

BCB 731:

# *Defense Against the Dark Arts*



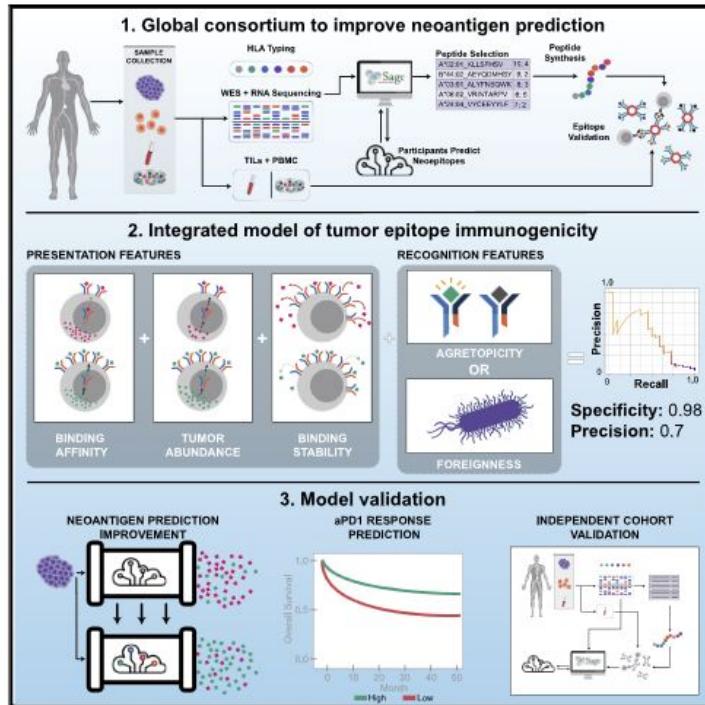
Optimist: Key Parameters of Tumor  
Epitope Immunogenicity Revealed  
Through a Consortium Approach  
Improve Neoantigen Prediction

*November 6th, 2023*



# Key Parameters of Tumor Epitope Immunogenicity Revealed Through a Consortium Approach Improve Neoantigen Prediction

## Graphical Abstract



## Authors

Daniel K. Wells, Marit M. van Buuren, Kristen K. Dang, ..., Ton N. Schumacher, Pia Kvistborg, Nadine A. Defranoux

## Correspondence

[dwells@parkerici.org](mailto:dwells@parkerici.org) (D.K.W.), [ndefranoux@parkerici.org](mailto:ndefranoux@parkerici.org) (N.A.D.)

## In Brief

Genomic tumor sequencing data with matched measurements of tumor epitope immunogenicity allows for insights into the governing parameters of epitope immunogenicity and generation of models for effective neoantigen prediction.

# *Background*



# Sean Parker (Napster)

napster v2.0 BETA 3 © 1999 napster Inc.

File Settings Help

Want Your Ad Here? Inquire Within

Chat Area Library Search Hot List Transfer Feedback

Online	Filename	Filesize	Bitrate	Frequency	Length
mathowie	MP3's\Huey Lewis & The News - The Power Of Love.mp3	3,788,382	128	44100	3:56
Redstone	MP3's\Incense & Peppermints - Strawberry Alarm Clock....	2,702,106	128	44100	2:48
	MP3's\JAMES GANG - WALK AWAY.mp3	3,415,272	128	44100	3:33
	MP3's\Jamiraquoi - Virtual Insanity.mp3	3,607,822	128	44100	3:45
	MP3's\Jimi Hendrix - Castles Made Of Sand.mp3	2,679,799	128	44100	2:47
	MP3's\Jimi Hendrix - Midnight.mp3	5,312,248	128	44100	5:32
	MP3's\Jimi Hendrix - Sweet Angel.mp3	3,765,184	128	44100	3:55
	MP3's\Jimi Hendrix - The Wind Cries Mary.mp3	3,203,367	128	44100	3:20
	MP3's\Joe Cocker - With a Little Help From My Friends....	4,825,896	128	44100	5:01
	MP3's\Joe Walsh - Funk #49.mp3	3,766,648	128	44100	3:55
	MP3's\John Fogerty - Centerfield.mp3	3,688,199	128	44100	3:50
	MP3's\Journey - Any Way You Want It.mp3	4,080,957	160	44100	3:24
	MP3's\Journey - Open Arms.mp3	3,168,868	128	44100	3:18
	MP3's\Kingmen - Louie Louie.mp3	2,658,059	128	44100	2:46
	MP3's\Kinks - Lola.mp3	3,932,840	128	44100	4:05
	MP3's\Kinks - You Really Got Me.mp3	2,156,435	128	44100	2:14
	MP3's\Kool and the Gang - Jungle Boogie.mp3	2,948,956	128	44100	3:04
	MP3's\Led Zeppelin - D'Yer Maker.mp3	4,206,759	128	44100	4:22
	MP3's\Led Zeppelin - Over The Hills And Far Away.mp3	4,633,496	128	44100	4:49
	MP3's\Led Zeppelin - Ramble On.mp3	13,936	128	44100	0:00

Add Computer Number Shared: 337 Total Megs Shared: 1,178 Ping Time Average:

Remove Computer Download Selected Song(s) Refresh Visible List Refresh Ping Time

Online (mathowie): Sharing 141 Songs. Currently 125,008 songs (505 gigabytes) available in 864 libraries.

# Sean Parker (Facebook)



# PICI: Parker Institute for Cancer Immunotherapy

## Here's why Napster billionaire Sean Parker just invested \$250 million in a new kind of cancer treatment

Lydia Ramsey Pflanzer Apr 13, 2016, 12:53 PM EDT

Another big name has joined the research effort for a new kind of cancer treatment called immunotherapy, which uses the immune system to fight cancer cells.



Sean Parker Spencer Platt/Getty Images

Sean Parker, the internet billionaire who co-founded Napster and is a former Facebook president, gave \$250 million on Wednesday to launch the Parker Institute that will help with the research and development of cancer immunotherapy treatments.



Reuters

**Sean Parker**

Connecting Cancer Research



Parker, formerly of Napster and Facebook, knows the power of networking, so he was surprised when he found that many cancer researchers didn't collaborate. With the Parker Institute for Cancer Immunotherapy, he supports and connects the world's best cancer doctors to speed new treatments. Its work led to the first approved gene immunotherapy for blood cancers, as well as a Nobel Prize this year for immune-based cancer drugs (*see Allison and Honjo*).  
—Alice Park

# *On the Case at Mount Sinai, It's Dr. Data*

By Steve Lohr

March 7, 2015

Share full article



Jeffrey Hammerbacher uses his finance and tech experience to understand diseases.  
Sam Hodgson for The New York Times

Jeffrey Hammerbacher is a number cruncher — a Harvard math major who went from a job as a Wall Street quant to a key role at Facebook to a founder of a successful data start-up.

But five years ago, he was given a diagnosis of bipolar disorder, a crisis that fueled in him a fierce curiosity in medicine — about how the body and brain work and why they sometimes fail. The more he read and talked to experts, the more he became convinced that medicine needed people like him: skilled practitioners of data science who could guide scientific discovery and decision-making.

# Hammerlab



His group's objective is to alter how doctors treat patients someday. For example, Mount Sinai medical researchers have done promising work on personalized cancer treatments. It involves the genetic sequencing of a patient's healthy cells and cancer tumor. Once the misbehaving gene cluster is identified and analyzed, it is targeted with tailored therapies, drugs or vaccines that stimulate the body's defenses.

# PICI's TESLA

## What We're Doing

### Tumor Neoantigen Selection Alliance (TESLA)

Improving cancer vaccines and cell therapies for patients through AI.

PICI and the Cancer Research Institute (CRI) did just that when they launched the Tumor Neoantigen Selection Alliance in fall 2016. This global bioinformatics consortium includes scientists from 36+ of the leading neoantigen research groups in academia, nonprofit and industry.

