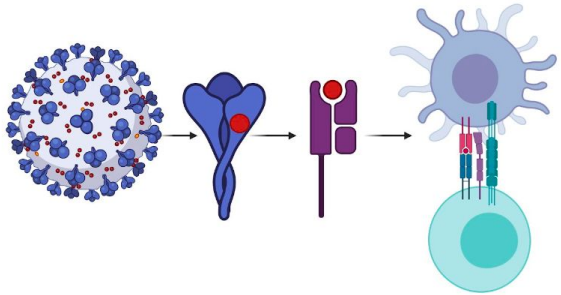
Precision vaccine design from cancer to SARS-CoV-2 and back



Alex Rubinsteyn
September 14th, 2020
LCCC Faculty Lunch

UNC
SCHOOL OF MEDICINE

Overview

- Cancer immunotherapy & personalized cancer vaccines
- Personalized cancer vaccine clinical trials at Mount Sinai
- OpenVax pipeline for selecting vaccines
- Do personalized cancer vaccines work?
- Peptide vaccines for SARS-CoV-2

Flavors of cancer immunotherapy

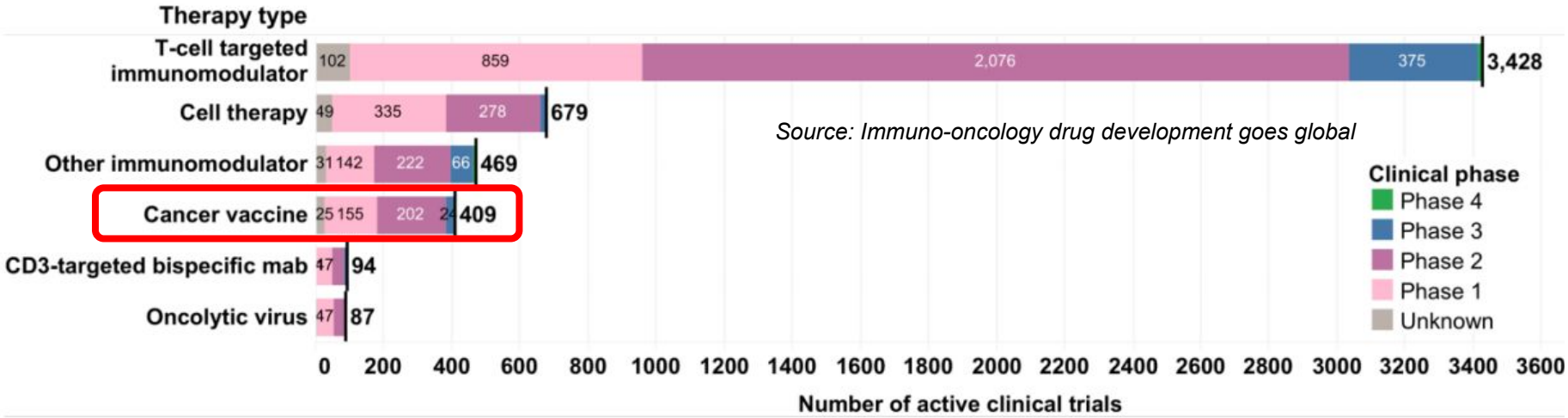
Checkpoint blockade	Cellular therapies	Vaccines
Disinhibit T-cells. Antigens responsible for tumor clearance typically unknown. <u>Success stories:</u> • αCTLA-4 (ipi) • αPD-1 (pembro, nivo, cemi) • αPD-L1 (atezo, ave, durva)	Ex-vivo expansion of patient T-cells after receptor engineering and/or selection. <u>Success stories:</u> • CAR T-cells for B-cell malignancies (CD19, CD20, CD22, BCMA)	Therapeutic vaccines against specific tumor antigens, including patient-specific mutated tumor antigens. <u>Success stories:</u> • ??? • Hints of efficacy in neoantigen vaccine trials

Shared antigen vaccines unsuccessful

Cancer type	Vaccine	Total patients	Patients responding
Melanoma	Tyrosinase + GMCSF	16	0
Melanoma	Peptides in IFA or on DC	26	3
Melanoma	MART-1 + IL-12	28	2
Prostate	Peptides	10	0
Melanoma	Peptides on PBMC + IL-12	20	2
Breast and prostate	Telomerase	7	0
Cervix	HPV16 E7	17	0
Colorectal	Peptides in IFA	10	0
Multiple	NY-ESO-1	12	0
Multiple	Ras in DETOX adjuvant	15	0
Multiple	Peptides in IFA	14	0
Prostate	Vaccinia-PSA	33	0
Prostate	Vaccinia-PSA	42	0
Colorectal	Vaccinia-CEA	20	0
Colorectal	Vaccinia-CEA and B7-1	18	0
Multiple	Avipox-CEA(IGMCSF)	60	0
Multiple	Avipox-CEA	15	0
Multiple	Vaccinia + avipox-CEA	18	0

Cancer immunotherapy: moving beyond current vaccines

...vaccines are back!

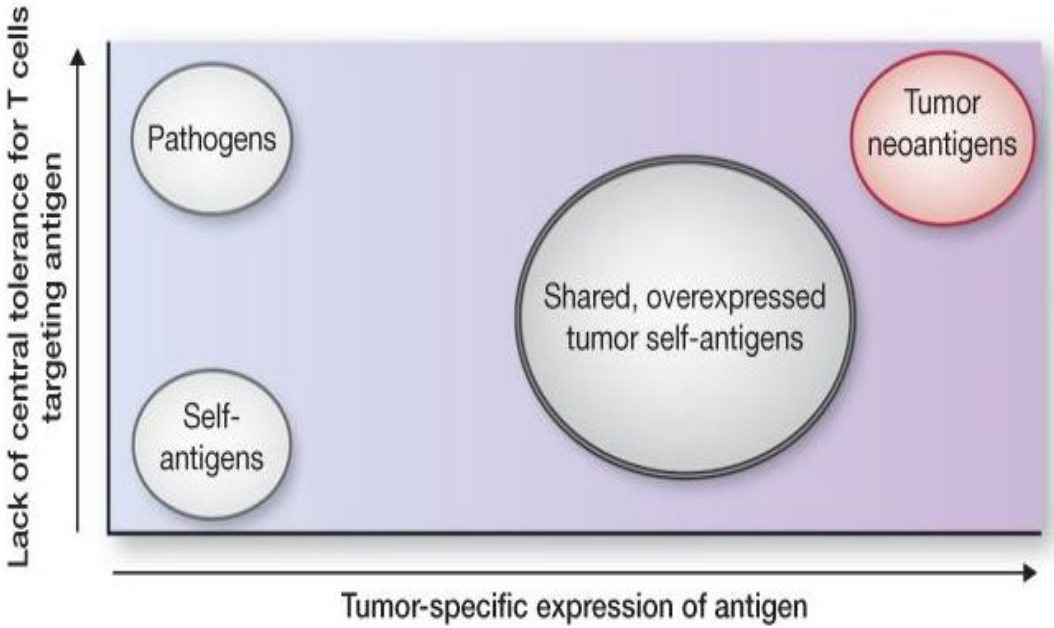


- >$1B invested in cancer vaccine startups, e.g.
  - Gritstone
  - BioNTech
  - Genocea

moderna
genocea BIOSCIENCES
GeneOS
NEON THERAPEUTICS
NEO-PV-01
BIONTECH
IVAC-Mutanome
gritstone
vaccibody
ADURO BIOTECH
agenus
Autosynvax
nousCom
NOUS-100
IMMUNOVATIVE Therapies Ltd.
ADVAXIS IMMUNOTHERAPIES
ADXS-NEO

Neoantigens

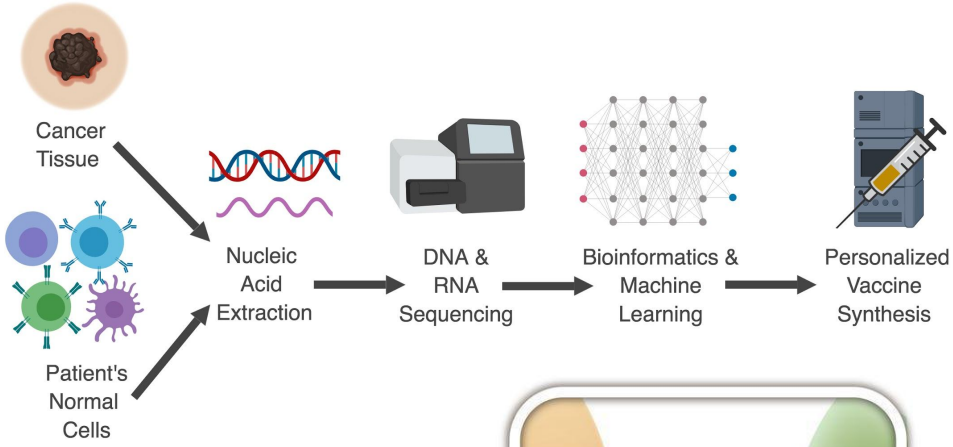
- No overlap with normal
  - mutations
  - abnormal splicing
  - abnormal post-translational modifications
- Unlikely to be shared between patients

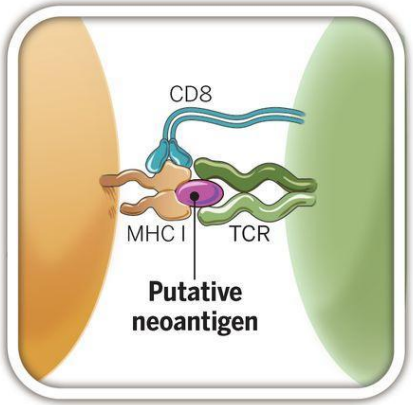


Getting Personal with Neoantigen-Based Therapeutic Cancer Vaccines

Neoantigen vaccination

- **Inputs**
  - Tumor + Normal DNA
  - Tumor RNA
- **Selection**
  - Predict mutated epitopes
  - *and/or*: identify MHC ligands w/ mass spec
  - *and/or*: screen for existing T cell responses
- **Vaccine**
  - Peptides + adjuvant, mRNA, DNA, viral vector, bacterial vector, &c





Schumacher & Schreiber 2015

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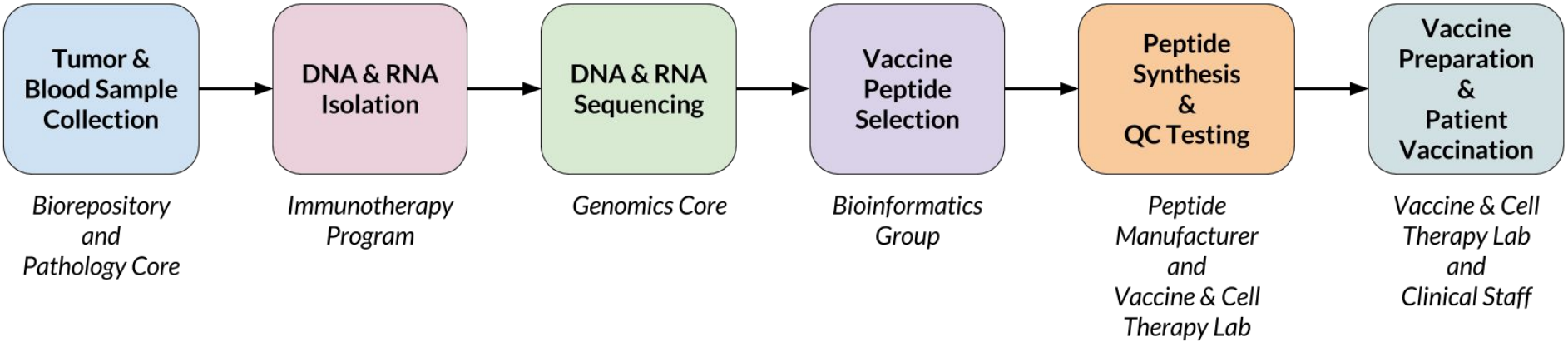
Clinical trials at Mount Sinai

