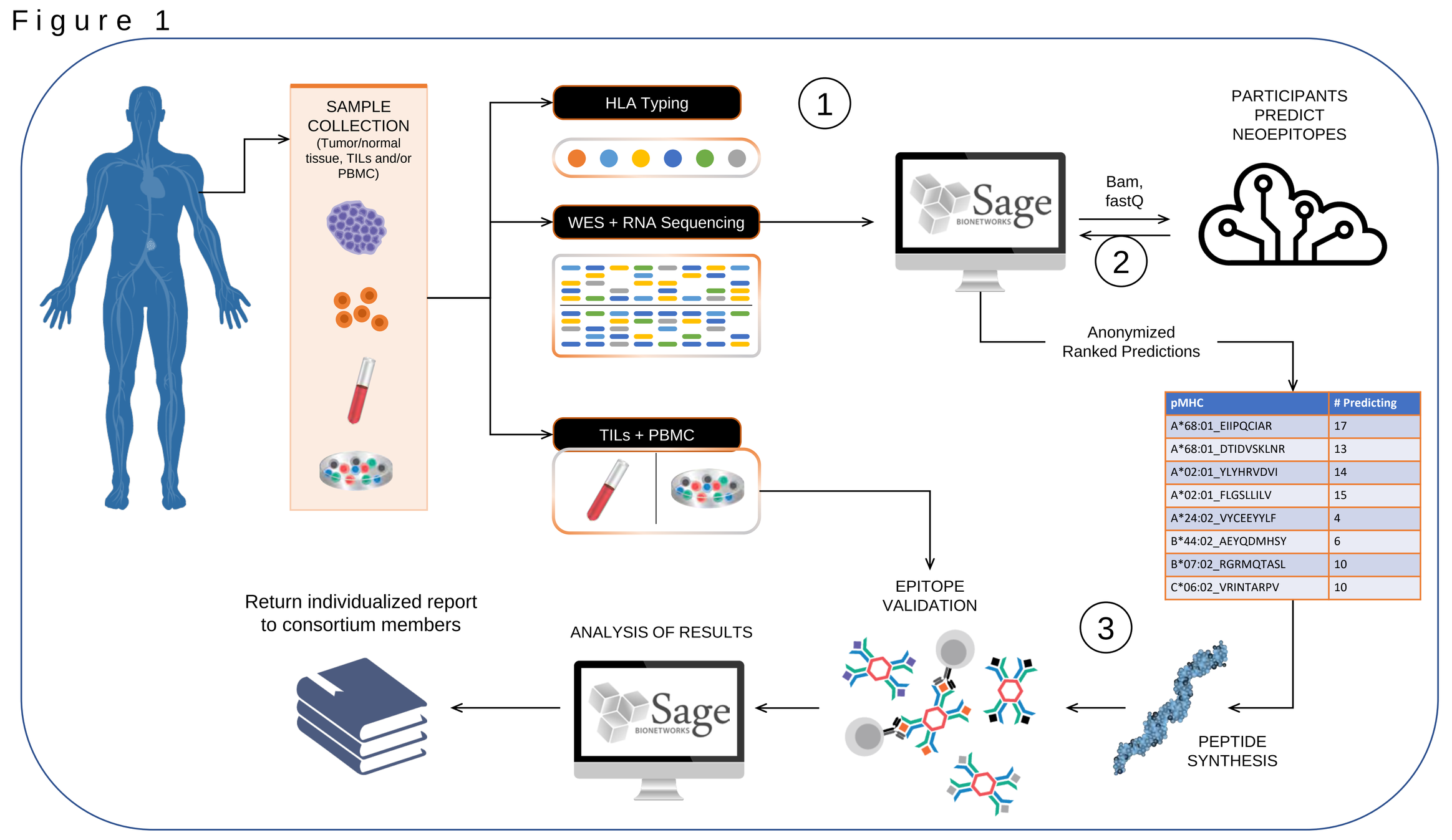
Background & Approach

- Therapeutic strategies targeting neoantigens hold the promise of being safe and effective anti-cancer therapies.
- Putative neoantigens are typically identified through *in silico* analyses of tumor tissue sequencing based on inferred rules of tumor epitope immunogenicity.
- However, there is no common reference data set with which these approaches can be compared, and the key parameters for effective neoantigen identification remain elusive.
- Here, we introduce a global consortium initiative, the Tumor nEoantigen SeLection Alliance (TESLA), and describe strategies through which the neoantigen prediction methods can be improved.