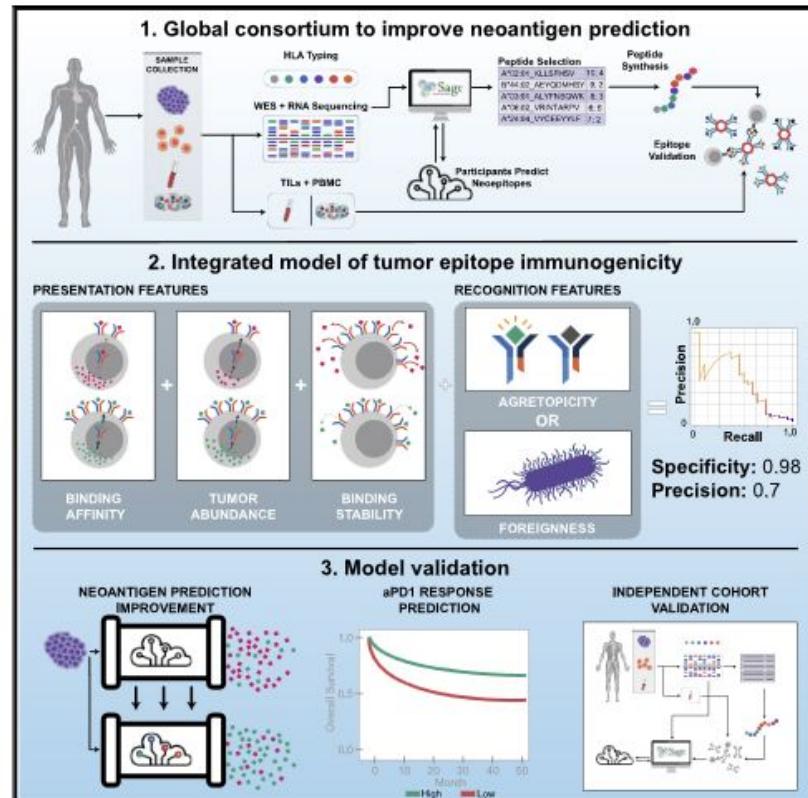
Through predictive algorithms and machine learning, the group is sniffing out which cancer neoantigens encoded in DNA can be recognized and stimulate an immune response.

Finding the right predictive algorithms for targeting neoantigens could allow scientists to create more cancer immunotherapy treatments tailored to each patient.

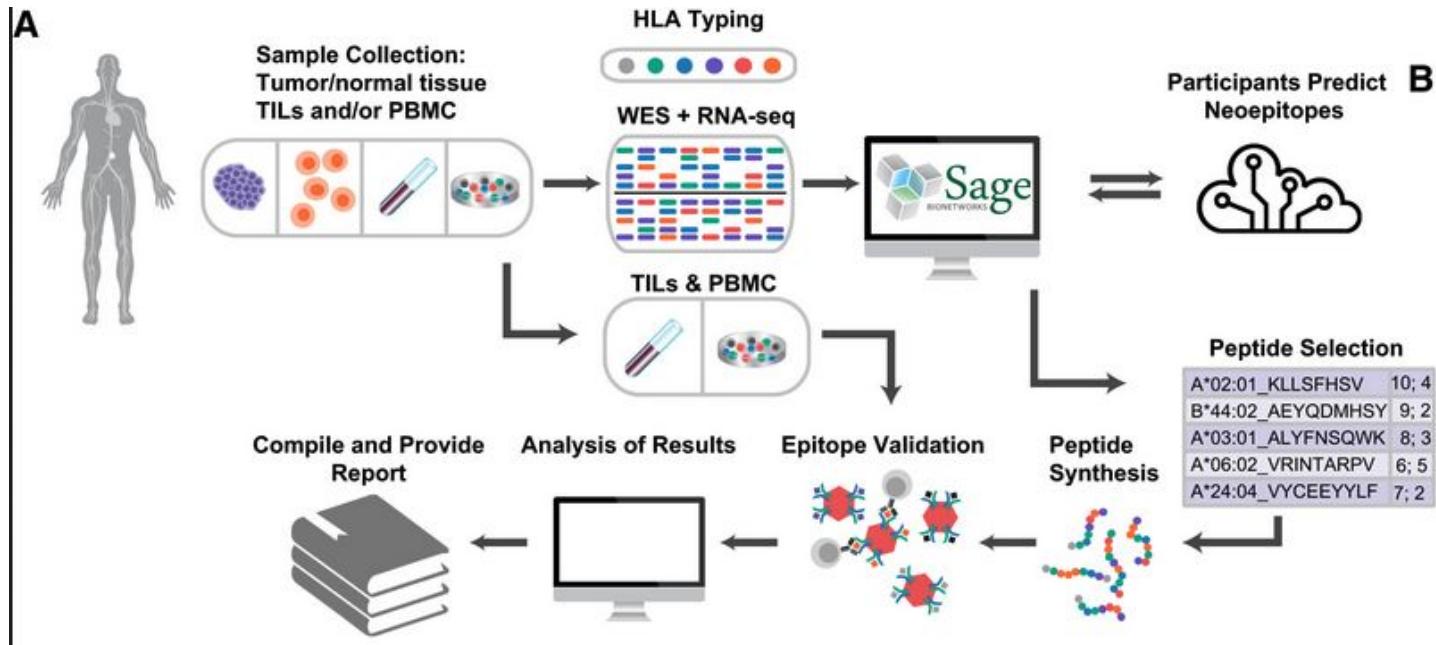
# *The Paper*

# Key Parameters of Tumor Epitope Immunogenicity Revealed Through a Consortium Approach Improve Neoantigen Prediction

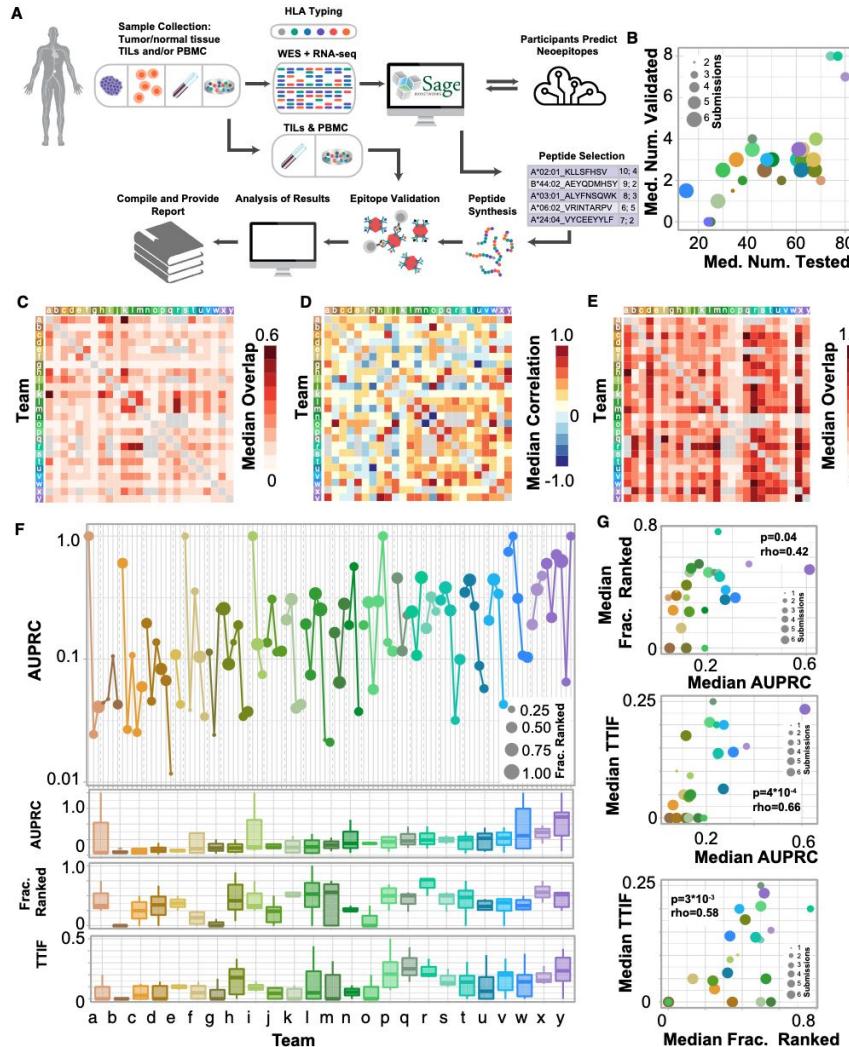
Daniel K. Wells,<sup>1,24,26,\*</sup> Marit M. van Buuren,<sup>2,3,24</sup> Kristen K. Dang,<sup>4,24</sup> Vanessa M. Hubbard-Lucey,<sup>5</sup> Kathleen C.F. Sheehan,<sup>6,7</sup> Katie M. Campbell,<sup>8</sup> Andrew Lamb,<sup>4</sup> Jeffrey P. Ward,<sup>9</sup> John Sidney,<sup>10</sup> Ana B. Blazquez,<sup>11</sup> Andrew J. Rech,<sup>1,12</sup> Jesse M. Zaretsky,<sup>8</sup> Begonya Comin-Anduix,<sup>1,13</sup> Alphonsus H.C. Ng,<sup>14</sup> William Chou,<sup>15</sup> Thomas V. Yu,<sup>4</sup> Hira Rizvi,<sup>16</sup> Jia M. Chen,<sup>8</sup> Patrice Manning,<sup>1</sup> Gabriela M. Steiner,<sup>1</sup> Xengie C. Doan,<sup>4</sup> The Tumor Neoantigen Selection Alliance, Taha Merghoub,<sup>1,17,18</sup> Justin Guinney,<sup>4,19</sup> Adam Kolum,<sup>1,5</sup> Cheryl Selinsky,<sup>1</sup> Antoni Ribas,<sup>1,8,9</sup> Matthew D. Hellmann,<sup>1,16,17,18</sup> Nir Hacohen,<sup>20,21</sup> Alessandro Sette,<sup>11,22</sup> James R. Heath,<sup>1,14</sup> Nina Bhardwaj,<sup>1,11</sup> Fred Ramsdell,<sup>1</sup> Robert D. Schreiber,<sup>1,6,7,25</sup> Ton N. Schumacher,<sup>23,25</sup> Pia Kvistborg,<sup>2,25</sup> and Nadine A. DeFranoux<sup>1,25,\*</sup>



