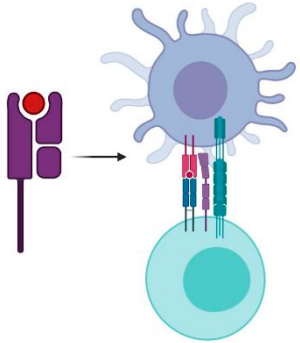
Neoantigens beyond SNVs

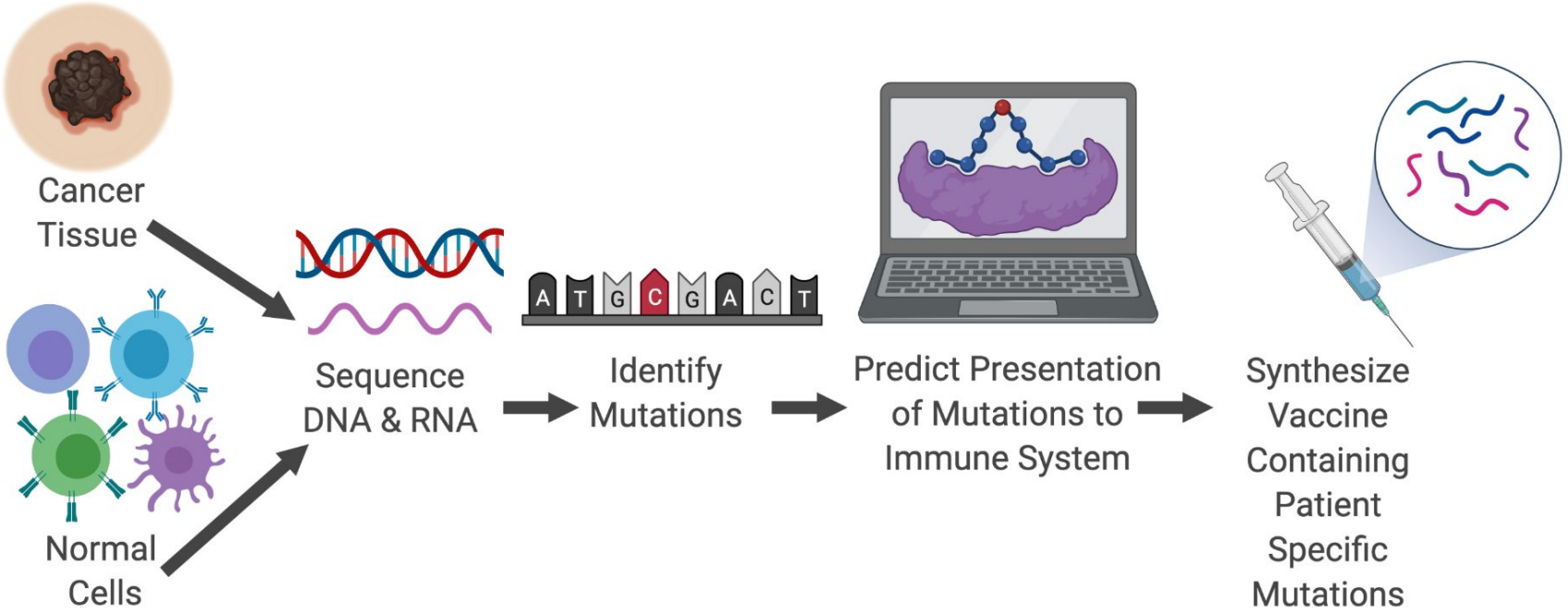
Embracing the full spectrum of antigenic dark matter in tumors