- **PGV001** (*Nina Bhardwaj*)
  - Solid cancers, multiple myeloma
  - Long peptides + poly-ICLC
  - 13 vaccinated
- **PGV for GBM** (*Adilia Hormigo*)
  - + TMZ, Tumor Treating Fields
  - 8 vaccinated
- **PGV for Bladder Cancer** (*Matt Galsky*)
  - + Atezolizumab (anti-PD-L1)
  - 3 vaccinated

Shared design:

- Up to 10 peptides
- Each peptide has up to 25 amino acids
- 10+ injections per trial over 6 months
- Adjuvant: poly-ICLC

Trial logistics



- 1-2 weeks from surgery to sequencing data
- 1 week to run computational pipeline and manually review results
- 6-8 weeks peptide synthesis
- 10 immunizations over 6 months

Overview

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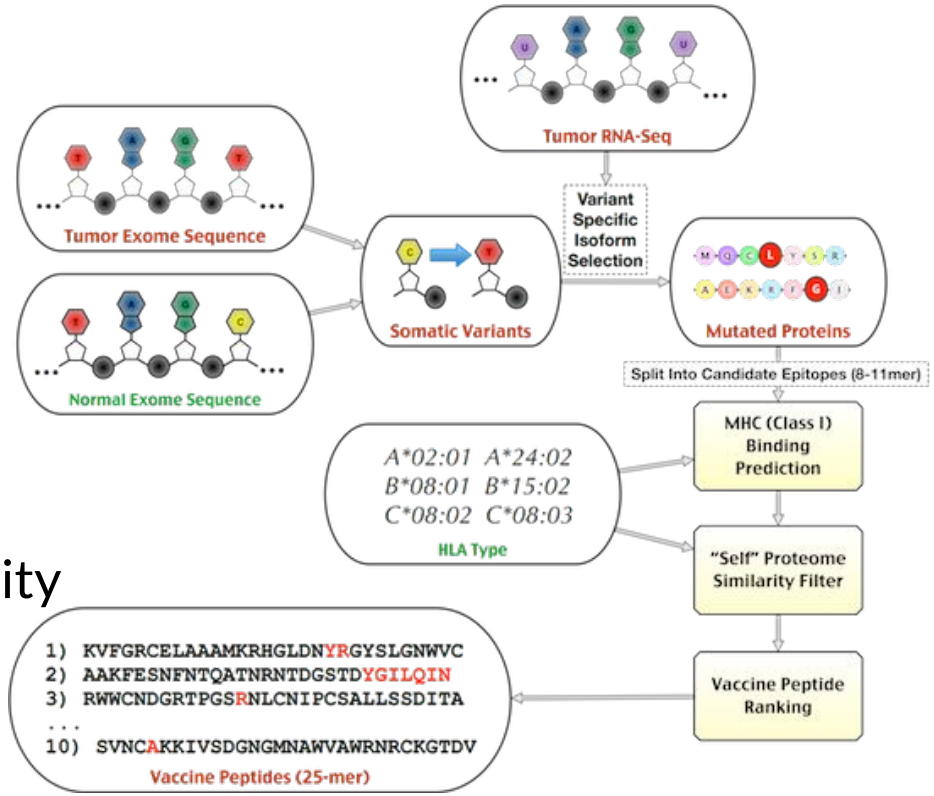
OpenVax Pipeline overview

- Tumor + normal DNA
  - Somatic variant calling
- Tumor RNA
  - Phase co-expressed variants
  - Mutant protein sequence
  - Quantify mut. allele expression
- Rank by expression and MHC-I affinity
- Select manufacturable peptides
- www.github.com/openvax/



Vaccine peptide ranking

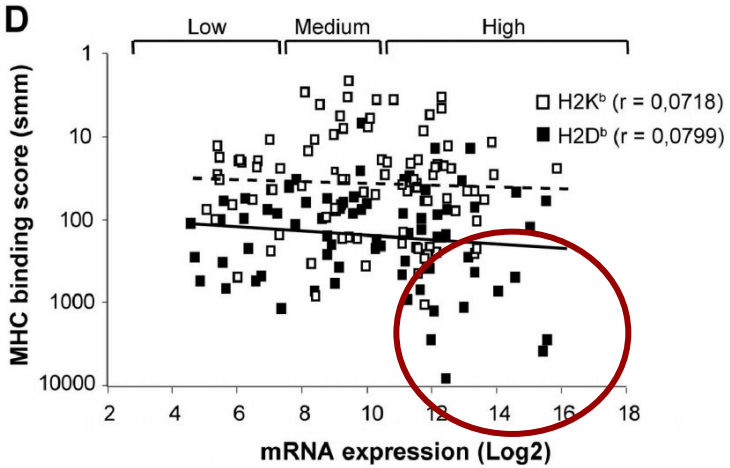
- Multiplicative ranking inspired by T cell epitopes which have low MHC affinity but high abundance

$$\text{TotalScore} = \text{ExpressionScore} \cdot \text{BindingScore}$$

$$\text{ExpressionScore} = \sqrt{\#\text{ supporting reads}}$$

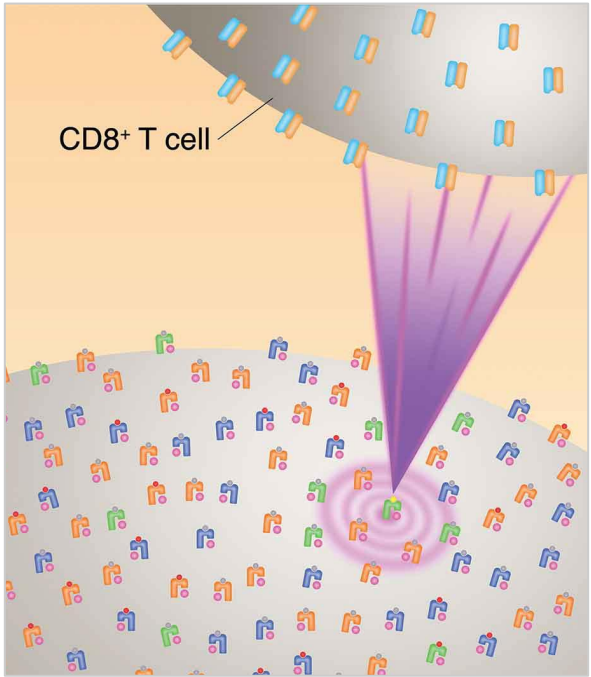
$$\text{BindingScore} = \sum_{p}^{\text{mutant peptides}} \sum_{mhc}^{\text{alleles}} \sigma(\text{IC50}(p, mhc))$$

$$\sigma(x) = \exp(-\frac{x - 150}{350})$$

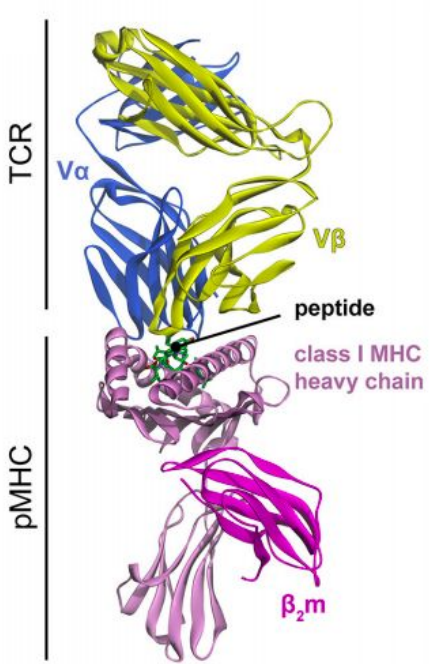


The MHC class I peptide repertoire is molded by the transcriptome (2008)

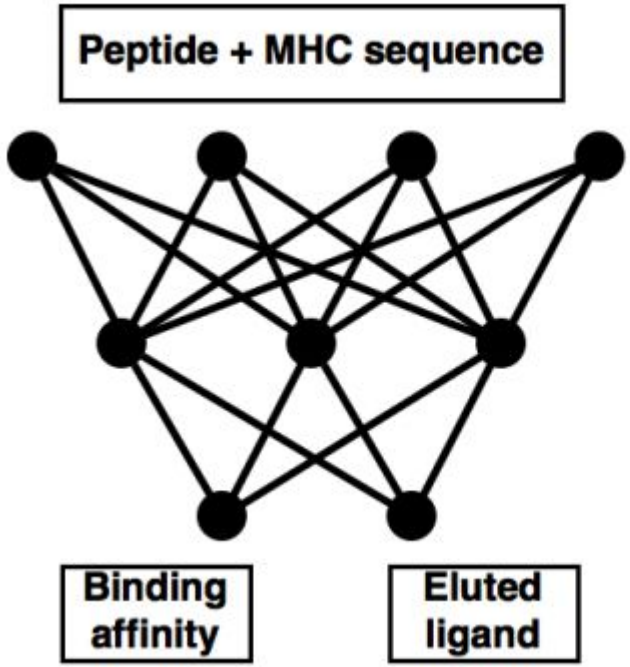
MHC binding prediction



Lost in the crowd: identifying targetable MHC class I neoepitopes for cancer immunotherapy

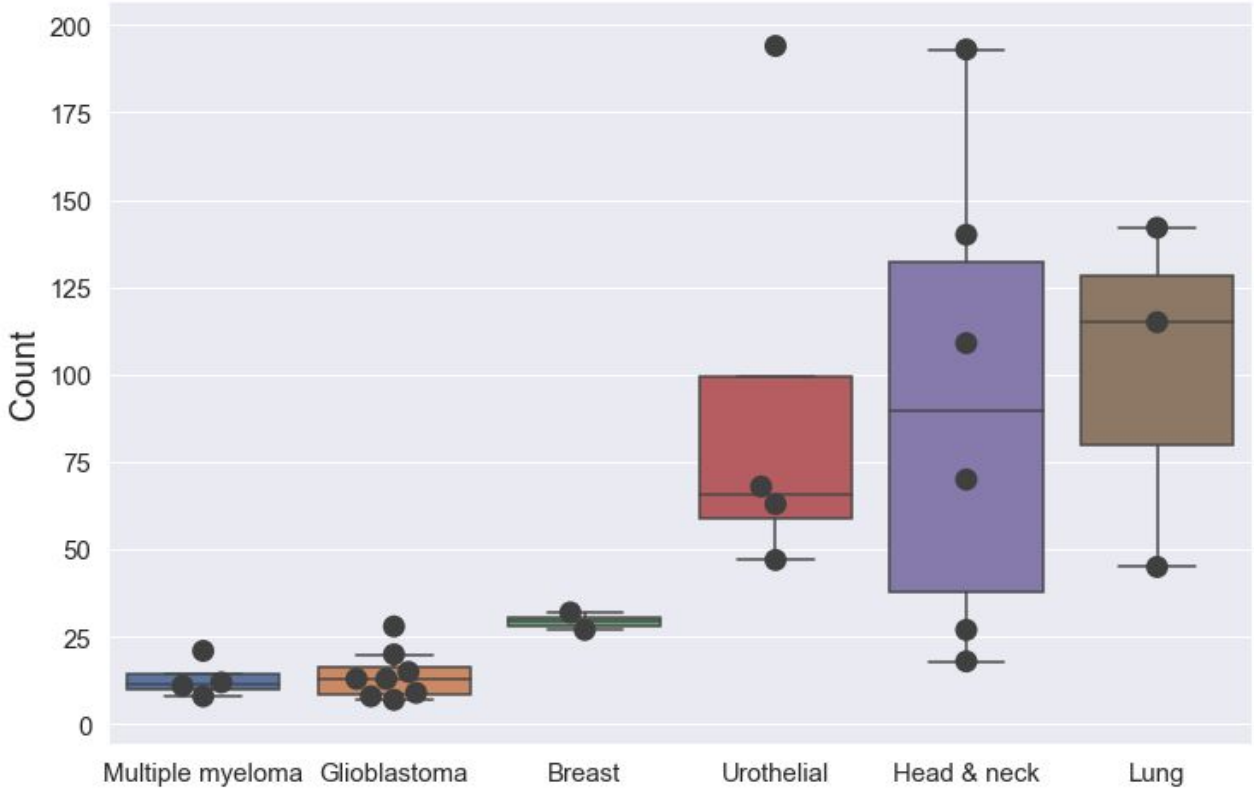


Using Global Analysis to Extend the Accuracy and Precision of Binding Measurements with T cell Receptors and Their Peptide/MHC Ligands



NetMHCpan 4.0

Do we get enough mutations?