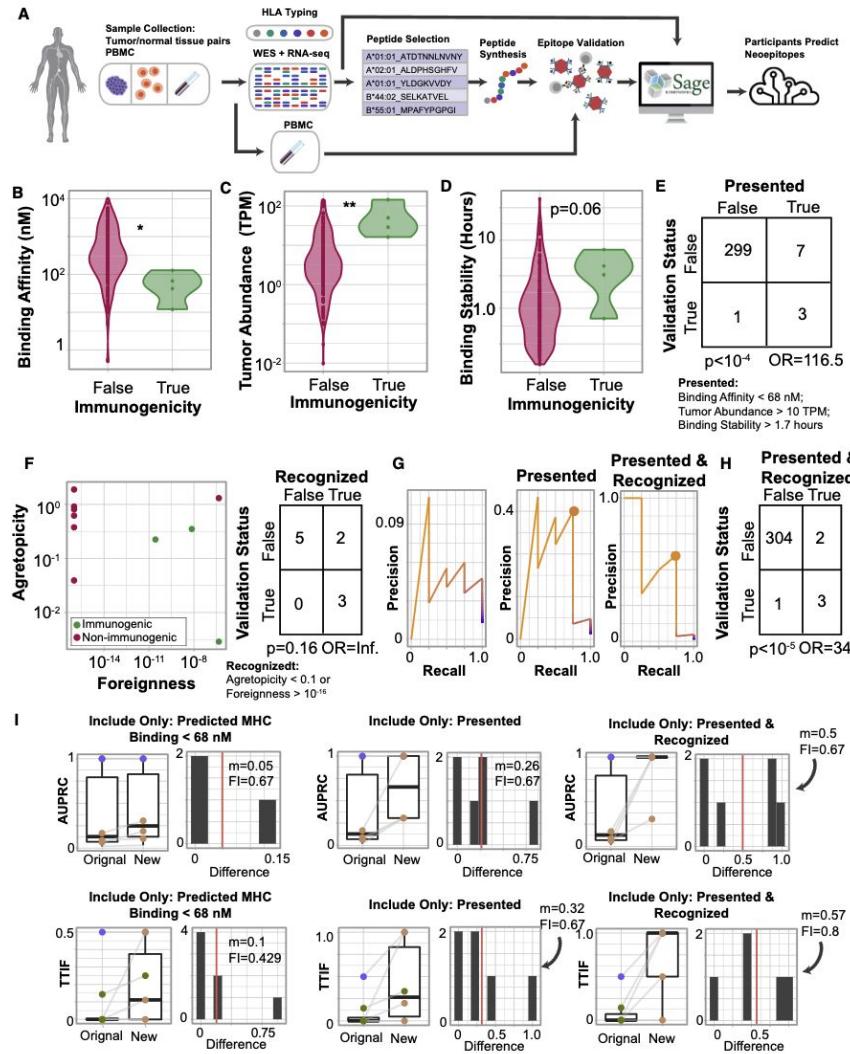
# Overall concept



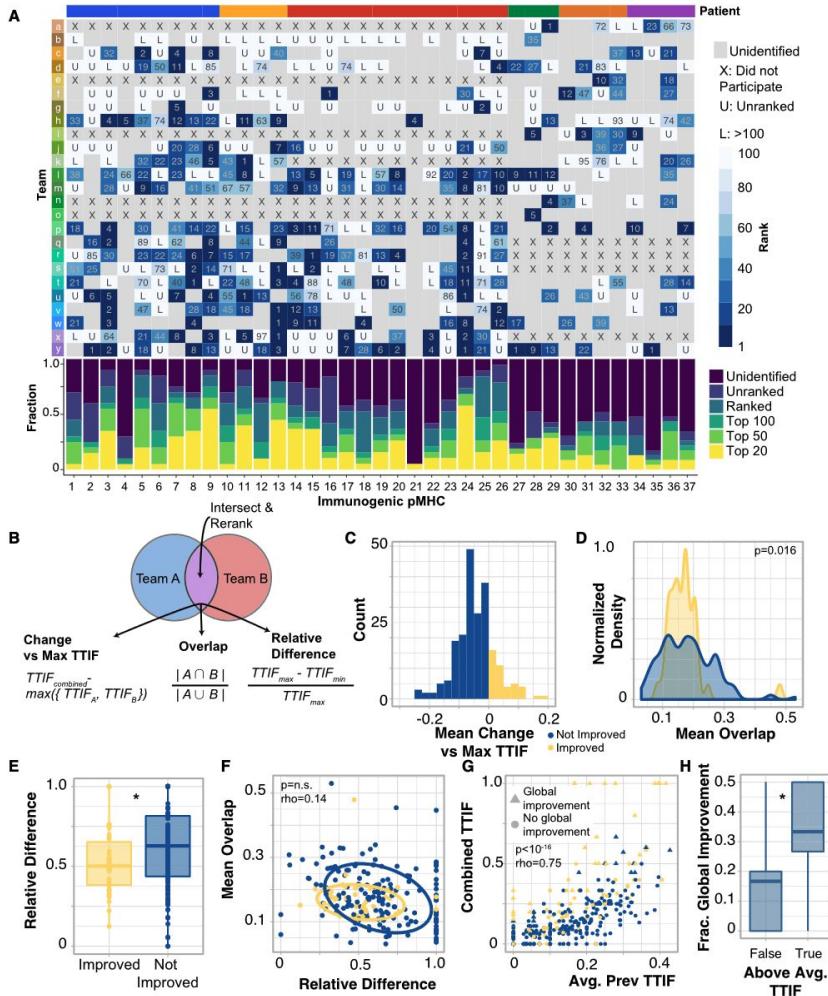
# Let's appreciate the figures



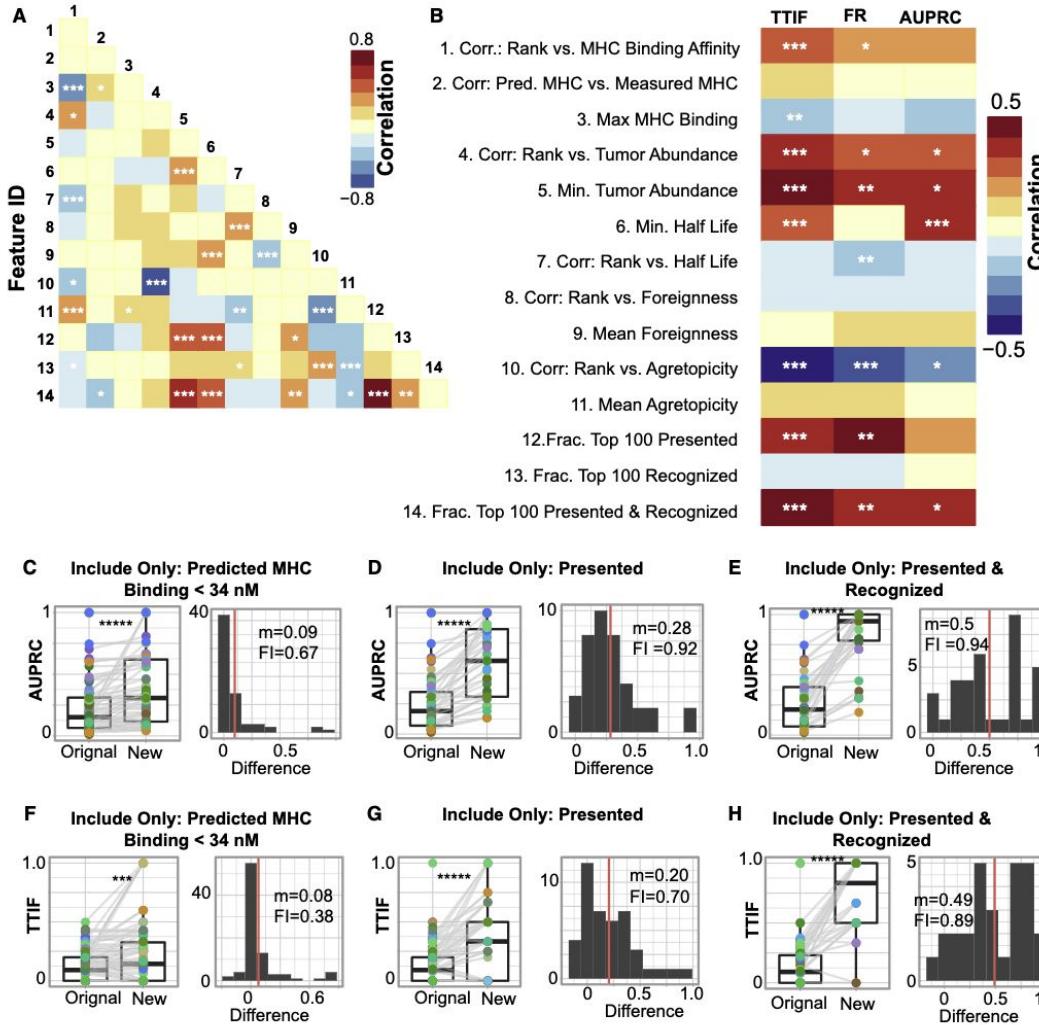
# Let's appreciate the figures



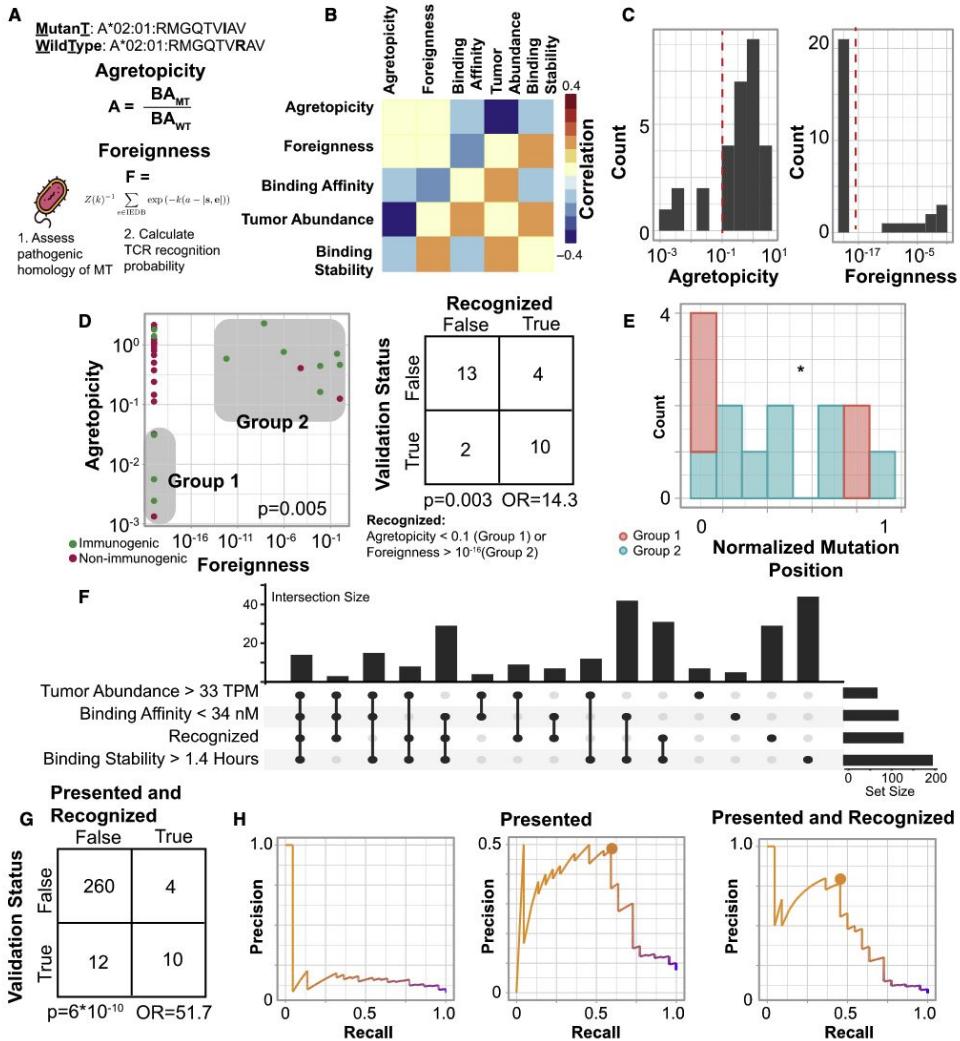
# Let's appreciate the figures



# Let's appreciate the figures



# Let's appreciate the figures



# Data: the “TESLA” cohort

## TESLA Participation and Immunogenicity Validation

### Results

Samples from six subjects were analyzed: 3 from subjects with metastatic melanoma and 3 from subjects with non-small cell lung cancer (NSCLC) (Table S3).

The study was conducted with samples from subjects with metastatic melanoma or non-small cell lung cancer (NSCLC), previously collected and stored at UCLA or MSKCC respectively, and for which adequate matched pre-treatment control/tumor biopsy were available along with baseline and/or on-treatment matched peripheral blood mononuclear cells (PBMCs; melanoma) or tumor lysates (NSCLC). Collection and genomic

1	Melanoma	Male	Metastatic	Epithelial	Skin	Pembrolizumab	-28	Partial Response
2	Melanoma	Male	Metastatic	Epithelial	Skin	Ipi + Nivo	-25	Partial Response
3	Melanoma	Male	Metastatic	Epithelial	Skin	Nivolumab	-11	Complete Response
10	NSCLC	Male	Primary	Epithelial	Lung	N/A	N/A	N/A
12	NSCLC	Male	Primary	Epithelial	Lung	N/A	N/A	N/A