Alex Rubinsteyn
CIMT
May 12th, 2021

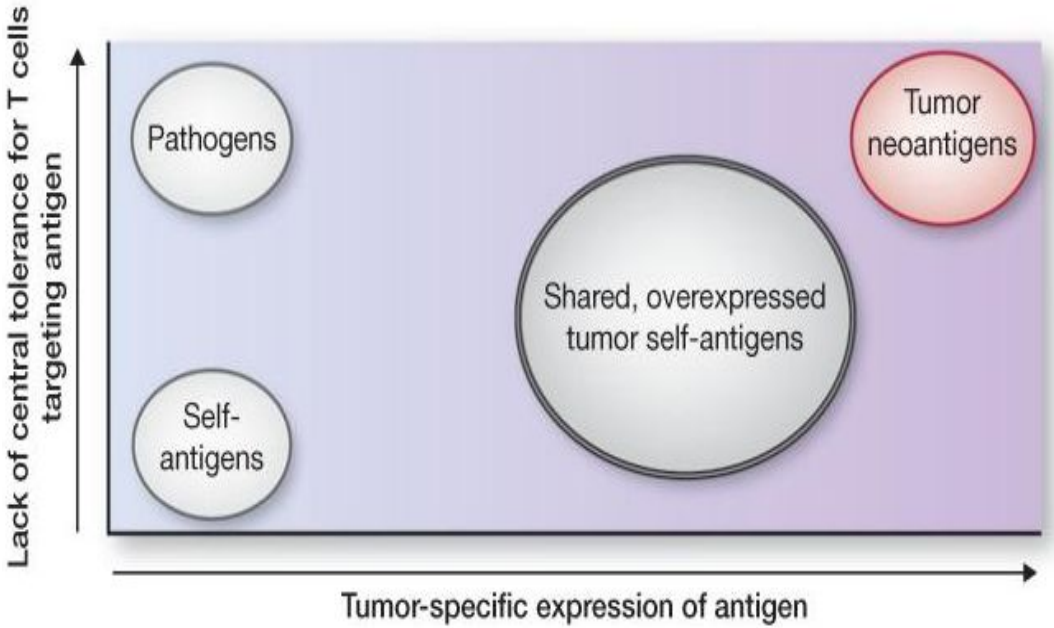
UNC
SCHOOL OF MEDICINE

Neoantigen vaccines: extremely personalized medicine



Neoantigens

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



Getting Personal with Neoantigen-Based Therapeutic Cancer Vaccines

PGV clinical trials at Mount Sinai

Personalized **G**enomic **V**accine

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

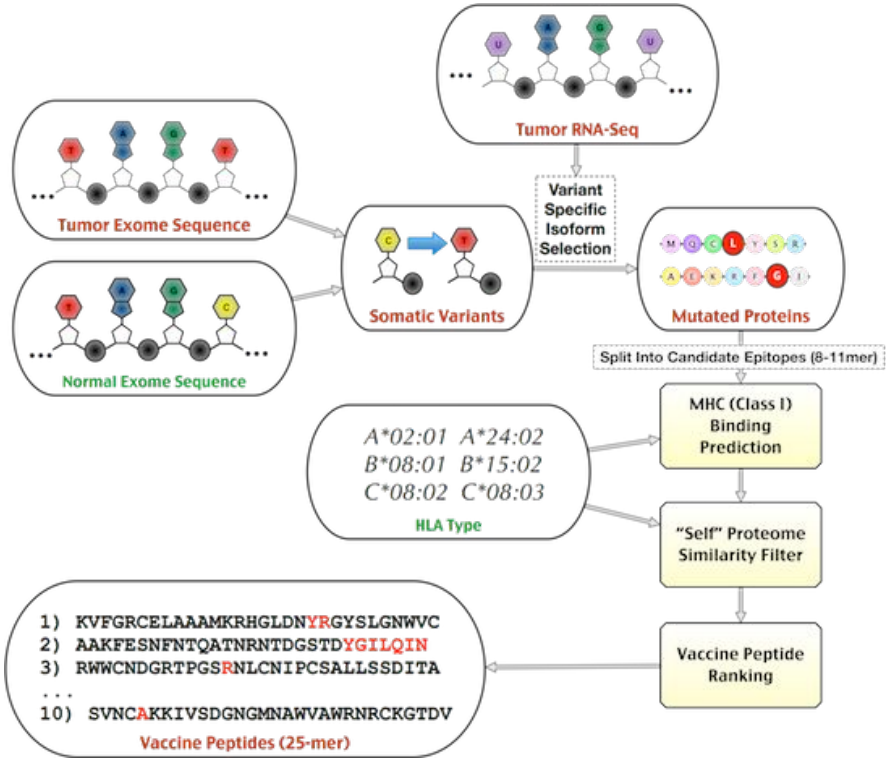


Shared design:

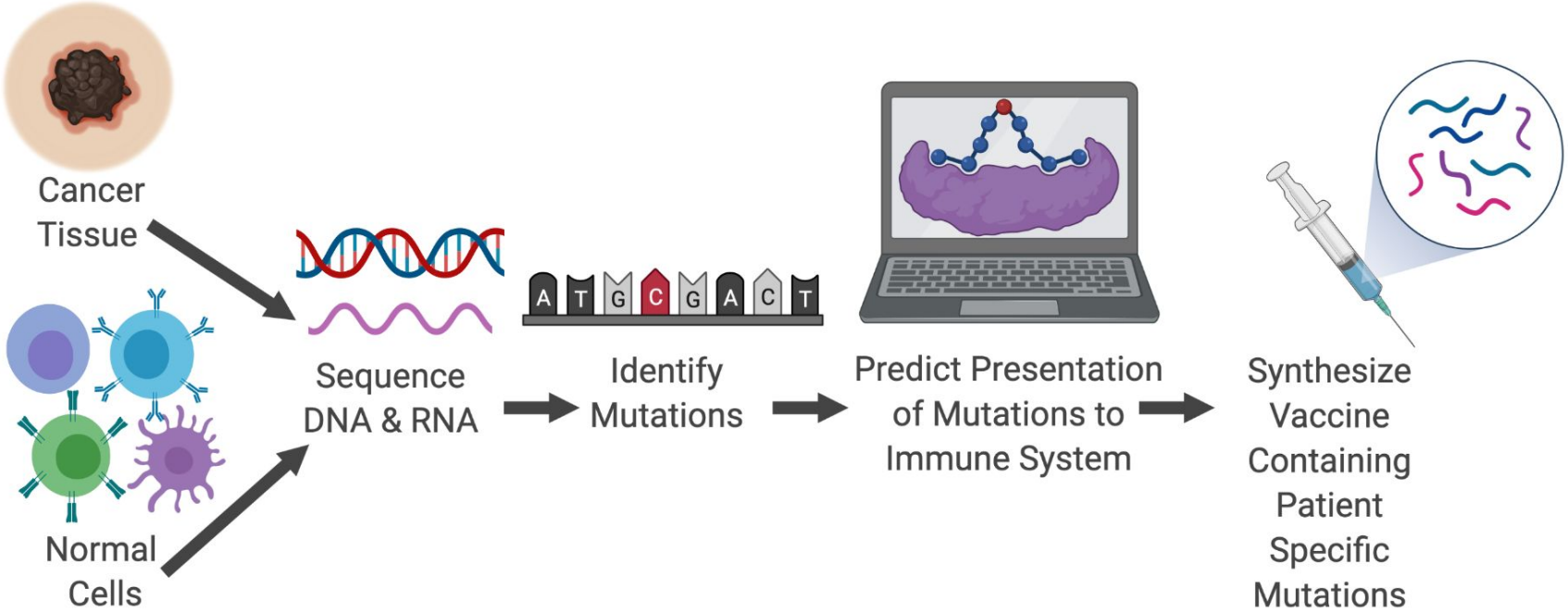
- Up to 10 peptides
- Peptide length: 25aa
- 10+ injections per trial over 6 months
- Adjuvant: poly-ICLC