Source: Julia Kodysh

Concordance of neoantigen pipelines

How many of the PGV001 trial vaccine variants (n=136) are predicted by different neoantigen prediction tools?

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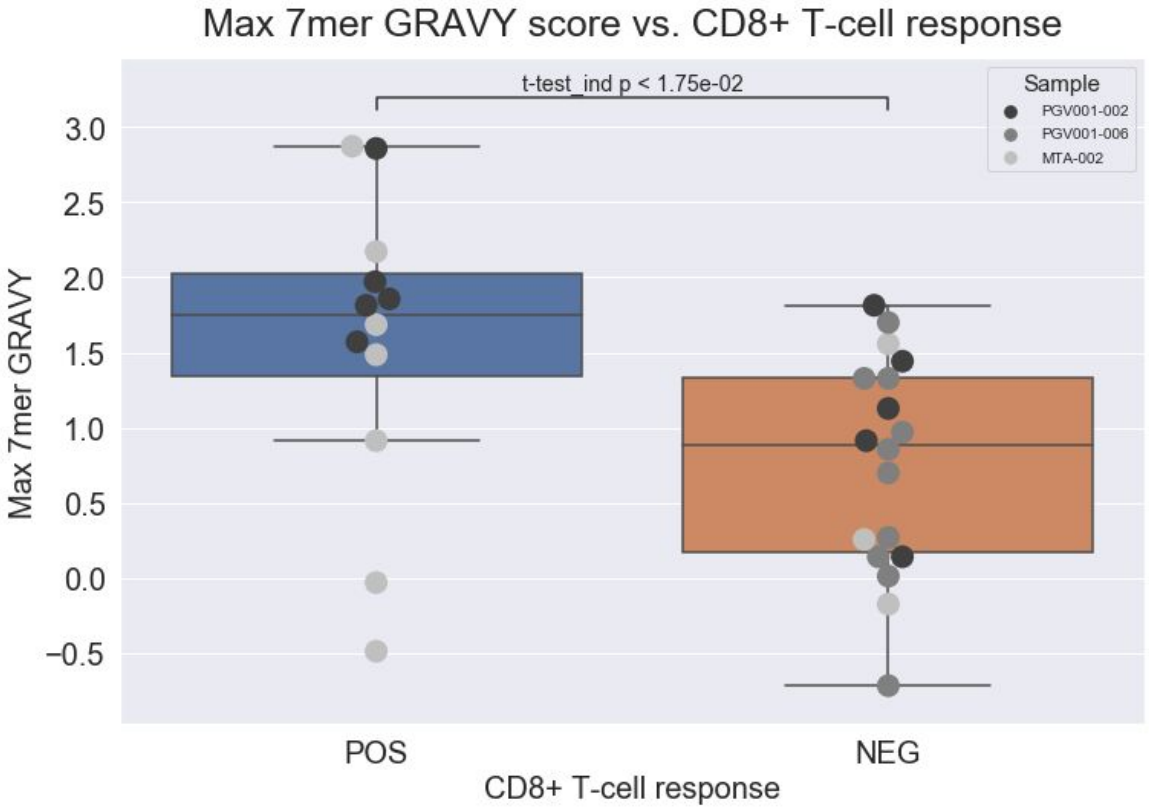
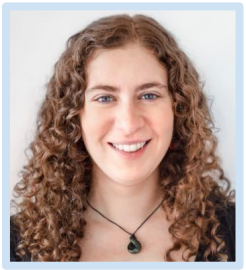
Hydrophobicity vs CD8+ response





Source: Julia Kodysh

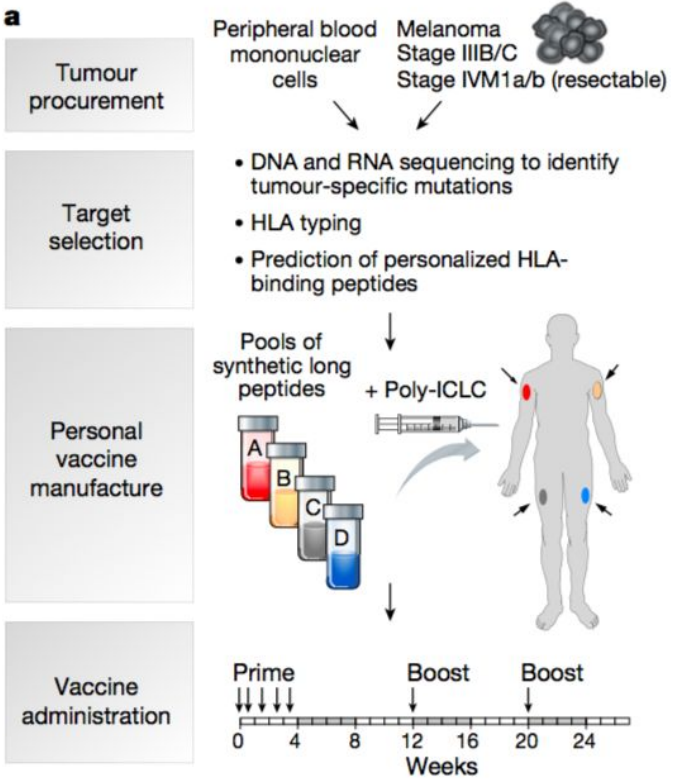
Peptides + poly-ICLC @ DFCI (2017)

An immunogenic personal neoantigen vaccine for patients with melanoma

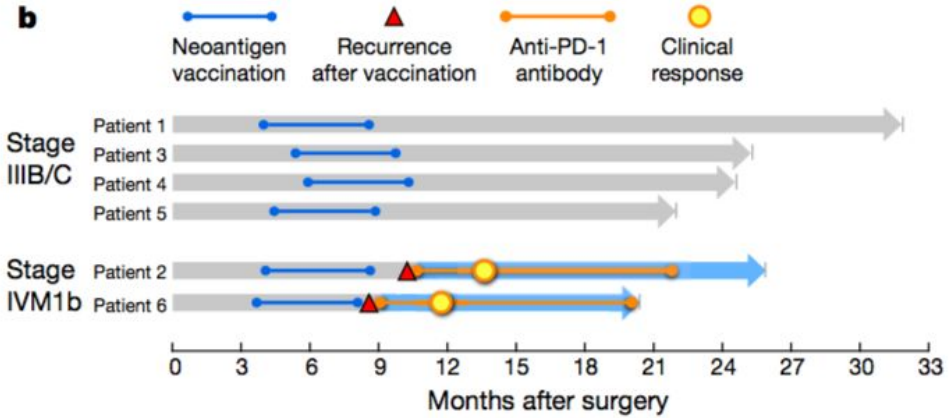
Patrick A. Ott[1,2,3]*, Zhuting Hu[1]*, Derin B. Keskin[1,3,4], Sachet A. Shukla[1,4], Jing Sun[1], David J. Bozym[1], Wandi Zhang[1], Adrienne Luoma[5], Anita Giobbie-Hurder[6], Lauren Peter[7,8], Christina Chen[1], Oriol Olive[1], Todd A. Carter[4], Shuqiang Li[4], David J. Lieb[4], Thomas Eisenhaure[4], Evisa Gjini[9], Jonathan Stevens[10], William J. Lane[10], Indu Javeri[11], Kaliappanadar Nellaiappan[11], Andres M. Salazar[12], Heather Daley[1], Michael Seaman[7], Elizabeth I. Buchbinder[1,2,3], Charles H. Yoon[3,13], Maegan Harden[4], Niall Lennon[4], Stacey Gabriel[4], Scott J. Rodig[9,10], Dan H. Barouch[3,7,8], Jon C. Aster[3,10], Gad Getz[3,4,14], Kai Wucherpfennig[3,5], Donna Neuberg[6], Jerome Ritz[1,2,3], Eric S. Lander[3,4], Edward F. Fritsch[1,4]†, Nir Hacohen[3,4,15] & Catherine J. Wu[1,2,3,4]

- 6 (stage III & IV) melanoma patients
- Up to 20 mutated peptides per vaccine
- Adjuvant: Poly-ICLC

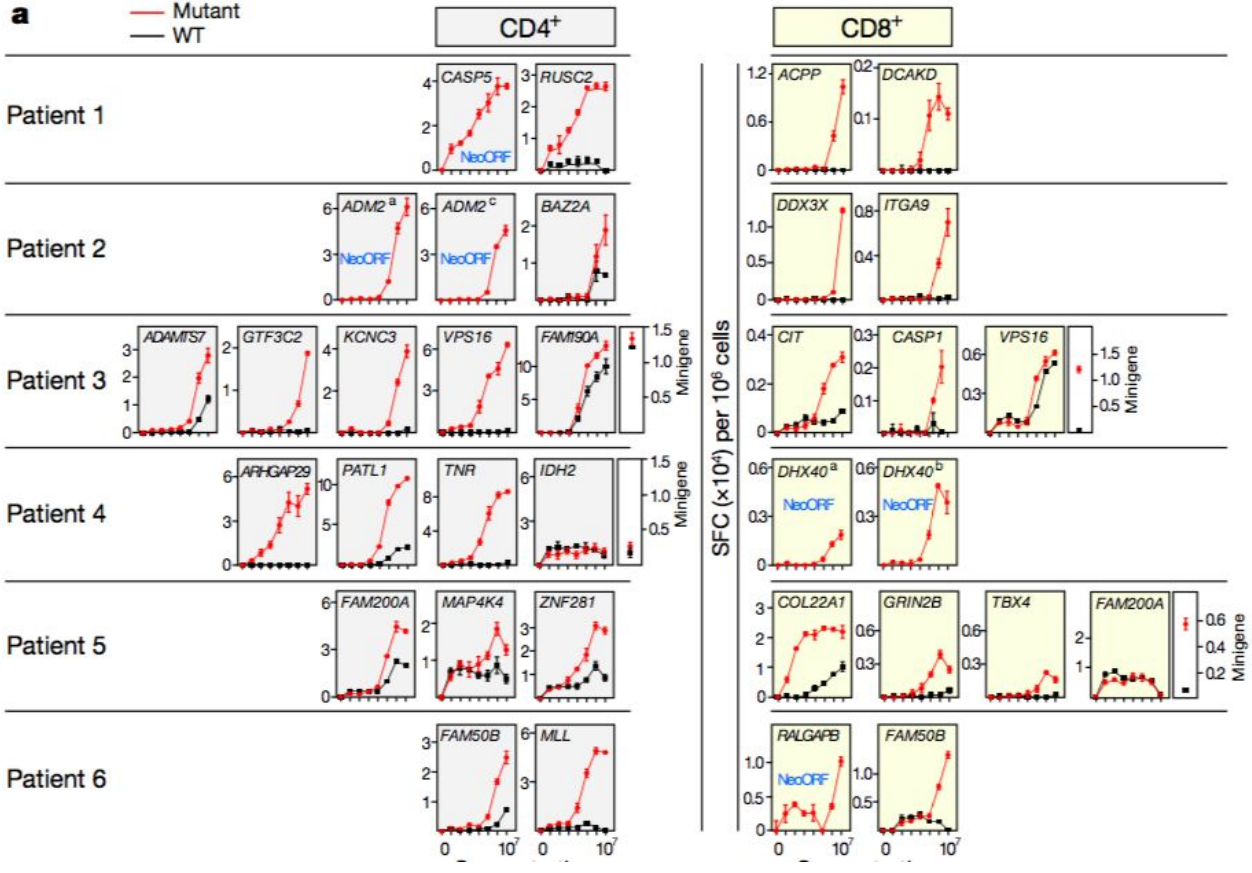
Peptides + poly-ICLC: Tumor control?



Of six vaccinated patients, four had no recurrence at 25 months after vaccination, while two with recurrent disease were subsequently treated with anti-PD-1 (anti-programmed cell death-1) therapy and experienced complete tumour regression, with expansion of the repertoire of neoantigen-specific T cells.