16	NSCLC	Female	Primary	Epithelial	Lung	N/A	N/A	N/A

# Data: the validation cohort

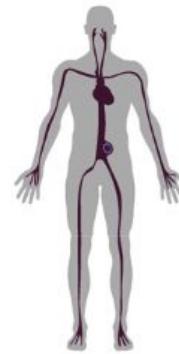
## Validation in an Independent Cohort

To assess whether the results identified herein are robust beyond our initial cohort, we identified an independent cohort of 3 melanoma patients with whole exome tumor-normal DNA sequencing and tumor RNA sequencing from tissue samples and for which 310 pMHC had been tested for immunogenicity using a tetramer-based assay in patient-matched PBMC samples ([Figure S7](#); [Table S7](#)). Of those pMHC tested, 4 were found to be immunogenic ([Figure 7A](#)). In this cohort, immunogenic

4	Melanoma	Female	Primary	Epithelial	Skin	N/A	N/A	N/A
8	Melanoma	Female	Primary	Epithelial	Skin	Ipilimumab	Unknown	Progressive Disease
9	Melanoma	Unknown	Primary	Epithelial	Skin	N/A	N/A	N/A

# Data: checkpoint blockade cohort

A 55 Melanoma Patients



<b>Primary Melanoma</b>	Cutaneous	55 (100%)
<b>Treatment</b>		
Nivolumab		20 (36%)
Pembrolizumab		35 (64%)
<b>Previous IpiLimumab</b>		
No		55 (100%)
<b>Sequencing</b>		
Has WES		55 (100%)
Has RNA-Seq		55 (100%)

**Calculate**

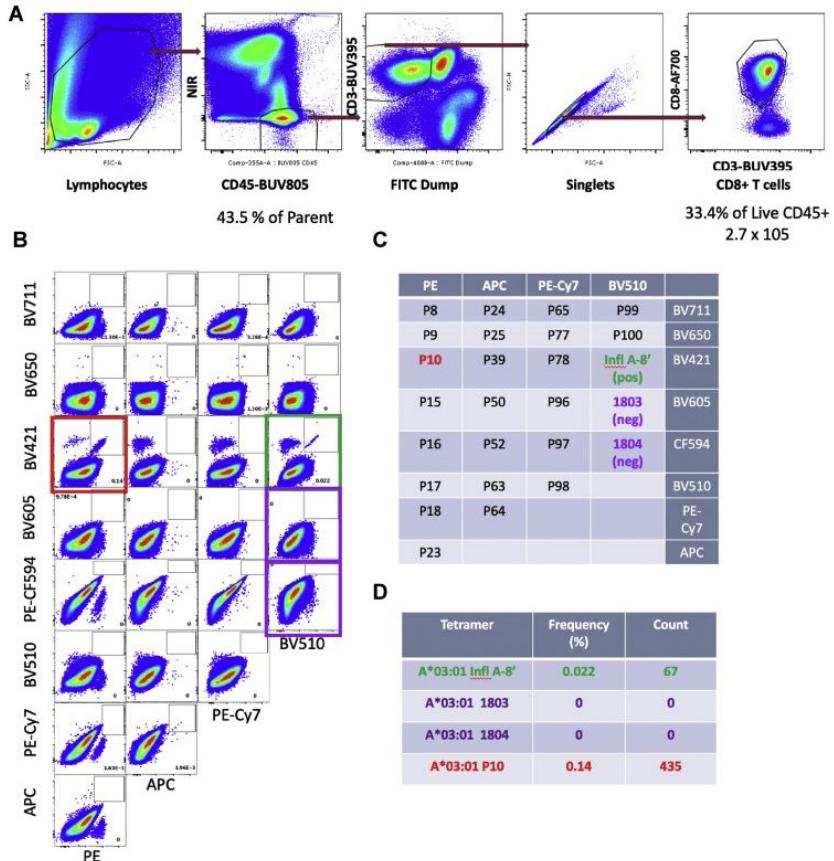
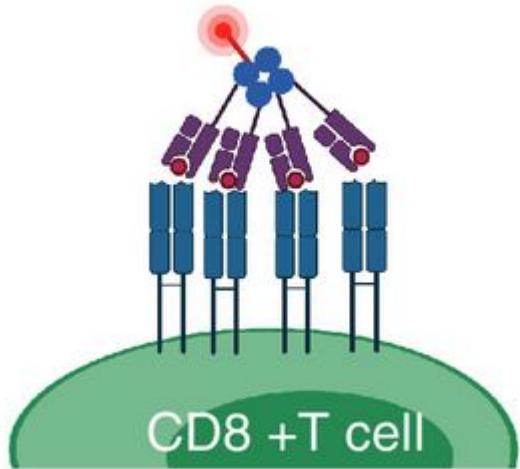
- Classical Neoantigen Burden
- Prestend Neoantigen Abundance
- Presented and Recognized Neoantigen Abundance