Consortium and Study Workflow (Figure 1)

Figure 1



- The consortium was formed 4 years ago and represents 40 teams of researchers from academia, industry, and non-profit groups total.

- **Workflow**:
  1. The teams are provided access to Tumor/normal whole exome sequencing (WES), tumor RNA-sequencing (fastQ and BAM files) and clinical-grade HLA typing, generated centrally and hosted on the Synapse platform.
  2. The teams generate neoepitope predictions based on their pipelines and return:
     - A **ranked** list of neoepitopes and associated HLA allele
     - An **unranked** list of filtered neoepitopes and associated HLA allele
     - A list of identified **variants**
     - The neoepitopes should be ranked based on their predicted ability to bind to the relevant **MHC class I** molecules (pMHC) and elicit an immune response.
     - To enable comparison across the different pipelines, participants are required to align sequence data to GRCh38 (Ensembl).
  3. A subset of peptides representing the top 5 ranked predicted neoepitopes from each team and the neoepitopes that were the most recurrently ranked in the top 50 across all teams are tested *in vitro* to determine MHC class I binding and the presence of pMHC-restricted T cells in subject-matched PBMCs or TILs.

Subjects, Treatment, and Specimens

Samples from six subjects were analyzed:

- 3 subjects with metastatic melanoma with matched pre-treatment control/tumor biopsy and on-treatment PBMC (samples previously collected and archived at UCLA)
- 3 subjects with non-small cell lung cancer (NSCLC) with matched pre-treatment control/tumor biopsy and pre-treatment Tumor Infiltrated Lymphocytes (TILs) from Lysates (samples previously collected and archived at MSKCC)

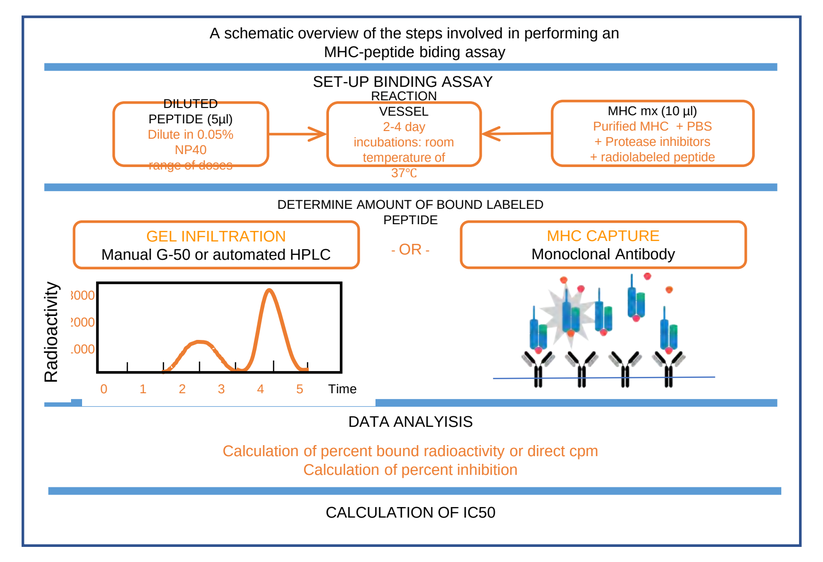
Subject ID	1	2	3	10	12	16
Site	UCLA			MSKCC		
IRB	11-001918 and 11-003066			06-107		
Tumor type	Melanoma			NSCLC		
Gender	Male	Male	Male	Male	Male	Female
Pathological Status	Metastatic	Metastatic	Metastatic	Primary	Primary	Primary
Cell type	Epithelial	Epithelial	Epithelial	Epithelial	Epithelial	Epithelial
Organ	Skin	Skin	Skin	Lung	Lung	Lung
Treatment (Check Point Inhibitor)	Pembrolizumab	Ipi + Nivo	Nivolimumab	N/A	N/A	N/A
Days on Treatment at time of Biopsy	-28	-25	-11	N/A	N/A	N/A
Response	Partial Response	Partial Response	Complete Response	N/A	N/A	N/A