Peptides + poly-ICLC: T Cell responses



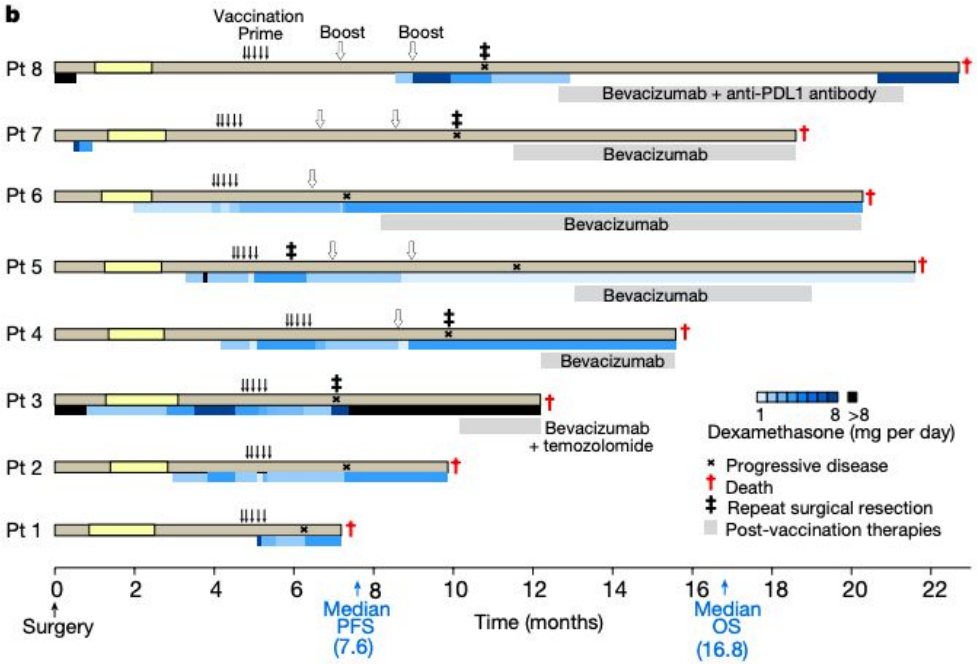
Peptides + poly-ICLC for GBM @ DFCI (2018)

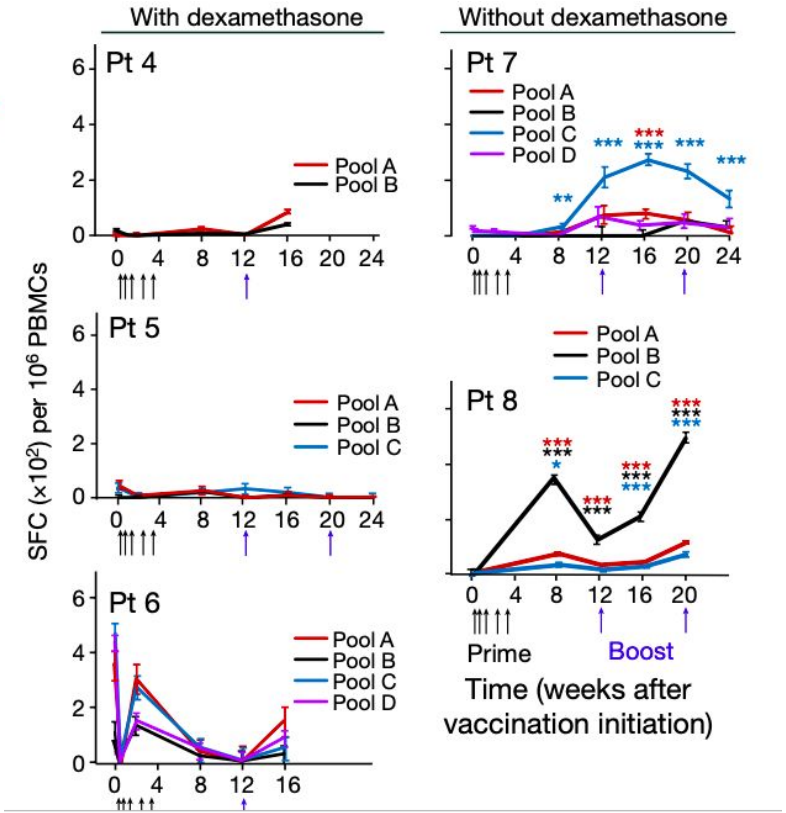
Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial

Derin B. Keskin[1,2,3,4,5,19], Annabelle J. Anandappa[1,4,19], Jing Sun[1,19], Itay Tirosh[3,6,19], Nathan D. Mathewson[4,7,19], Shuqiang Li[3,5], Giacomo Oliveira[1], Anita Giobbie-Hurder[8], Kristen Felt[9], Evisa Gjini[9], Sachet A. Shukla[1,5], Zhuting Hu[1], Letitia Li[1], Phuong M. Le[1], Rosa L. Allesøe[1,10], Alyssa R. Richman[3,4,11,12], Monika S. Kowalczyk[3], Sara Abdelrahman[9], Jack E. Geduldig[13], Sarah Charbonneau[13], Kristine Pelton[13], J. Bryan Iorgulescu[1,4,14], Liudmila Elagina[3], Wandi Zhang[1], Oriol Olive[1], Christine McCluskey[1], Lars R. Olsen[10], Jonathan Stevens[14], William J. Lane[4,14], Andres M. Salazar[15], Heather Daley[1], Patrick Y. Wen[1,4,16], E. Antonio Chiocca[4,17], Maegan Harden[3], Niall J. Lennon[3], Stacey Gabriel[3], Gad Getz[3,4,12], Eric S. Lander[3], Aviv Regev[3], Jerome Ritz[1,2,4], Donna Neuberg[8], Scott J. Rodig[4,9,14], Keith L. Ligon[3,4,13,14], Mario L. Suvà[3,4,11,12], Kai W. Wucherpfennig[4,7], Nir Hacohen[3,4,12], Edward F. Fritsch[1,3,18], Kenneth J. Livak[1,5], Patrick A. Ott[1,2,4], Catherine J. Wu[1,2,3,4] & David A. Reardon[1,2,4]*

- 10 enrolled glioblastoma patients, 8 w/ enough mutations
- All eight vaccinated patients eventually died
- 6/8 were given steroids during priming: no T-cell responses!

GBM 2018: steroids during priming = bad





mRNA vaccine @ BioNTech (2017)

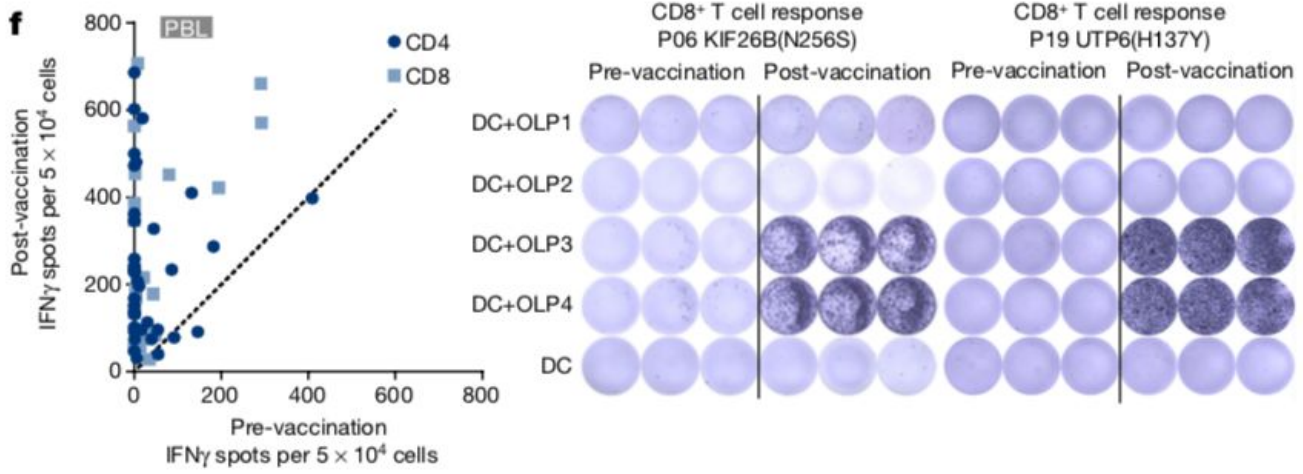
Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer

Ugur Sahin[1,2,3], Evelyna Derhovanessian[1], Matthias Miller[1], Björn-Philipp Kloke[1], Petra Simon[1], Martin Löwer[2], Valesca Bukur[1,2], Arbel D. Tadmor[2], Ulrich Luxemburger[1], Barbara Schrörs[2], Tana Omokoko[1], Mathias Vormehr[1,3], Christian Albrecht[2], Anna Paruzynski[1], Andreas N. Kuhn[1], Janina Buck[1], Sandra Heesch[1], Katharina H. Schreeb[1], Felicitas Müller[1], Inga Ortseifer[1], Isabel Vogler[1], Eva Godehardt[1], Sebastian Attig[2,3], Richard Rae[2], Andrea Breitkreuz[1], Claudia Tolliver[1], Martin Suchan[2], Goran Martic[2], Alexander Hohberger[3], Patrick Sorn[2], Jan Diekmann[1], Janko Ciesla[4], Olga Waksmann[4], Alexandra-Kemmer Brück[1], Meike Witt[1], Martina Zillgen[1], Andree Rothermel[2], Barbara Kasemann[2], David Langer[1], Stefanie Bolte[1], Mustafa Diken[1,2], Sebastian Kreiter[1,2], Romina Nemecek[5], Christoffer Gebhardt[6,7], Stephan Grabbe[3], Christoph Höller[5], Jochen Utikal[6,7], Christoph Huber[1,2,3], Carmen Loquai[3]* & Özlem Türeci[8]*

- 13 (stage III & IV) melanoma patients
- 10 mutated sequences encoded in mRNA
- Ultrasound guided injection of mRNA into lymph nodes

mRNA 2017: T cell responses

- ~20% mutations had ex vivo CD4+ responses
- ~50% mutations had CD4+ responses after in vitro stim
- ~25% mutations had CD8+ responses after in vitro stim



mRNA 2017: Tumor control

- 8/13 patients had no measurable lesions before vaccination
  - Remained disease free throughout monitoring period
- 5 patients had growing lesions before vaccination
  - 1 patient: complete response
  - 1 patient: stable disease
  - 1 patient: complete response after treatment with anti-PD1
  - 1 patient had partial response until **tumor cells lost B2M**
- ~20% mutations had ex vivo CD4+ responses
- ~50% mutations had CD4+ responses after in vitro stim
- ~25% mutations had CD8+ responses after in vitro stim

Personalized Cancer Summary

- Existing vaccines elicit (weak) T cell responses vs. neoAg
  - CD4+ responses much stronger than CD8+
- Hint of efficacy, especially with αPD-1 after vaccination
  - NEO-PV-01 had αPD-1 before, drowns out effect of vaccine
- Past clinical trials focused on SNVs + small indels, field looking more at “dark matter” (SVs, splicing, hERVs, &c)
- Hard to compare neoantigen selection algorithms until vaccine platforms improve