## Integrative molecular and clinical modeling of clinical outcomes to PD1 blockade in patients with metastatic melanoma

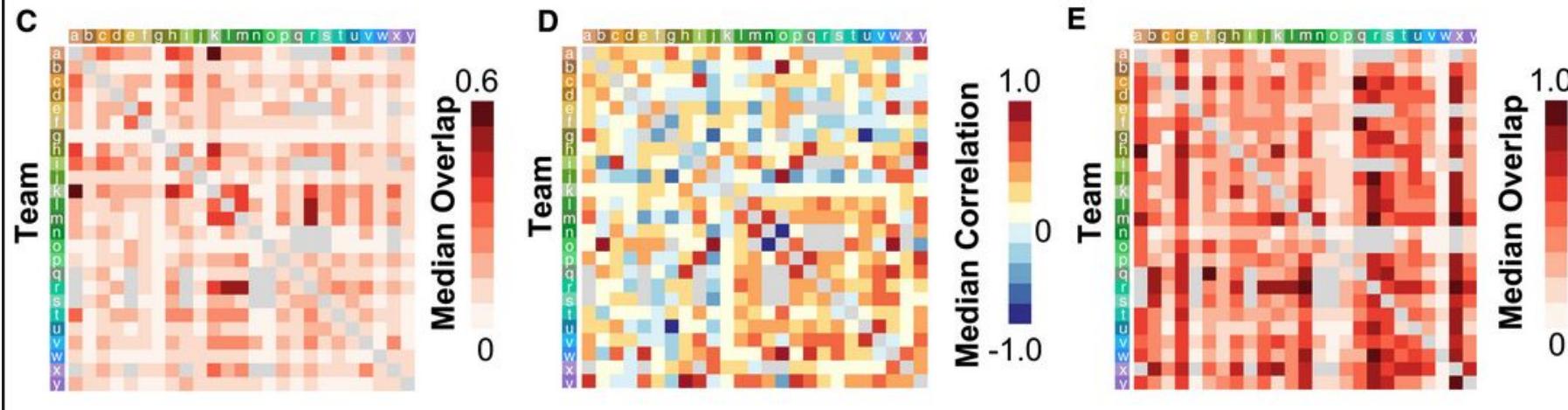
David Liu<sup>1,2,25</sup>, Bastian Schilling<sup>3,4,5,25</sup>, Derek Liu<sup>1,2,6</sup>, Antje Sucker<sup>4,5</sup>, Elisabeth Livingstone<sup>4,5</sup>,

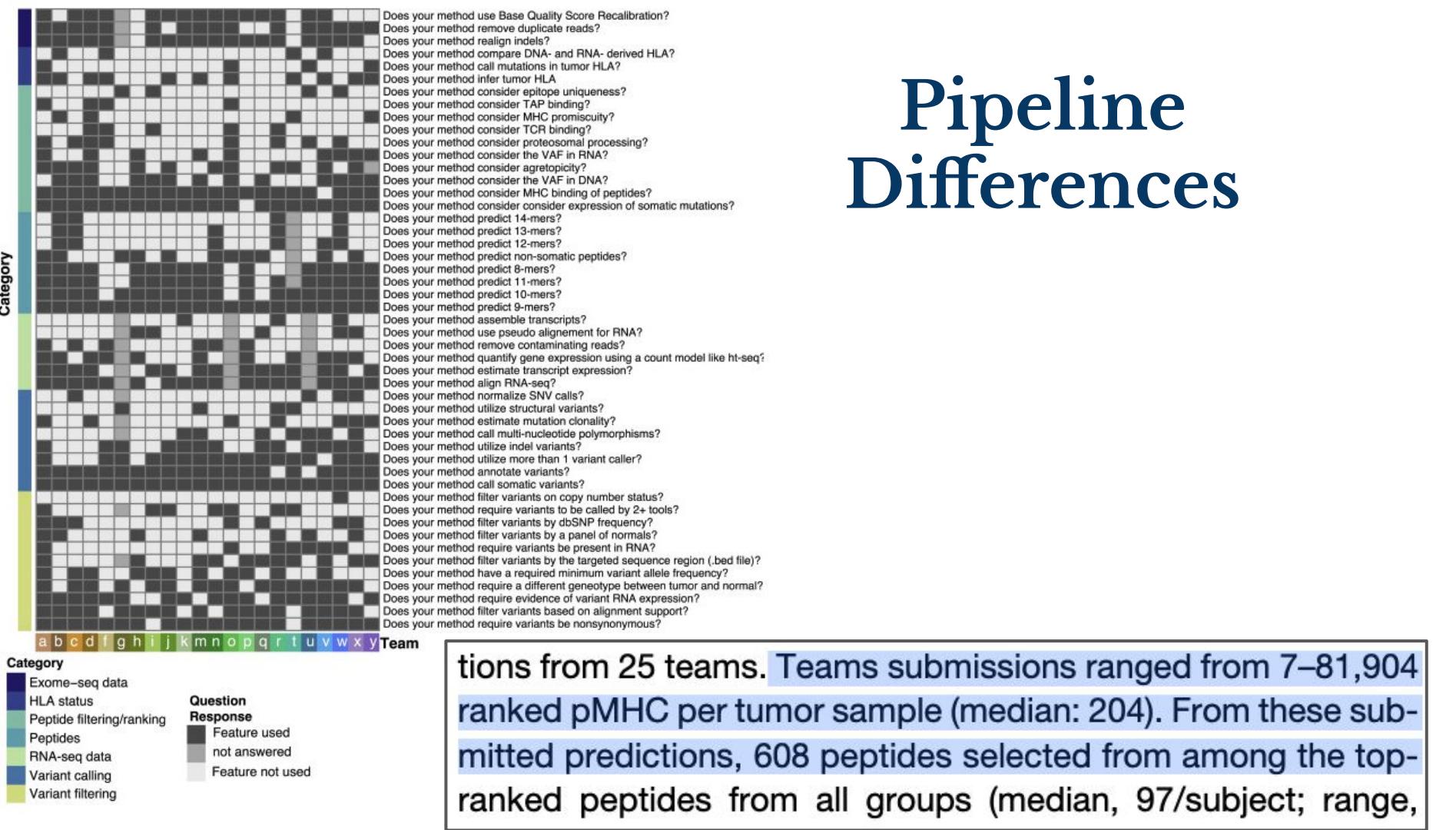
# How to validate neoantigens?

(A) pMHC class I tetramer



# Meet The Teams





# Which peptides get validated?

## Process for Choosing Neoepitopes to Test in Validation Assays

For each sample used in TESLA there is capacity to test between 100-200 neoepitopes for immunogenicity. Given that each participating team submitted 5-100,000 peptides for each sample, a sub-set of peptides for validation had to be selected. To do this, we focused on ensuring that each participant would have roughly the same number and quality (ranking) of peptides validated (“fairness”) while also making sure that, if there are differences between the algorithms, we would be able to detect them (“distinguishability”). These principles were constrained by the fact that some peptides are too hydrophobic (or otherwise hard to make/use) to be used in our assays, as well as the fact that not every neoepitope can be tested in every assay due to HLA restriction requirements. In practice, we used the following guidelines to choose peptides:

- To the extent possible, each participant had their top 5 neoepitopes selected, taking into account the MHC for which they are restricted
- Additionally, neoepitopes that were the most recurrently ranked in the top 50 by all participants were also selected taking into consideration the amount of biological material available for testing, the demand for each assay and the MHC constraints for all four assays.

50 was chosen as a cutoff to reflect the upper bound on the current number of epitopes that can be included in a personalized therapeutic approach.