Validation of Predicted Neoepitopes

Figure 2

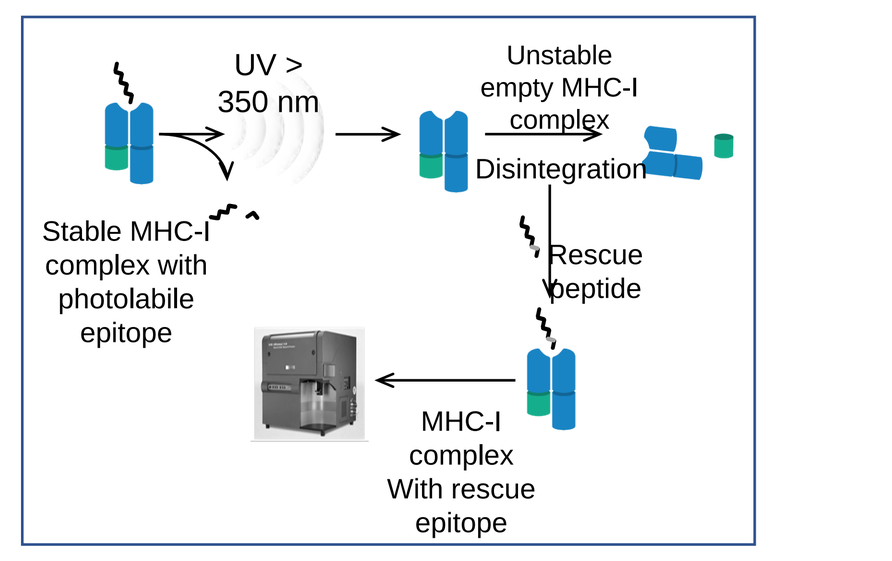
- Validation aims to determine whether an immune response has been generated against the predicted neoepitopes and can be detected in sample-matched T cells.
- For this study, a mutant peptide is called 'immunogenic' if it is recognized by T cells in the context of the right HLA.

Peptide:MHC Binding



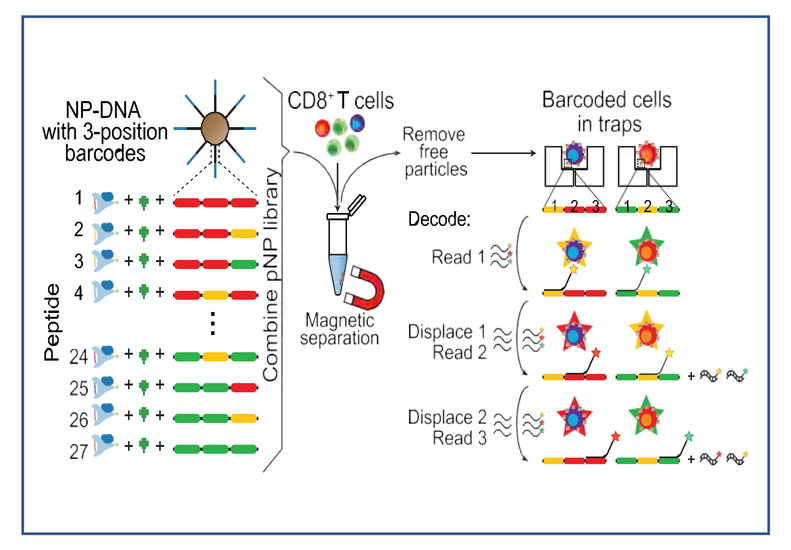
Sidney J., et al. (2013). Curr. Protoc. Immunol *100*, 18.3.1-18.3.36. [Pubmed]

Tetramer-Flow



Toebes M, et al. (2006). Nature medicine 12:246–251. [PubMed]
Andersen RS, et al. (2012). Nature protocols 7:891–902. [PubMed]
Kvistborg P, et al. (2012) Oncoimmunology 1:409–418. [PubMed]