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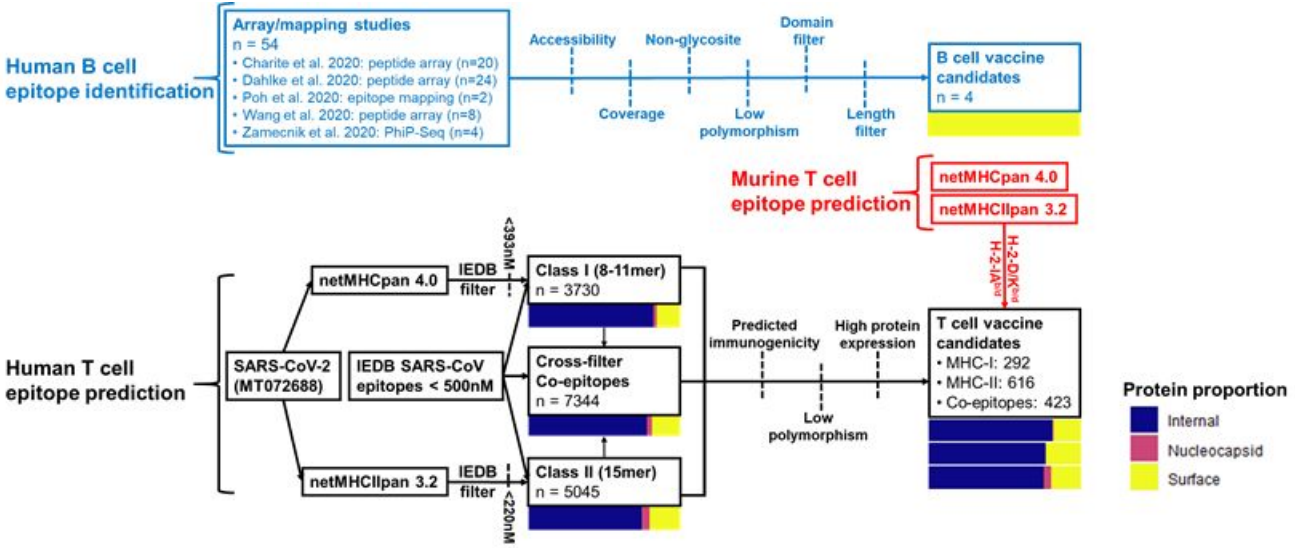
Peptide vaccines for pathogens

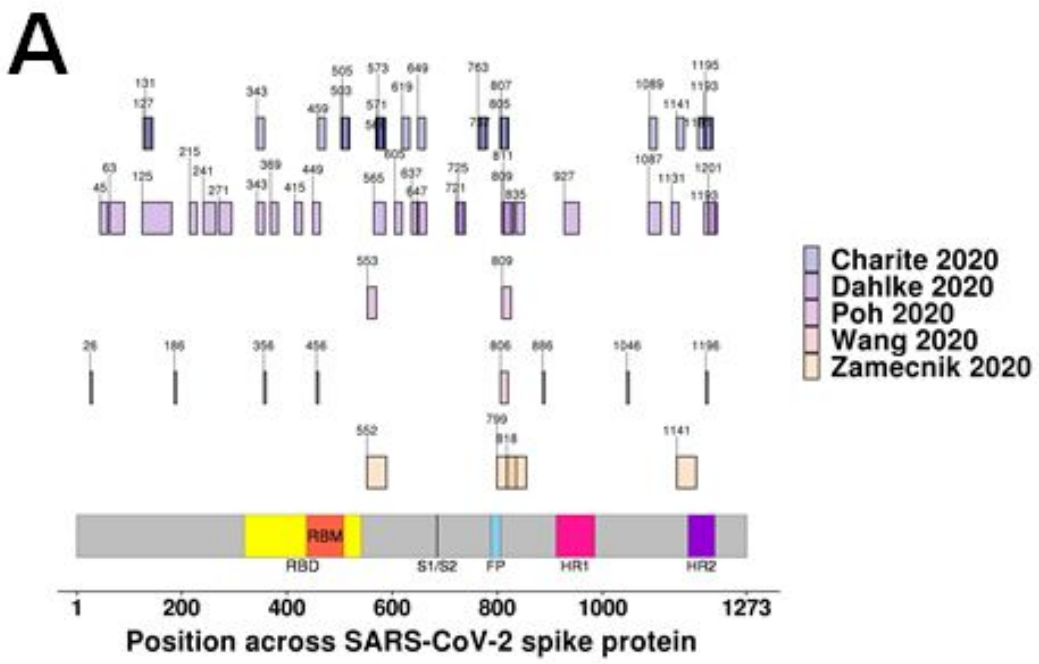
- Potential problems with whole virus or whole protein vaccination:
  - Diffuse T-cell responses; will immunodominant epitopes match presented epitopes of infected cells?
  - Responses to polymorphic regions of virus
  - Unlikely (but worrying) possibility of antibody dependent enhancement (ADE), mediated by non-neutralizing antibodies
- Potential benefits of peptide vaccines:
  - Fine-grained selection of antigenic content
- Limits:
  - Can’t target conformational B-cell epitopes! (only linear)
  - Only a few effective prophylactic peptide vaccines (e.g. FMDV)

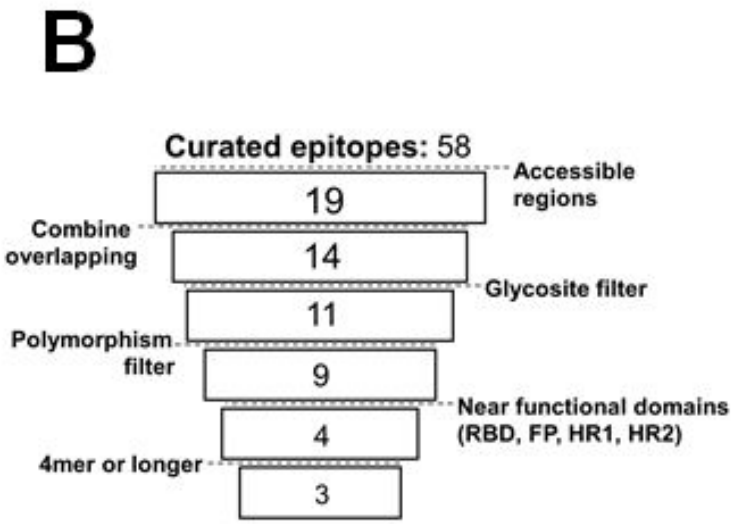
Integrating Predicted T-Cell Epitopes With Measured Linear B-cell Epitopes

- Predict SARS-CoV-2 MHC binding for Class I & II alleles covering US population
  - Filter by predicted T-cell immunogenicity, protein abundance, polymorphic sites
- Combine w/ measured B-cell epitopes from convalescent patient plasma
  - Filter by accessibility, non-glycosylation, annotated functional regions on spike protein



Curated Linear B-cell Epitope Data Sources

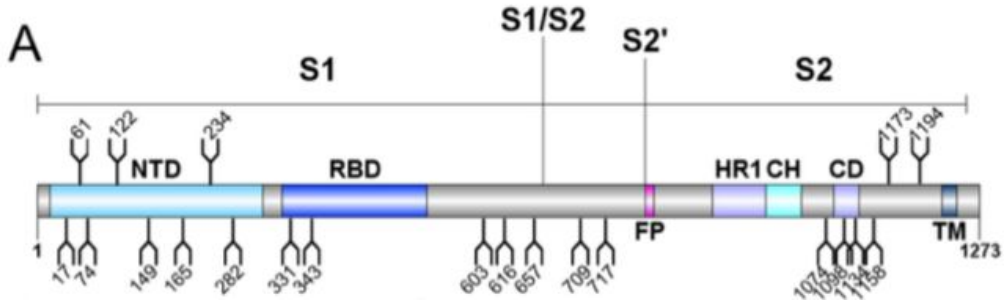


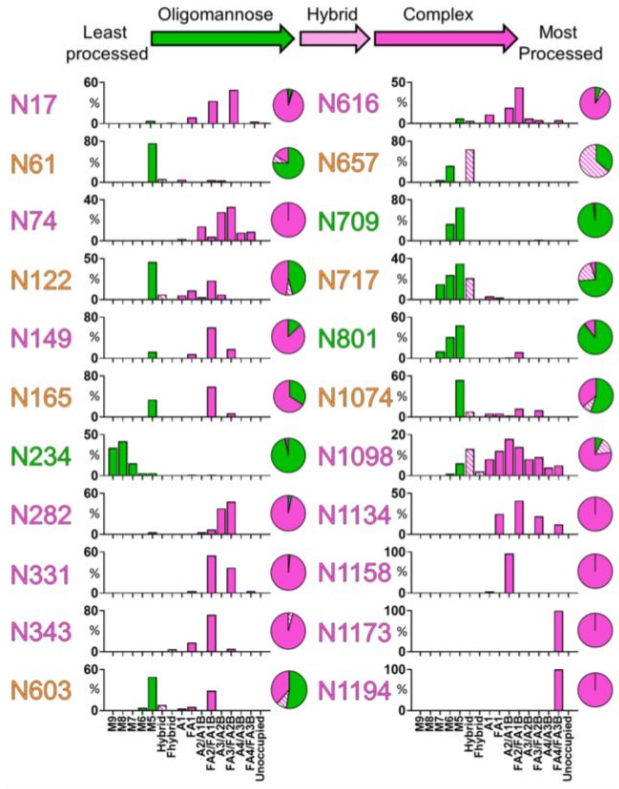


Source for Glycosites (Watanabe et al.)

Site-specific analysis of the SARS-CoV-2 glycan shield

Yasunori Watanabe[1,2,3#], Joel D. Allen[1#], Daniel Wrapp[4], Jason S. McLellan[4], Max Crispin[1*]





Polymorphic Sites

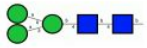
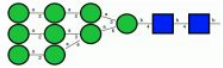
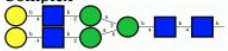
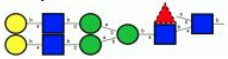
- Collected all SARS-CoV-2 sequences in Nextstrain
- >0.1% frequency
- 28 sites
- Most common: D614G (~50%)

Source for Accessibility (Grant et al.)

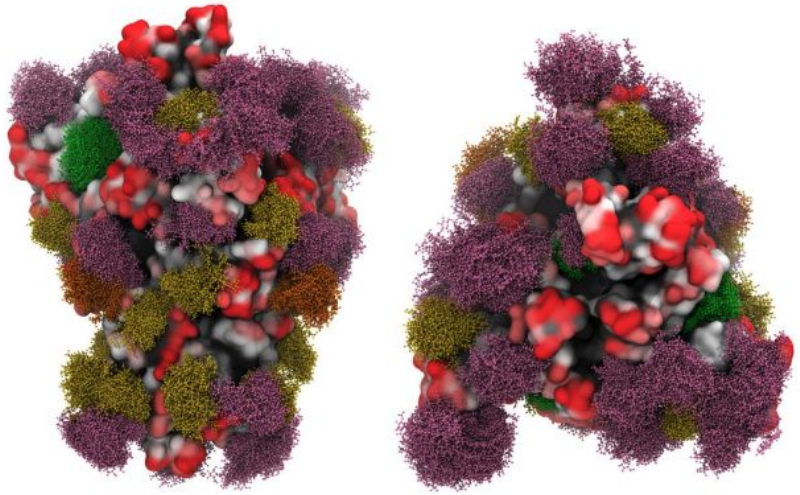
3D Models of glycosylated SARS-CoV-2 spike protein suggest challenges and opportunities for vaccine development