# 37/608 peptides immunogenic

predictions from 25 teams. Teams submissions ranged from 7–81,904 ranked pMHC per tumor sample (median: 204). From these submitted predictions, 608 peptides selected from among the top-ranked peptides from all groups (median, 97/subject; range, 73–144, see [Table S4](#) for complete list of tested peptides) were tested for immunogenicity by pMHC multimer-based assays and 37 (6%) of those were found to be immunogenic, a validation rate similar to what has previously been reported ([Yadav et al., 2014](#)). Each TESLA team had a median of 51 of their submitted peptides tested of which on median 3 (6%) were immunogenic ([Figure 1B](#)).

# How did the teams do?

## Area Under the Precision Recall Curve (AUPRC)

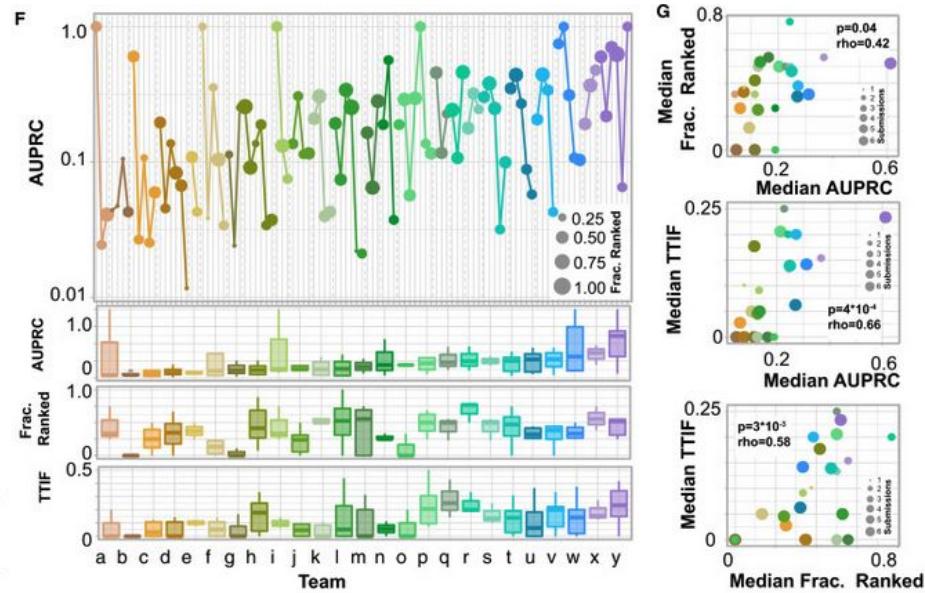
AUPRC was calculated using the 'pr.curve' function in the PRROC package ([Grau et al., 2015](#)) with default parameters.

## Fraction Ranked (FR)

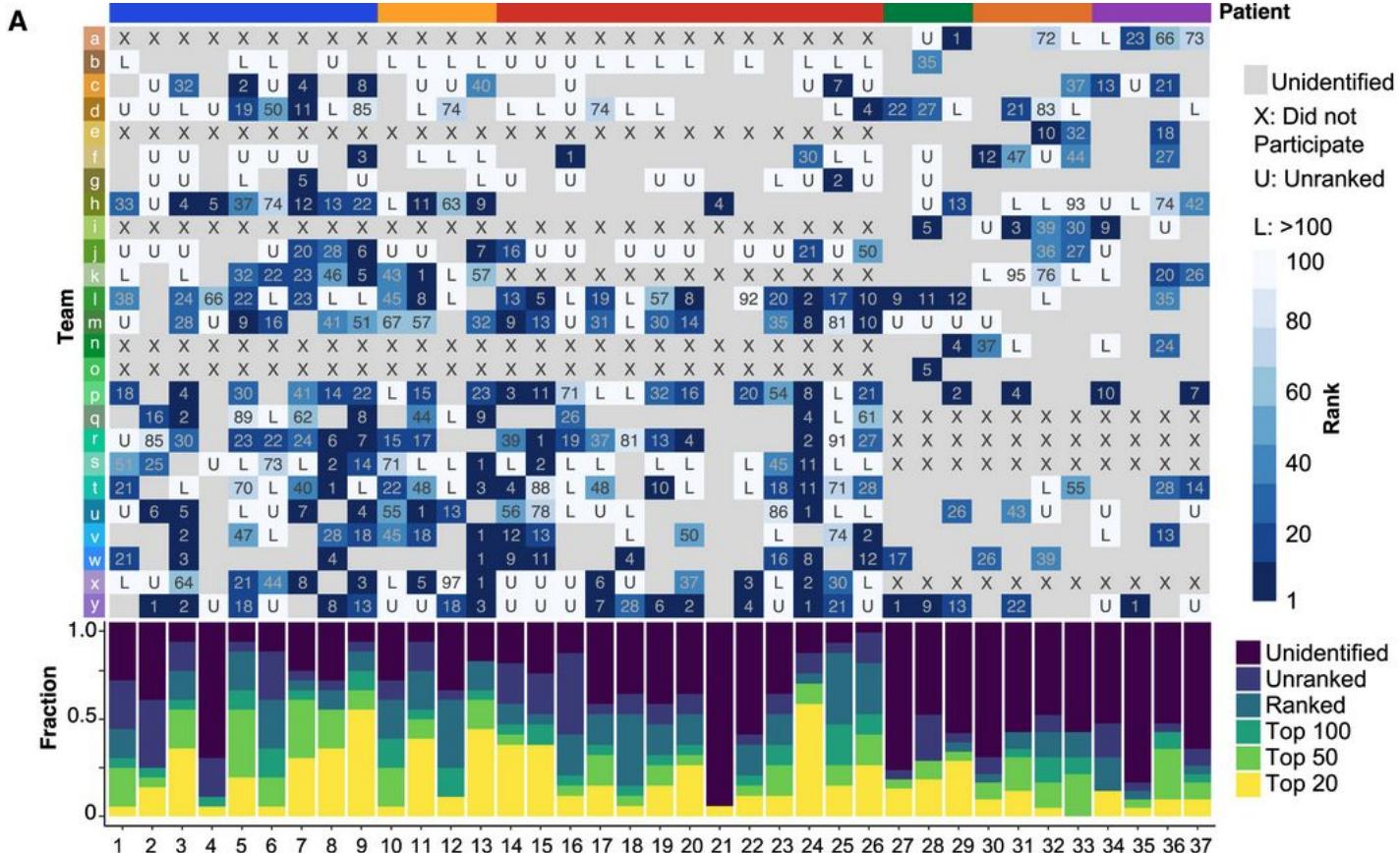
Fraction ranked is calculated as the fraction of all peptides with detected immunogenicity for a particular subject that were included in the top 100 ranked pMHC by a participant. A cutoff of 100 peptides was chosen to calculate FR to normalize for differing numbers of pMHC submitted by each team. The value of 100 was chosen in particular to ensure consistency with other analyses and to be reflective of the total number of epitopes which might be considered for inclusion in a personalized therapeutic.

## Top-20 Immunogenic Fraction (TTIF)

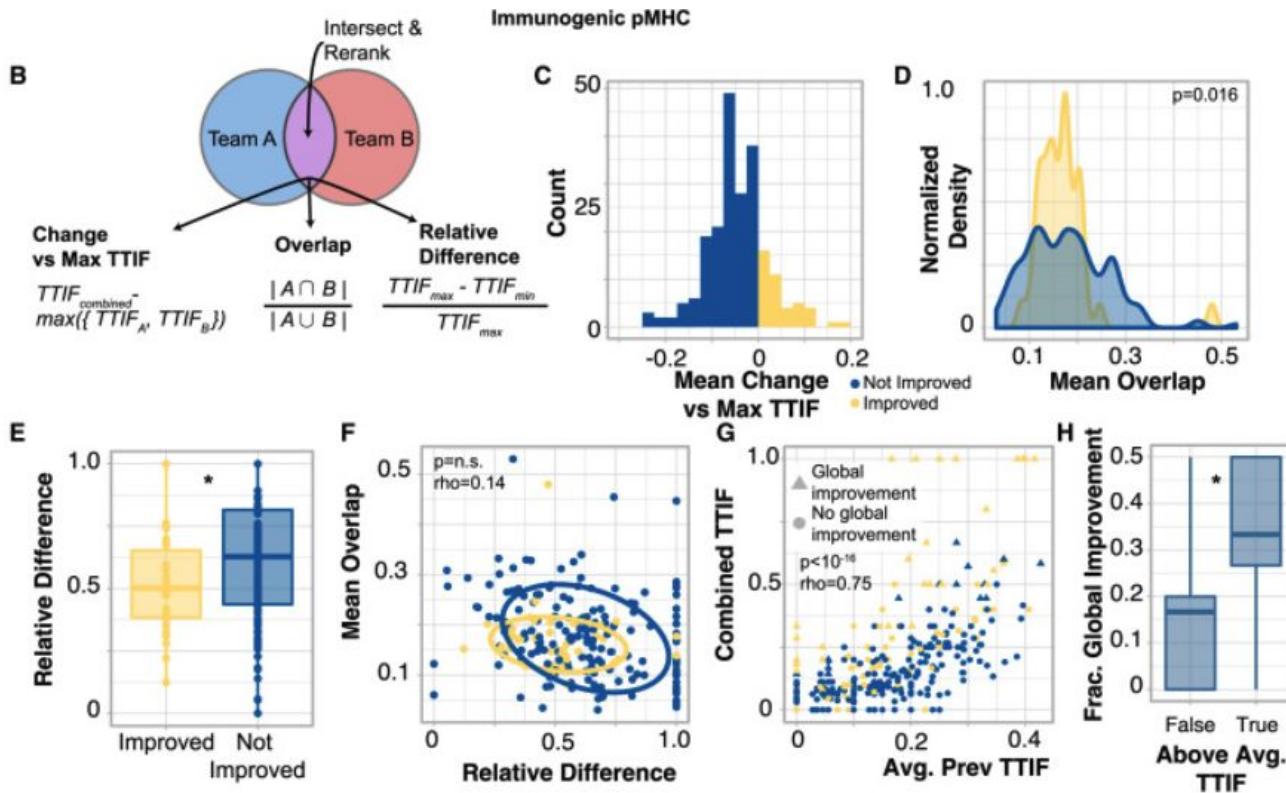
Top-20 immunogenic fraction for a particular subject and participant is calculated as the ratio of the top-20 ranked pMHC with detected immunogenicity to the total number of top-20 pMHC which were tested for immunogenicity. As TTIF was designed to assess the therapeutic efficacy of a specific peptide set, the threshold of 20 was selected since therapeutic vaccine platforms reported to date have included ~20 neoepitopes ([Ott et al., 2017; Sahin et al., 2017](#)).