Nano-Particle-barcoded Assay

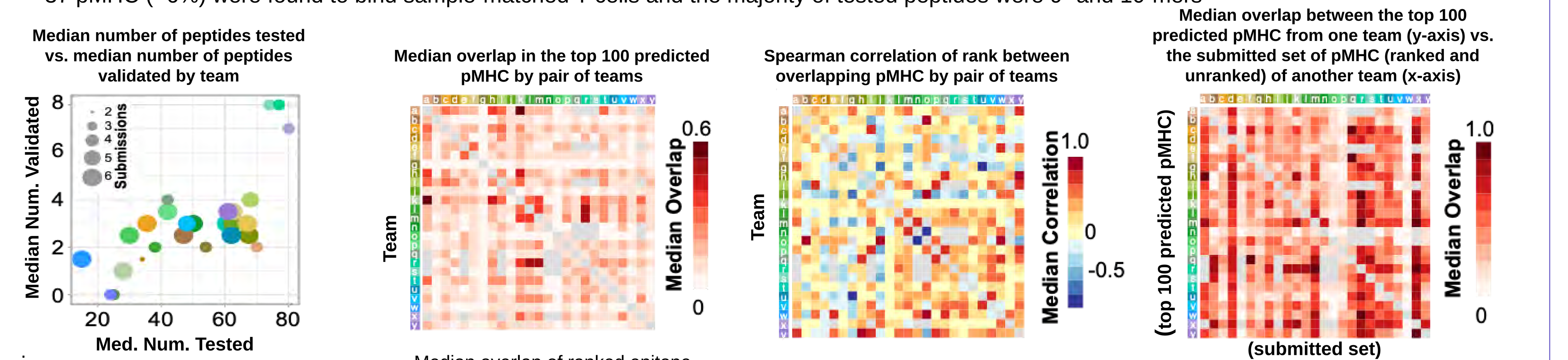


Peng S., et al. (2019) Cell Reports 28, 2728–2738. [Pubmed]

Participation & Predictions

Figure 3

- 25 teams submitted predictions for at least two melanoma or NSCLC patients
- 608 pMHC were tested for T-cell binding
- 37 pMHC (~6%) were found to bind sample-matched T cells and the majority of tested peptides were 9- and 10-mers



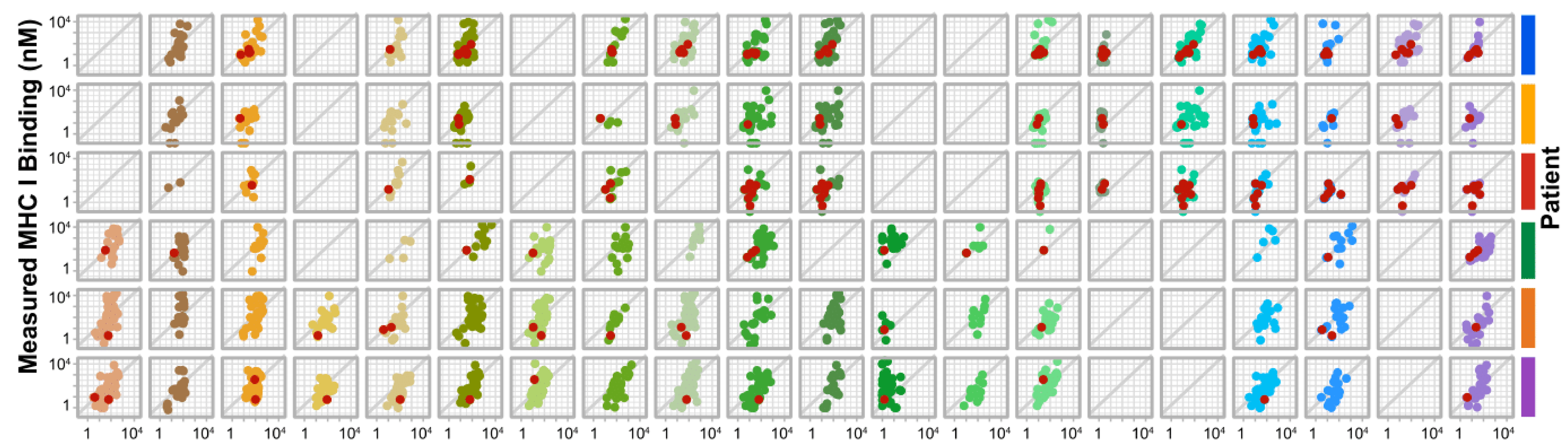
Colors indicate teams. Most teams had ~50 peptides tested by patient and ~3 validated peptides.

Median overlap of ranked epitope being less than 20%.

Overall there is a limited overlap of predicted neoepitope and top ranked epitopes between the teams.