Oliver C. Grant, David Montgomery, Keigo Ito, Robert J. Woods*

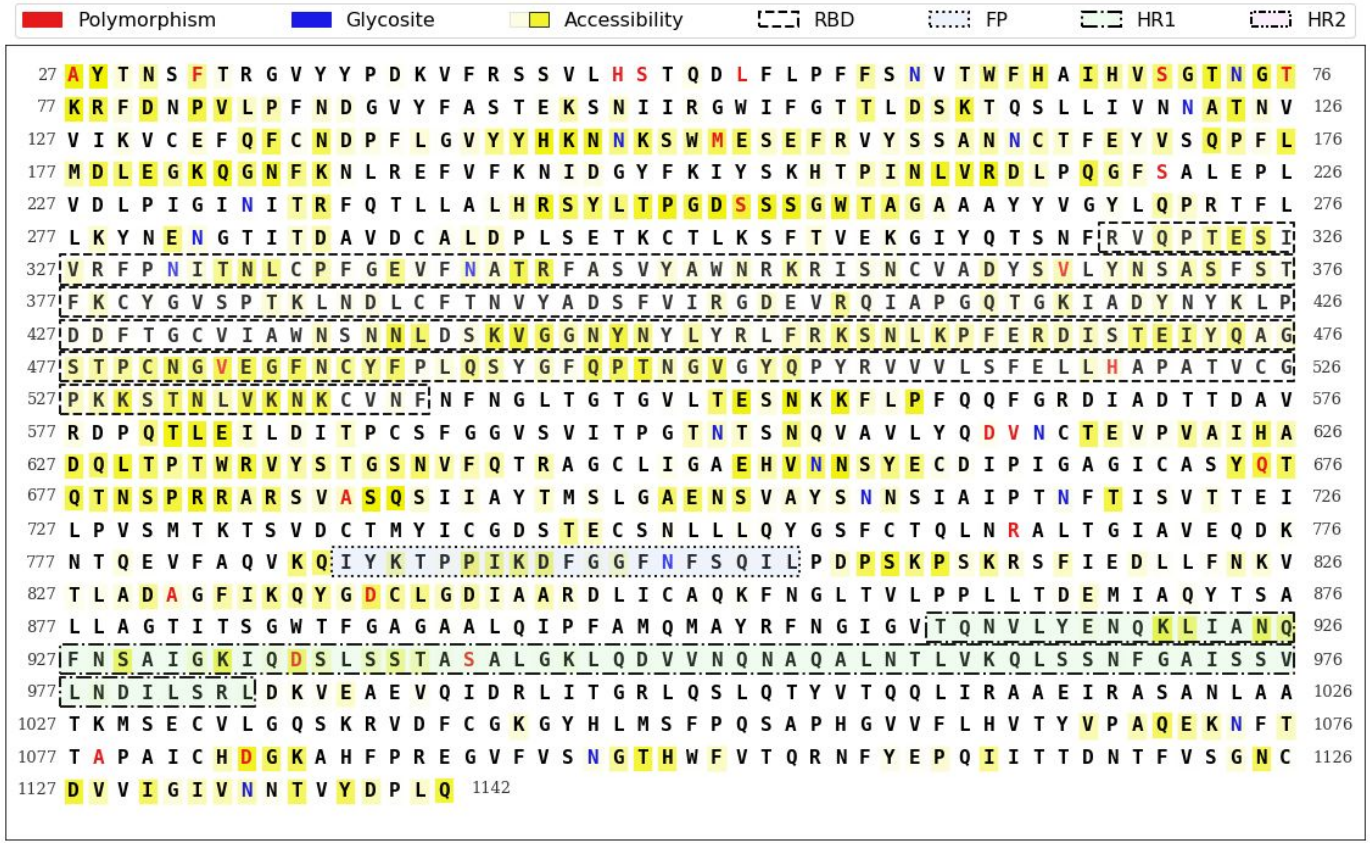
Table 1. SARS-CoV-2 S glycoprotein antigenic surface areas (Å^2) as a function of glycoform.

Glycoform	Average antibody accessible surface area (AbASA)[a]	Exposed fraction of AbASA
M3 	58,579 ± 2.8%	0.71
M9 	44,184 ± 1.1%	0.53
Complex 	45,571 ± 1.6%	0.55
Complex Core F 	43,943 ± 2.0%	0.53
HEK293 site-specific glycosylation	48,322 ± 0.7%	0.58
Non-glycosylated	83,041 ± 2.8%	1.00

[a]Surface areas were computed with the Naccess software [68], version 2.1.1.



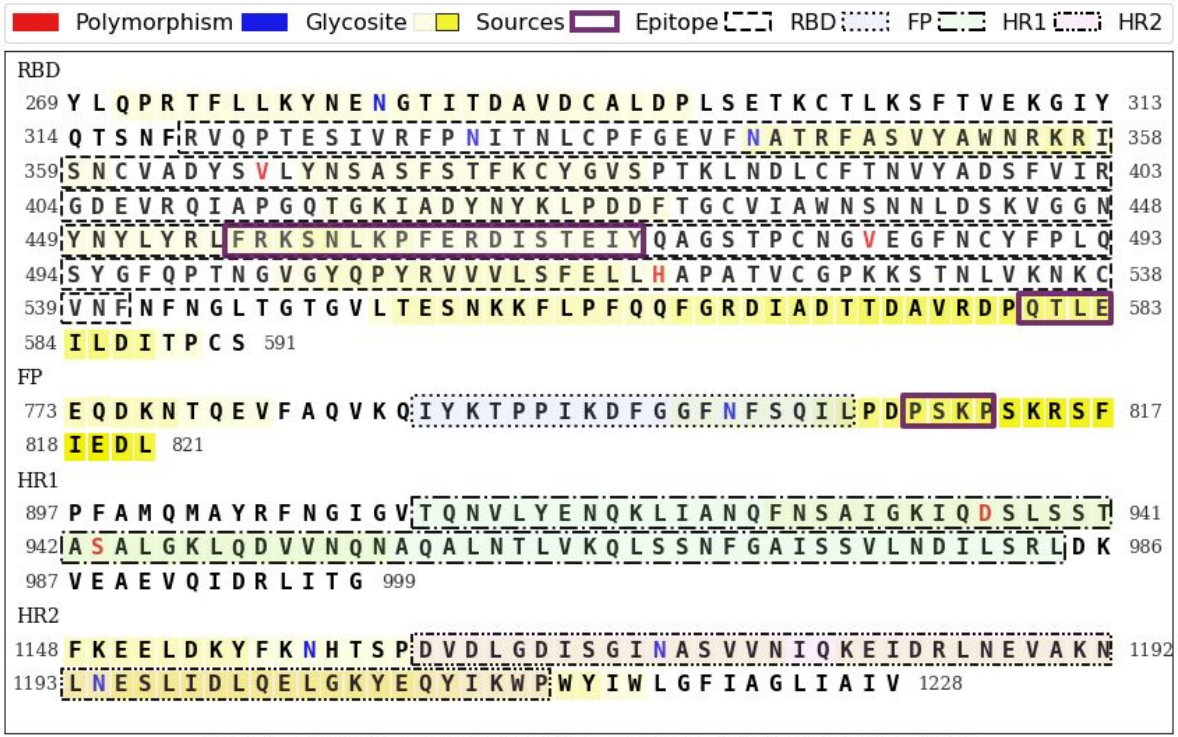
Accessible residues near functional features



Only 3 B-cell linear epitopes regions

Filters:

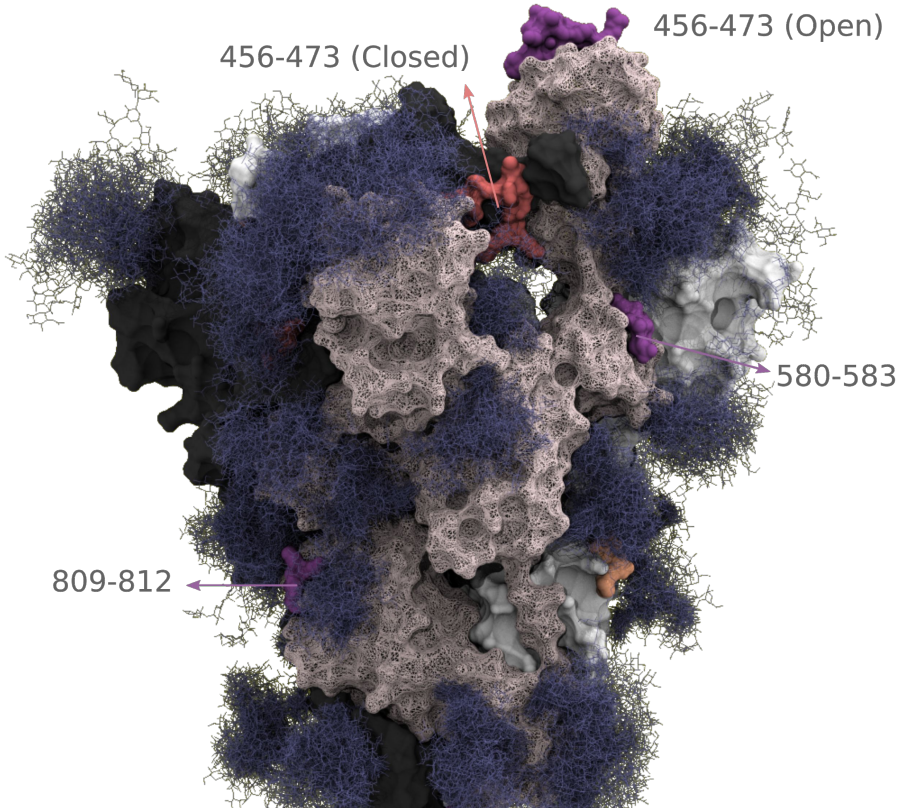
- >=4mer region
- Accessibility > 25%
- Does not contain glycosites
- Does not contain polymorphic sites
- Within 50aa of RBD or 15aa of fusion peptide (FP) or HR1/HR2 regions



Number of data sources supporting each residue as antibody epitope

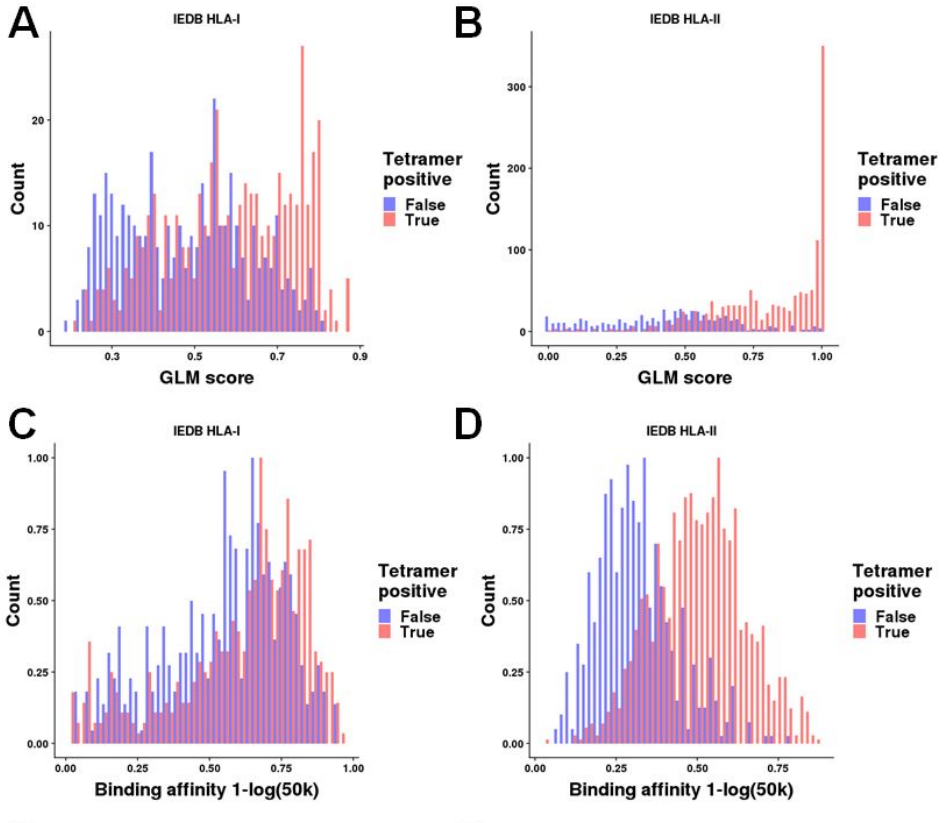
Location of predicted linear B-cell epitopes

- **S580-583**: downstead of RBD, target of known neutralizing antibody
- **S809-812**: adjacent to fusion peptide, occurs in a 5 B-cell epitope datasets
- **S456-473**: RBM loop which contacts ACE2, only accessible when RBD in open conformation



T-Cell Immunogenicity Prediction

- Constructed CD4+ & CD8+ immunogenicity models from IEDB tetramer data
  - Model = logistic regression
- Features
  - % amino acids {aromatic, acidic, basic, cyclic, thiols}
  - MHC binding & presentation
    - CD8+: NetMHCpan & MHCflurry
    - CD4 +: NetMHCIIpan
  - CD8+: MHCflurry processing score