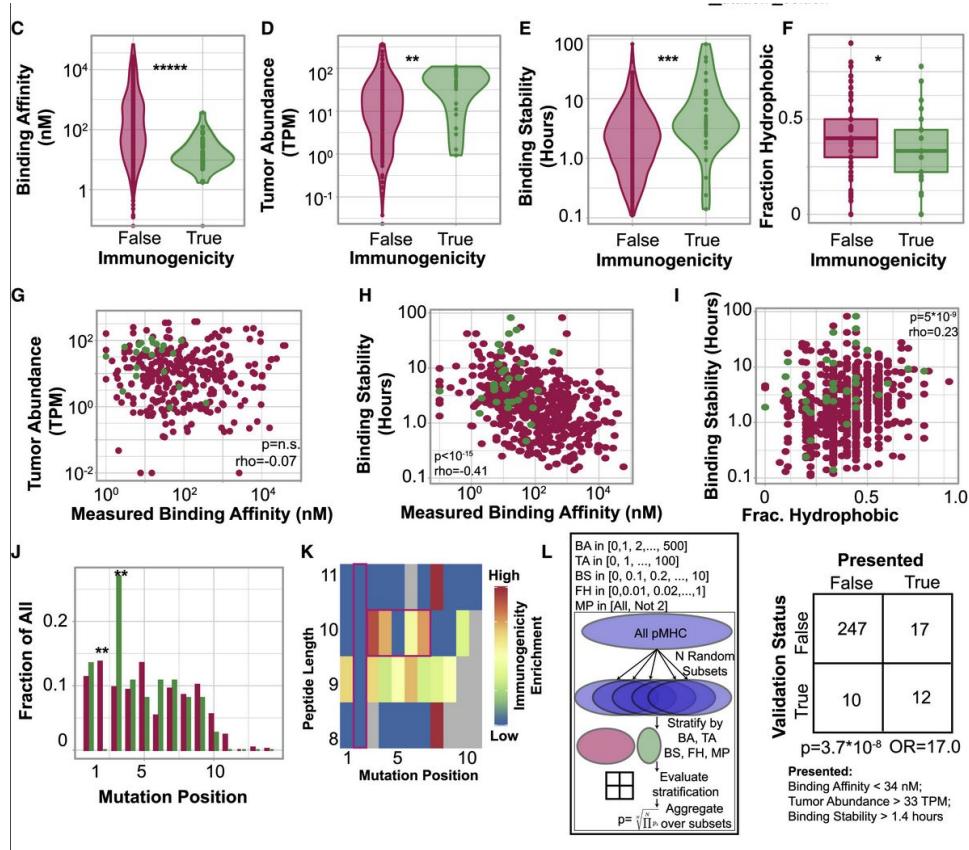
# No pipeline gets more than 20 right



# Let's combine pipelines?

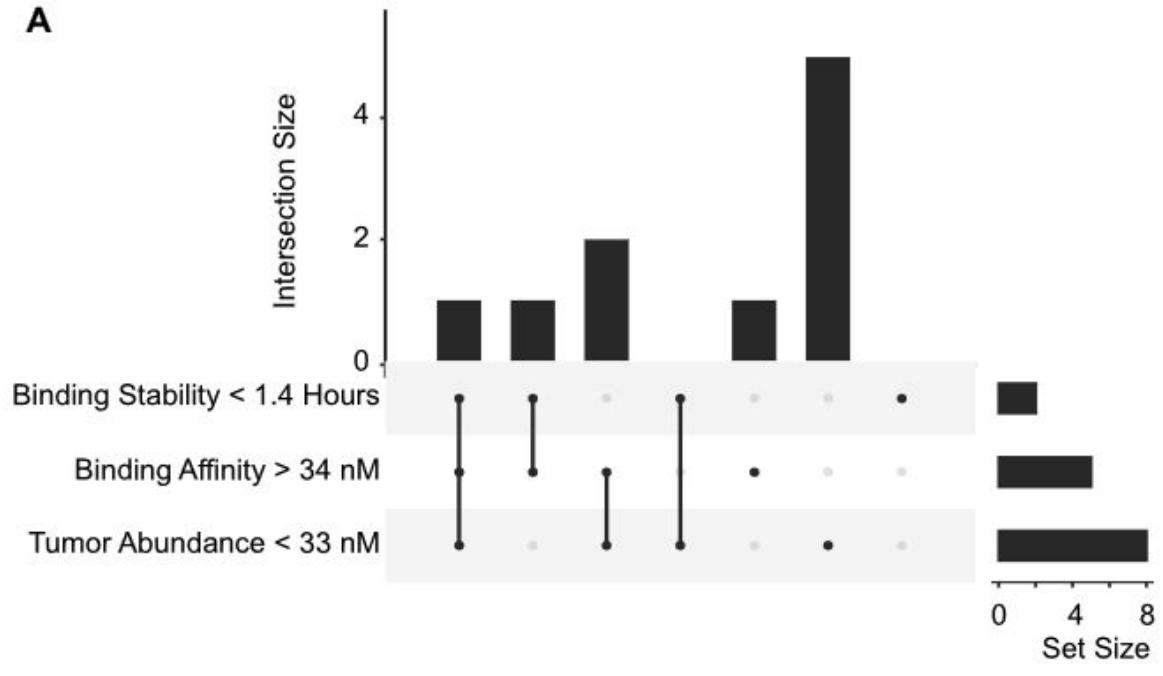


# Let's stratify by immunogenicity

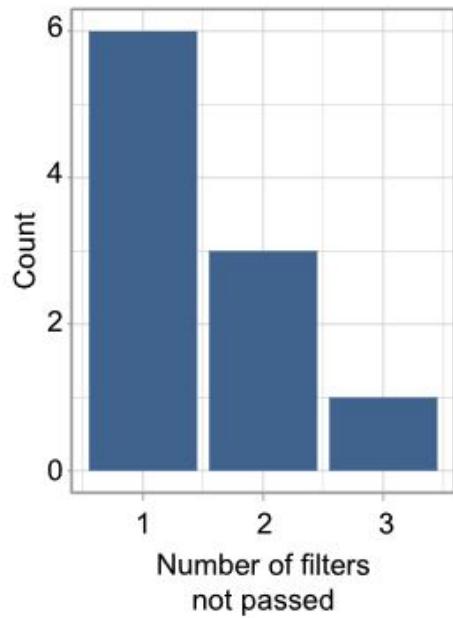


# A simple presentation rule!

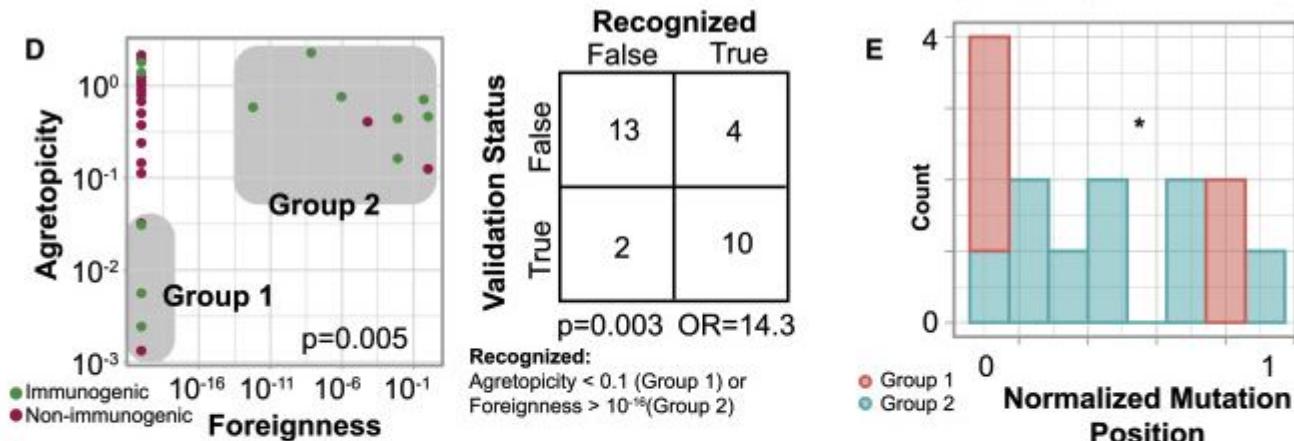
A



B

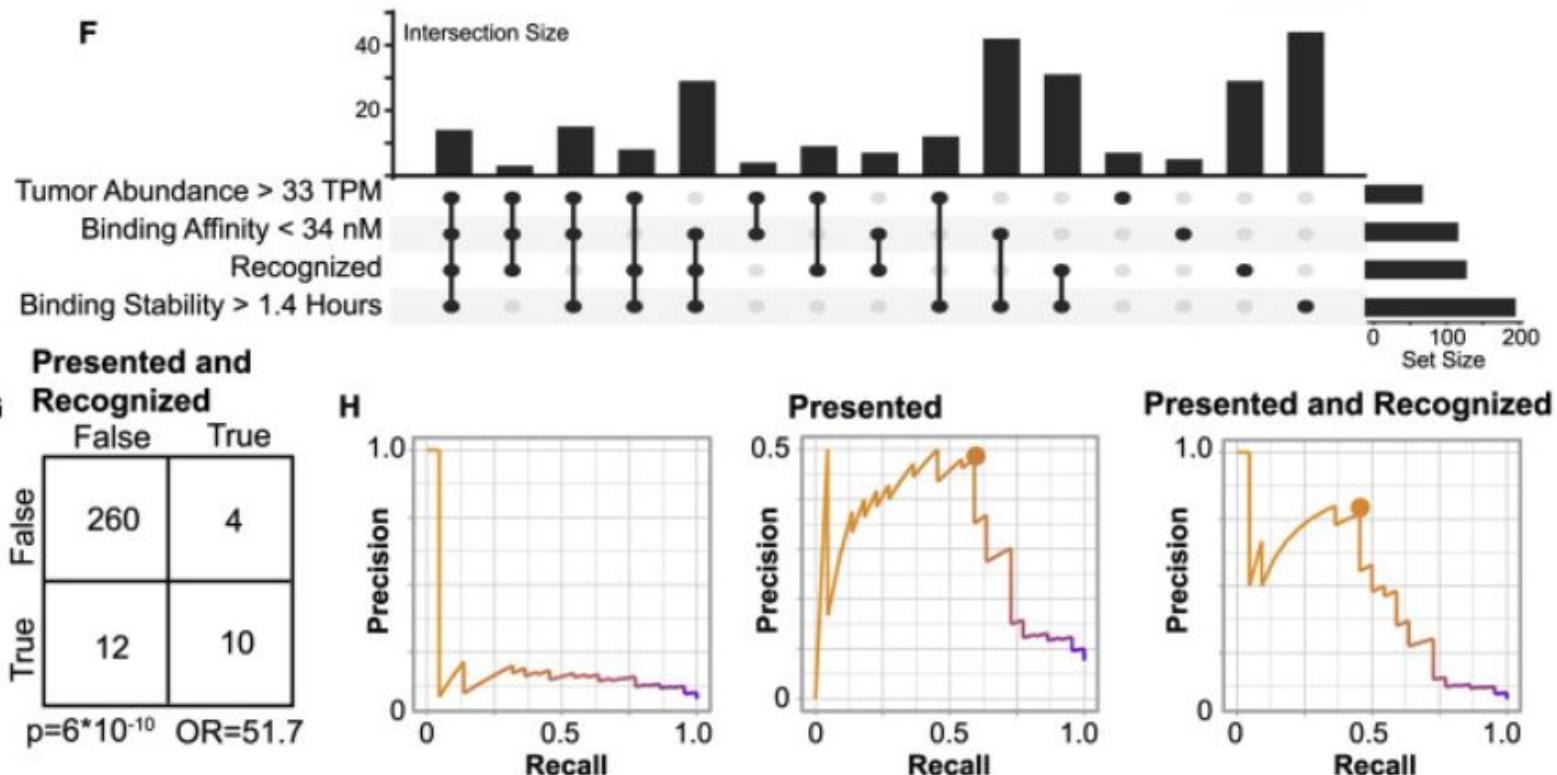


# ...and for recognition...



associated parameters (Figure 4B). Furthermore, we observed that the majority of these pMHC had agretopicity greater than 0.1 and foreignness less than  $10^{-16}$  (Figure 4C), while smaller subsets were found to have values that were orders of magnitude smaller (agretopicity, “group 1”) or larger (foreignness, “group 2”). These subsets of low agretopicity or high foreignness pMHC were mutually exclusive ( $p=0.005$ , binomial test). We term the presence of either low agretopicity or high foreignness as “recognition” and term any peptide in either group 1 or group 2 as “recognized.” Importantly, recognition is defined here as purely a property of a peptide, and is not *a priori* associated with the immunogenicity of that peptide in a particular patient.

# Works on the validation set



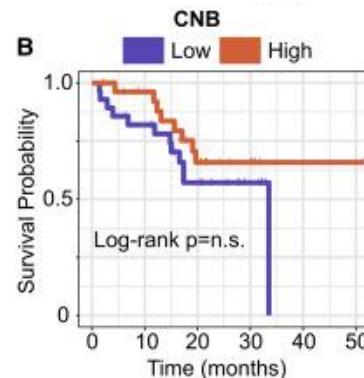
# Predicts checkpoint blockade response!

## Predicted Neoantigen Abundance

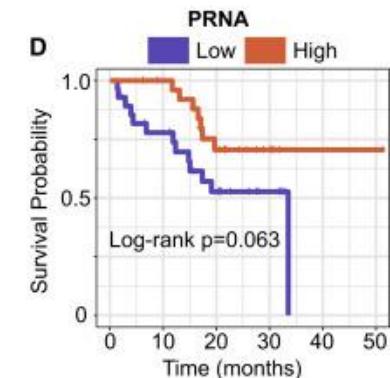
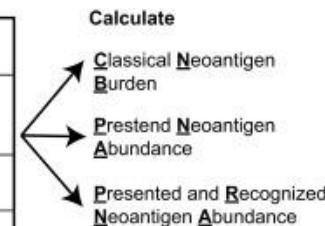