Predicted vs Measured pMHC Binding

Figure 4



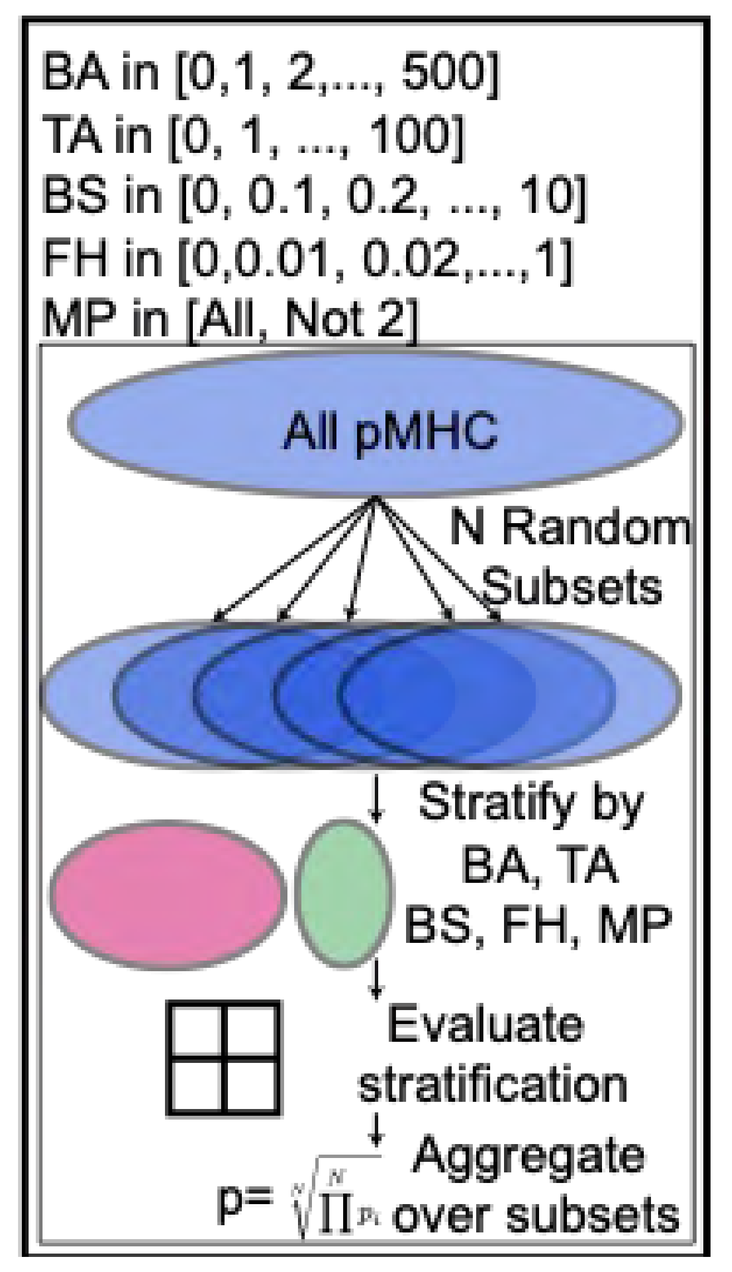
Red dots indicate pMHC which validated in a tetramer based assay. Twenty of the 25 teams analyzed submitted predicted MHC binding

There are no substantial differences between teams in how well they predict MHC binding strength.

Differentiating Immun. vs Non-Immun. Peptides

Figure 6

Schematic of cross-validation scheme to select feature and threshold set



BA: Binding Affinity; TA: Tumor Abundance; BS: Binding Stability; FH: Fraction Hydrophobic; MP: Mutation Position.