Compact peptide sets for different selection criteria

Symbol	Set	# Peptides	HLA-I Coverage	HLA-II Coverage	Total Coverage	# B-cell Epitope Regions
⊛	CD4+/CD8+	4	92.2%	88.5%	81.6%	0
$⊛^{d}$	CD4+/CD8+ ($H2^{d}$ ligands)	4	93.8%	84.7%	79.5%	0
$⊛^{b}$	CD4+/CD8+ ($H2^{b}$ ligands)	3	92.2%	84.7%	78.1%	0
$⊛^{bd}$	CD4+/CD8+ ($H2^{b}$ and $H2^{d}$ ligands)	4	92.1%	84.7%	78.0%	0
○	CD4+	3	91.3%	88.5%	80.8%	0
$○^{d}$	CD4+ ($H2^{d}$ ligands)	3	91.3%	88.5%	80.8%	0
$○^{b}$	CD4+ ($H2^{b}$ ligands)	3	76.8%	84.7%	65.0%	0
$○^{bd}$	CD4+ ($H2^{b}$ and $H2^{d}$ ligands)	3	92.2%	84.7%	78.1%	0
*	CD8+	3	95.8%	61.3%	58.7%	0
$*^{d}$	CD8+ ($H2^{d}$ ligands)	3	95.1%	76.2%	72.5%	0
$*^{b}$	CD8+ ($H2^{b}$ ligands)	3	95.8%	61.3%	58.7%	0
$*^{bd}$	CD8+ ($H2^{b}$ and $H2^{d}$ ligands)	3	94.7%	72.6%	68.8%	0
⊛	B-Cell/CD4+/CD8+	3	88.9%	62.7%	55.7%	3
⊙	B-Cell/CD4+	3	88.9%	62.7%	55.7%	3
$⊙^{d}$	B-Cell/CD4+ ($H2^{d}$ ligands)	1	66.2%	39.9%	26.4%	1
$⊙^{b}$	B-Cell/CD4+ ($H2^{b}$ ligands)	2	64.8%	39.4%	25.5%	2
⊛	B-Cell/CD8+	3	90.8%	57.7%	52.4%	3
$⊛^{d}$	B-Cell/CD8+ ($H2^{d}$ ligands)	1	81.8%	38.4%	31.4%	1
$⊛^{b}$	B-Cell/CD8+ ($H2^{b}$ ligands)	2	89.4%	46.5%	41.5%	2
$⊛^{bd}$	B-Cell/CD8+ ($H2^{b}$ and $H2^{d}$ ligands)	1	81.8%	38.4%	31.4%	1
□	B-Cell	3	81.8%	52.8%	43.2%	3

Combined vaccine peptide set

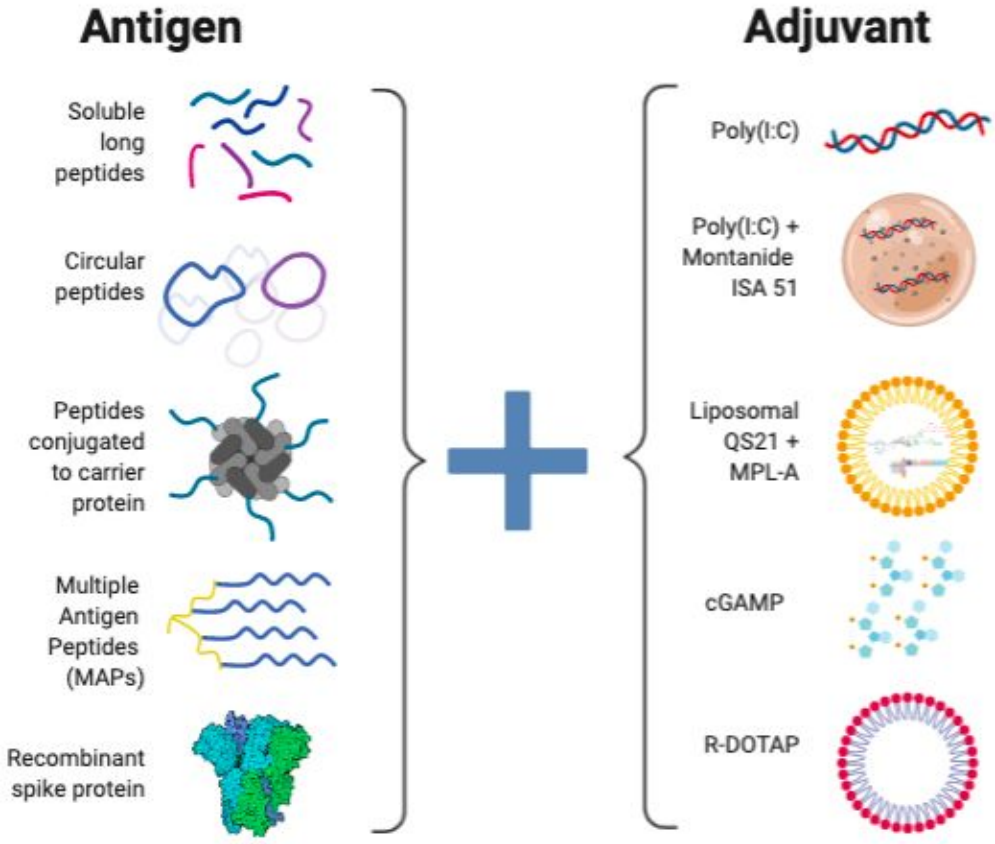
	Sequence	Protein	Start	End	B-cell Epitope Region	HLA-I Coverage	HLA-II Coverage	$H2^b$ I	$H2^b$ II	$H2^d$ I	$H2^d$ II	Selection Sets
1	LLQFAYANRNRFLYIIKLIFLWLLWPV	M	34	60		89.0%	36.0%	+	+	+	+	
2	PVTLACFVLAAVYRINWITGGIAIAMA	M	59	85		42.0%	76.0%	+	+	-	+	
3	YFIASFRLFARTRSMWSFNPETNILLN	M	95	121		78.0%	53.0%	+	+	+	+	
4	KDLSPRWYFYYLGTGPEAGLPYGANKD	N	102	128		49.0%	39.0%	+	+	+	-	
5	WPQIAQFAPSASAFFGMSRIGMEVTPS	N	301	327		63.0%	61.0%	+	+	+	+	
6	AQFAPSASAFFGMSRIGMEVTPSGTWL	N	305	331		71.0%	57.0%	+	+	+	-	
7	SASAFFGMSRIGMEVTPSGTWLTYTGA	N	310	336		76.0%	45.0%	+	-	+	-	
8	VTPSGTWLTYTGAIKLDDKDPNFKDQV	N	324	350		50.0%	62.0%	+	+	-	-	
9	PQRQKKQQTVTLLPAADLDDFSKQLQQ	N	383	409		11.0%	52.0%	-	-	-	+	
10	YPDKVFRSSVLHSTQDLFLPFFSNVTW	S	38	64		44.0%	52.0%	-	+	+	+	
11	GAAAYYVGYLQPRTFLLKYNENGTITD	S	261	287		88.0%	38.0%	+	+	+	-	
12	SETKCTLKSFTVEKGIYQTSNFRVQPT	S	297	323		54.0%	52.0%	-	-	+	-	
13	GLTVLPPLLTDEMIAQYTSALLAGTIT	S	857	883		66.0%	73.0%	+	+	+	+	
14	SVLNDILSRLDKVEAEVQIDRLITGRL	S	975	1001		72.0%	28.0%	+	-	-	-	
15	RLQSLQTYVTQQLIRAAEIRASANLAA	S	1000	1026		54.0%	81.0%	-	+	+	+	
16	GNYNYLYRLFRKSNLKPFERDISTEIY	S	447	473	456-FRKSNLKPFERDISTEIY-473	82.0%	38.0%	+	-	+	-	
17	YLYRLFRKSNLKPFERDISTEIYQAGS	S	451	477	456-FRKSNLKPFERDISTEIY-473	78.0%	46.0%	+	-	-	-	
18	FRKSNLKPFERDISTEIYQAGSTPCNG	S	456	482	456-FRKSNLKPFERDISTEIY-473	46.0%	30.0%	-	+	-	-	
19	KFLPFQQFGRDIADTTDAVRDPQTLEI	S	558	584	580-QTLE-583	0.0%	0.0%	-	-	-	-	
20	PQTLEILDITPCSFGGVSVITPGTNTS	S	579	605	580-QTLE-583	13.0%	21.0%	-	-	-	-	
21	IYKTPPIKDFGGFNFSQILPDPSKPSK	S	788	814	809-PSKP-812	35.0%	23.0%	-	+	-	-	
22	PSKPSKRSFIEDLLFNKVTLADAGFIK	S	809	835	809-PSKP-812	66.0%	40.0%	+	-	-	+	

Validation in Multiple SARS-CoV-2 T-cell Studies

	Sequence	Protein	Start	End	B-cell Epitope Region	HLA-I Coverage	HLA-II Coverage	$H2^b$ I	$H2^b$ II	$H2^d$ I	$H2^d$ II	Selection Sets
1	LLQFAYANRNRFLYIIKLIFLWLLWPV	M	34	60		89.0%	36.0%	+	+	+	+	$*^b\ *^d\ *^{bd}$ $\circ\ \circ^d\ \circ^{bd}\ \circledast$ $\circledast^d\ \circledast^b\ \circledast^{bd}$
2	PVTLACFVLAAVYRINWITGGIAIAMA	M	59	85		42.0%	76.0%	+	+	-	+	$\circ^b$
3	YFIASFRLFARTRSMWSFNPETNILLN	M	95	121		78.0%	53.0%	+	+	+	+	$\circledast^{bd}$
4	KDLSPRWYFYYLGTGPEAGLPYGANKD	N	102	128		49.0%	39.0%	+	+	+	-	$*\ *^b\ *^d$
5	WPQIAQFAPSASAFFGMSRIGMEVTPS	N	301	327		63.0%	61.0%	+	+	+	+	$\circ^{bd}\ \circledast^d\ \circledast^{bd}$
6	AQFAPSASAFFGMSRIGMEVTPSGTWL	N	305	331		71.0%	57.0%	+	+	+	-	$\circledast\circledast^b$
7	SASAFFGMSRIGMEVTPSGTWLTYTGA	N	310	336		76.0%	45.0%	+	-	+	-	$*^{bd}$
8	VTPSGTWLTYTGAIKLDDKDPNFKDQV	N	324	350		50.0%	62.0%	+	+	-	-	$\circ^b$
9	PQRQKKQQTVTLLPAADLDDFSKQLQQ	N	383	409		11.0%	52.0%	-	-	-	+	$\circ\ \circ^d\ \circledast$
10	YPDKVFRSSVLHSTQDLFLPFFSNVTW	S	38	64		44.0%	52.0%	-	+	+	+	$\circledast^d$
11	GAAAYYVGYLQPRTFLLKYNENGTITD	S	261	287		88.0%	38.0%	+	+	+	-	$*^{bd}$
12	SETKCTLKSFTVEKGIYQTSNFRVQPT	S	297	323		54.0%	52.0%	-	-	+	-	$*^d$
13	GLTVLPPLLTDEMIAQYTSALLAGTIT	S	857	883		66.0%	73.0%	+	+	+	+	$\circledast\circledast^d\ \circledast^b\circledast^{bd}$
14	SVLNDILSRLDKVEAEVQIDRLITGRL	S	975	1001		72.0%	28.0%	+	-	-	-	$**^b$
15	RLQSLQTYVTQQLIRAAEIRASANLAA	S	1000	1026		54.0%	81.0%	-	+	+	+	$\circ\ \circ^d\ \circ^b\circ^{bd}$
16	GNYNYLYRLFRKSNLKPFERDISTEIY	S	447	473	456-FRKSNLKPFERDISTEIY-473	82.0%	38.0%	+	-	+	-	$\boxplus\boxplus^d\ \boxplus^b\boxplus^{bd}$
17	YLYRLFRKSNLKPFERDISTEIYQAGS	S	451	477	456-FRKSNLKPFERDISTEIY-473	78.0%	46.0%	+	-	-	-	$\square\ \boxdot\ \boxplus$
18	FRKSNLKPFERDISTEIYQAGSTPCNG	S	456	482	456-FRKSNLKPFERDISTEIY-473	46.0%	30.0%	-	+	-	-	$\boxdot^b$
19	KFLPFQQFGRDIADTTDAVRDPQTLEI	S	558	584	580-QTLE-583	0.0%	0.0%	-	-	-	-	$\square$
20	PQTLEILDITPCSFGGVSVITPGTNTS	S	579	605	580-QTLE-583	13.0%	21.0%	-	-	-	-	$\boxplus\ \boxdot\ \boxplus$
21	IYKTPPIKDFGGFNFSQILPDPSKPSK	S	788	814	809-PSKP-812	35.0%	23.0%	-	+	-	-	$\square\boxdot^b$
22	PSKPSKRSFIEDLLFNKVTLADAGFIK	S	809	835	809-PSKP-812	66.0%	40.0%	+	-	-	+	$\boxplus\boxplus^b\ \boxdot\boxdot^d$ $\boxplus$

Can we make precise vaccination work for SARS-CoV-2?