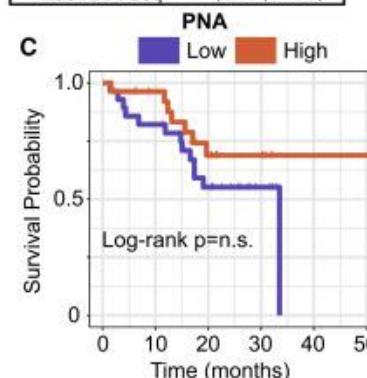
Potential immunogenic peptides were generated using a previously generated set of mutation calls (Liu et al., 2019) and predicted MHC binding affinity was assigned using NetMHCpan4.0. Predicted neoantigen abundance was taken as the sum of the normalized transcripts per million (TPM) of the mutations which passed all “presented” filters from Figure 3L (excluding the abundance filter) – specifically, MHC binding affinity stronger than 34 nM and MHC binding stability longer than 1.4 hours, and mutational position not 2.

## Predicted and Recognized Neoantigen Abundance

Potential immunogenic peptides were generated using a previously generated set of mutation calls (Liu et al., 2019) and predicted MHC binding affinity was assigned using NetMHCpan4.0. Predicted and recognized neoantigen abundance was taken as the sum of the normalized transcripts per million (TPM) of the mutations which passed all “presented” features (excluding the abundance filter, identical to predicted neoantigen abundance) and the “recognized” filters from Figure 4D – specifically, peptide agretopicity less than 0.1 or peptide foreignness greater than  $10^{-16}$ .



<b>Primary Melanoma</b>	
Cutaneous	55 (100%)
<b>Treatment</b>	
Nivolumab	20 (36%)
Pembrolizumab	35 (64%)
<b>Previous Ipilimumab</b>	
No	55 (100%)
<b>Sequencing</b>	
Has WES	55 (100%)
Has RNA-Seq	55 (100%)



 *Fin*