Contingency table using the optimal stratification parameters

Validation Status	Presented: False	Presented: True
False	250	17
True	10	12

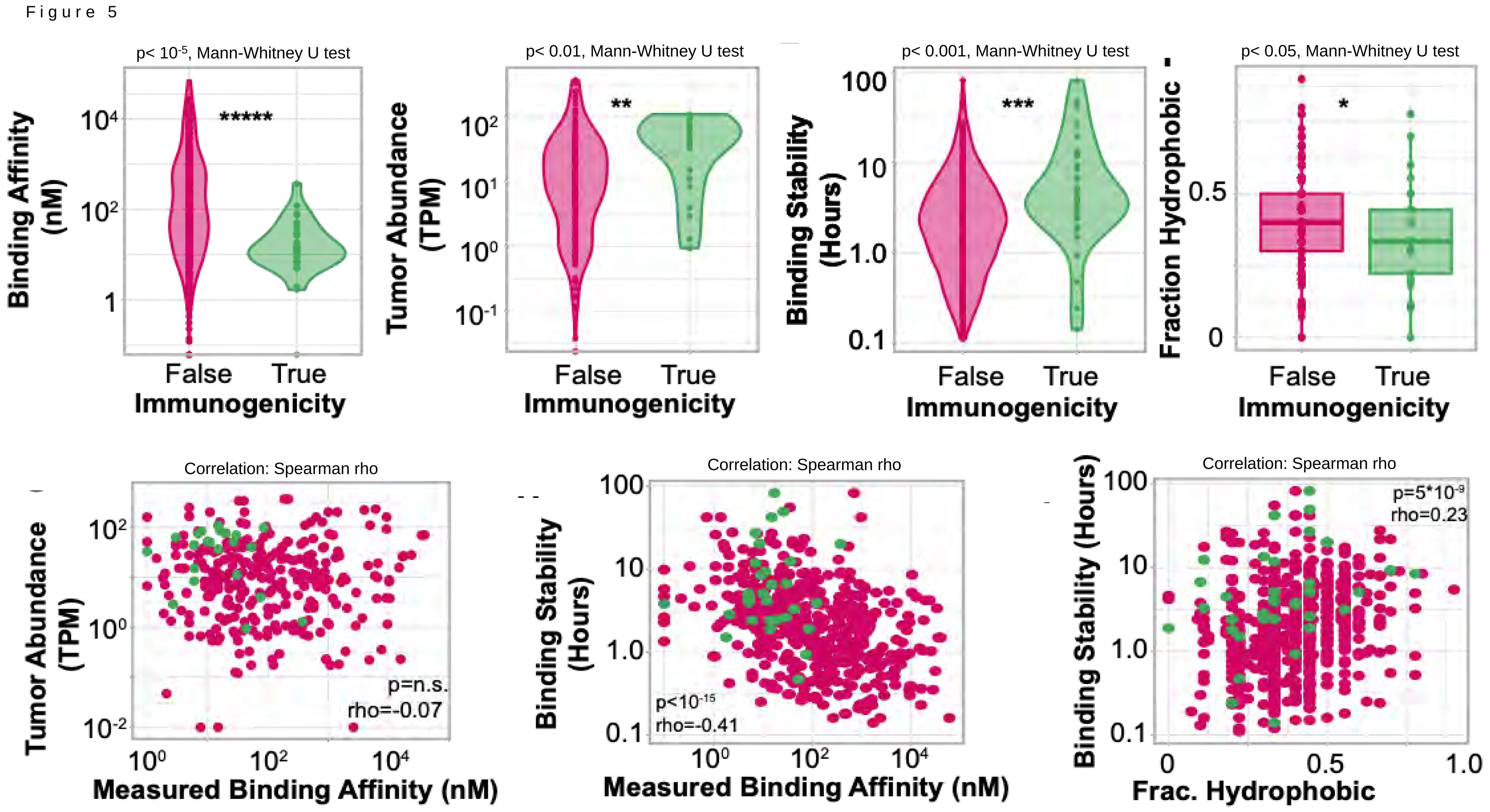
p=3.7*10^{-8} OR=17.0
p: Fisher exact test.

Optimal parameters identified by a repeated-random-subsample-based method :

- **Binding Affinity:** <34 nM
- **Tumor Abundance :** >33 TPM
- **Binding Stability:** >1.4H

Characterization of the Peptides Associated with Detectable Antigen-specific T Cells

Figure 5



- Immunogenic pMHC had significantly stronger measured binding affinity, significantly higher tumor abundance and significantly higher calculated binding stability.
- There is no correlation between MHC binding affinity and tumor abundance: high tumor abundance did not compensate for weak pMHC binding affinity.
- MHC binding affinity was significantly inversely correlated with binding stability.

SUMMARY

- We assembled a global consortium. Each participant predicted immunogenic epitopes from a set of 6 subjects' tumor sequencing data.
- There was a limited overlap of predicted neoepitopes and top ranked epitopes between the teams.
- 608 epitopes predicted to bind to MHC class I, were assessed for T-cell binding in patient-matched samples.
- Features that differed significantly between immunogenic and non-immunogenic peptides included: MHC binding affinity, expression of the originating gene (aka "tumor abundance") and MHC binding stability.
- A new model which includes MHC binding affinity (<34nM), tumor abundance (>33TPM) and pMHC biding stability (>1.4H) filtered out 93% of non-immunogenic peptides while maintaining 55% of immunogenic ones.
- These results were validated in an independent cohort (not shown).
- It is the intent of the TESLA consortium to make these datasets available to the scientific community as a reference dataset.

ACKNOWLEDGMENTS

We thank all the subjects who contributed to this study through donation of tumor and blood samples, as well as the research staff at UCLA and MSKCC for sample collection and processing. We acknowledge Olga Malkova, Diane E. Bender, Likui Yang and Tammi Vickery for their work on Class I Peptide Binding Assay; Jeff Bluestone, Jeff Hammerbacher and Robert Vonderheide for helpful and supportive comments and David Liu and Eliezer van Allen for help in obtaining access to published data.

Abbreviations: MHC: Major Histocompatibility Complex; pMHC: peptide-MHC complex; nM: nanomolar

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