- Baseline vaccines:
  - Soluble long peptides (or recombinant spike) + Poly(I:C)
- Find better adjuvant + antigen combination
  - Circular peptides more stable, restricted conformations
  - MAPS = branched peptides



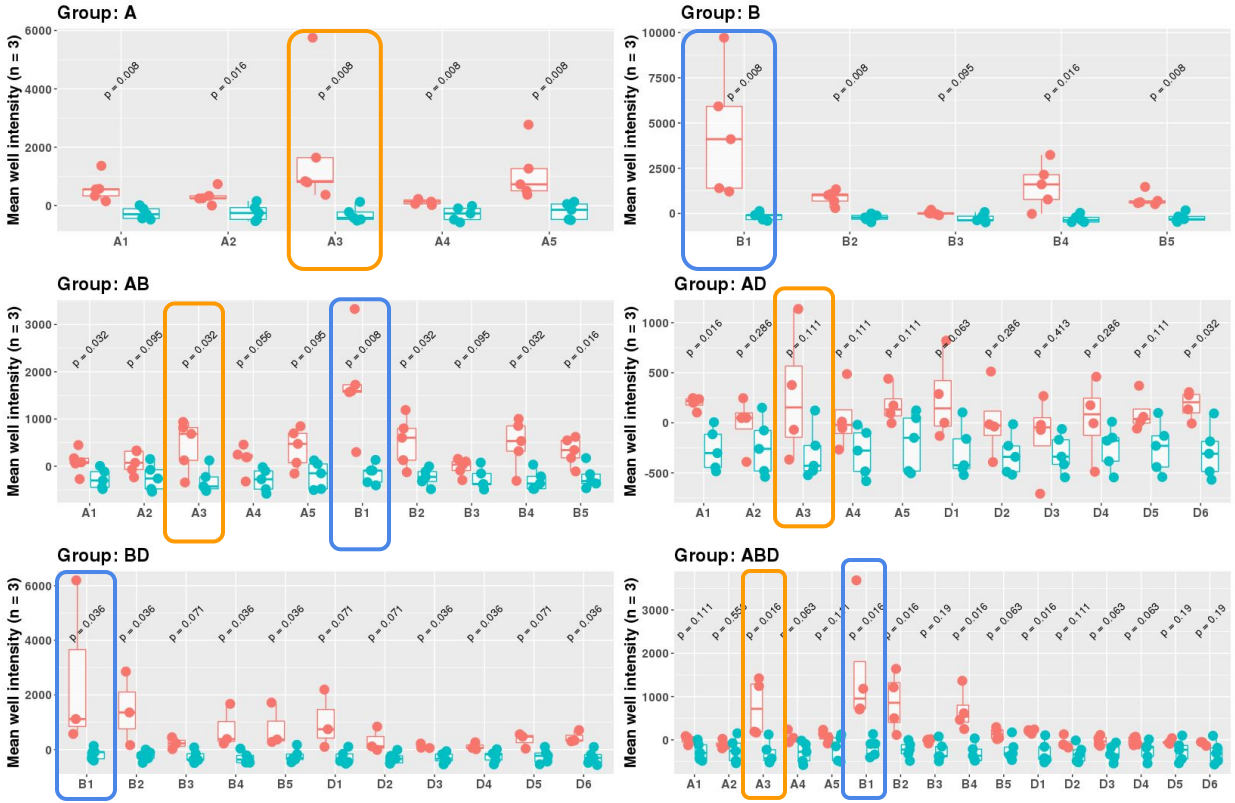
First Experiments (w/ Vincent Lab)

- 27mer peptides + Poly(I:C)
- BALB/c mice
- T-cell responses (ICS) & Ab binding to spike (ELISA)
- Do vaccine peptides compete other?
  - A (n=5): T-cell
  - B (n=5): T-cell
  - C (n=10): A+B
  - D (n=6): B-cell

	SARSCOV2_20-121.0_002.5			N	Day				
					1	7	14		21
	Group	Peptide	Adjuvant		↓	↓	↓		↓
T Cell	1	Set A	Poly IC + M	6	Vaccinate	Cheek Bleed	Cheek Bleed	Boost	Sacc
T Cell	2	Set B	Poly IC + M	6					
T Cell	3	Set C (Set A + B)	Poly IC + M	6					
						↓ Serum	↓ Serum		↓ Serum ↓ Spleen
						Freeze	Freeze		Freeze Elispot
T Cell + B Cell	4	Set A + Set D	Poly IC + M	6	Vaccinate	Cheek Bleed	Cheek Bleed	Boost	Sacc
T Cell + B Cell	5	Set B+ Set D	Poly IC + M	6					
T Cell + B Cell	6	Set C + Set D	Poly IC + M	6					
Controls	Measles	Measles	Poly IC + M	6					
Controls	Adjuvant Only	None	Poly IC + M	6					
Controls	Control	PBS		3					
				Tissue		↓ Serum	↓ Serum		↓ Serum ↓ Spleen
				Assay		ELISA (peptide)	ELISA (peptide)		ELISA (peptide) Elispot
									ELISA

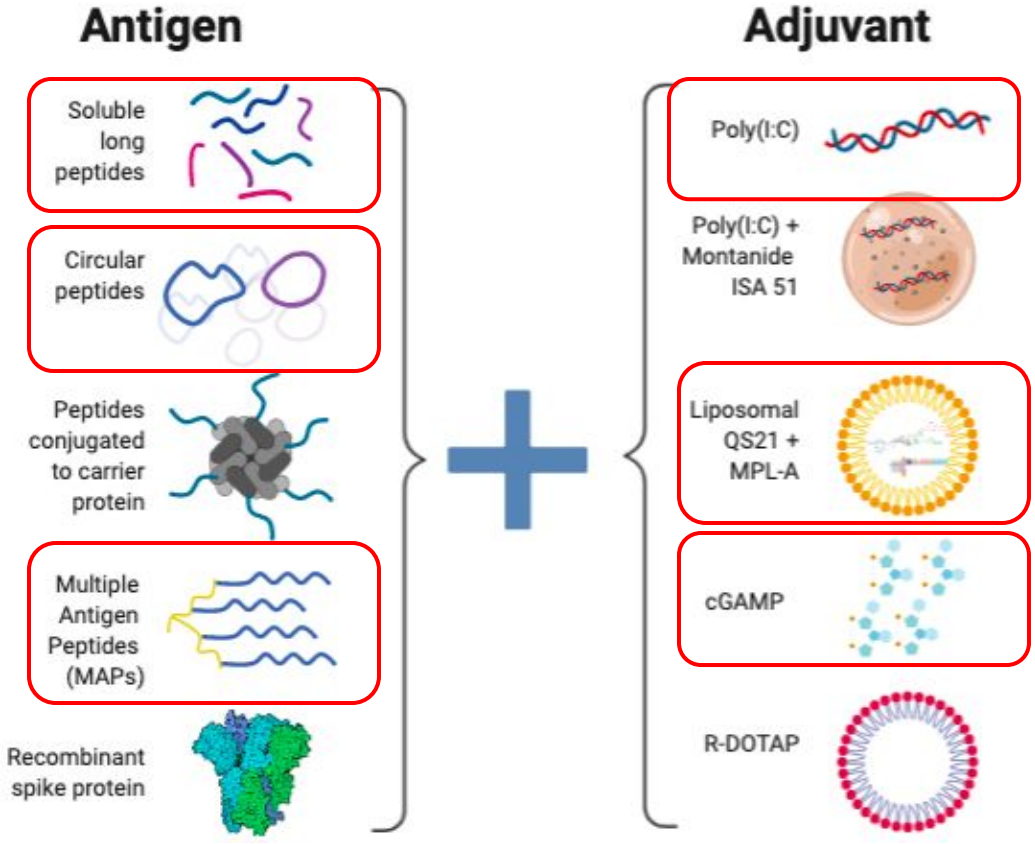
Preliminary ELISpot Results

- Highlighted strongest responses in n=5 peptide groups
  - A3: **N**310-336
  - B1: **M**95-121
- Still highest when combined w/ other peptides
  - ~3x-4x reduction in mean well intensity



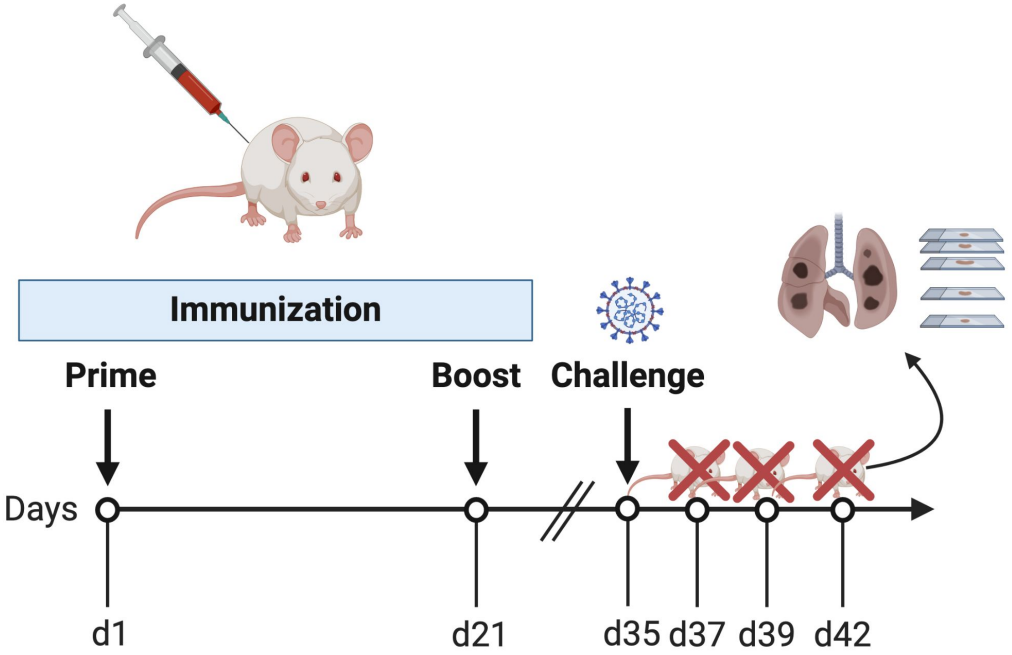
Future Experiments

- Find best adjuvant for each antigen
  - small # of mice
- Compare antigens to each other + recombinant spike



Challenge / Protection (Heise Lab)

- Vaccine candidates with strong T-cell or B-cell responses repeated and tested for:
  - Neutralization
  - Protection from challenge with murine adapted SARS-CoV-2



- Vaccine & Cell Therapy Lab at Mount Sinai interested in starting a trial based on successful candidates, but hopefully not necessary

Beyond SARS-CoV-2

- **Other coronaviruses**: If protective against SARS-CoV-2 in mice, will same formulation & selection algorithm work for MERS?
- **Improve T-cell epitope prediction**: Large amounts of emerging T-cell epitope mapping from convalescent Covid-19 patients (ground truth)
  - Collaboration with Colin Raffel's to apply modern NLP deep learning techniques to antigen processing and T-cell epitope prediction
  - "Modern techniques" = Transformer neural network architecture, self-attention, semi-supervised and contrastive learning
- **Back to cancer vaccines**: Use improved algorithms and vaccine formulation for personalized cancer vaccine (PANDA-VAC 2.0?)

Fin