PGV sequencing & informatics

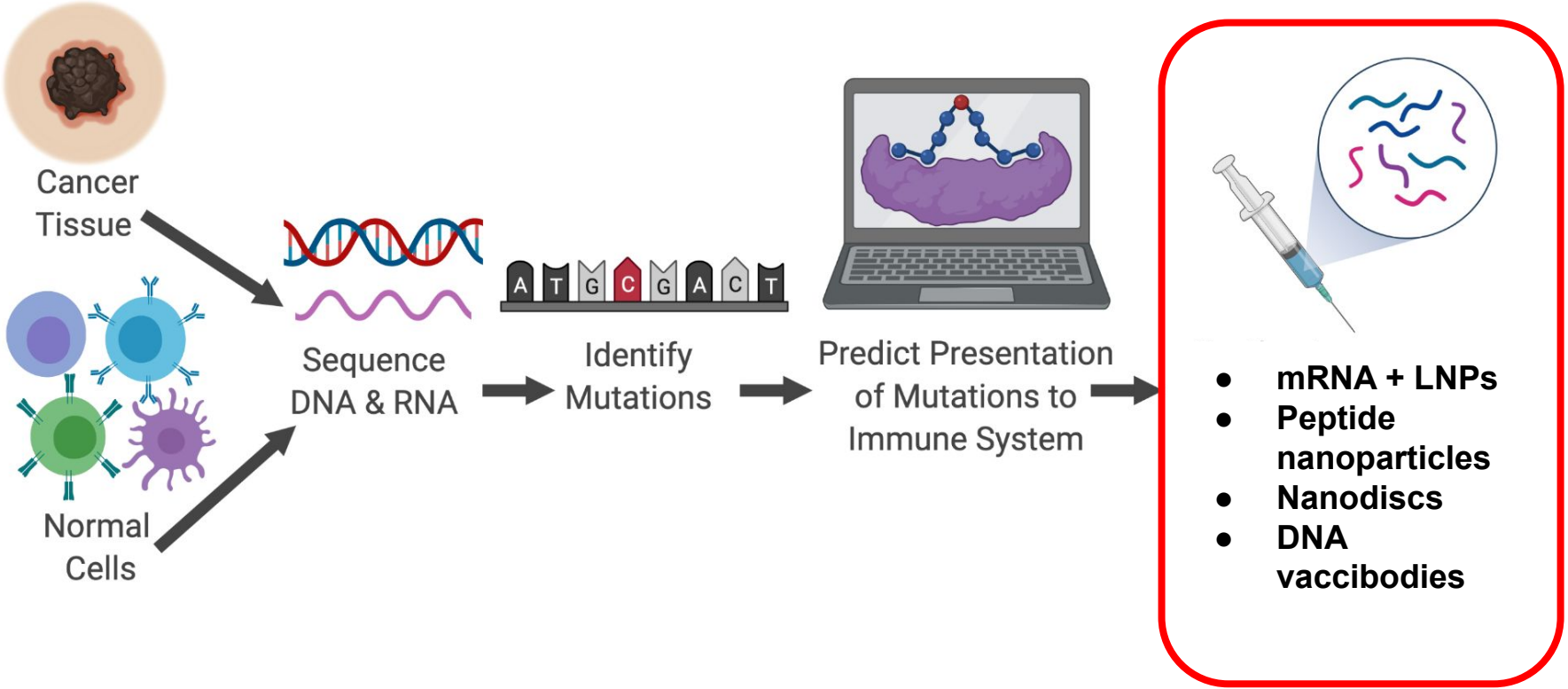
- **Sequencing**
  - Tumor/normal WES
  - Tumor mRNA-seq
- **OpenVax pipeline**
  - Identify tumor-specific mutations
  - Predict patient HLA binding of mutant peptides
  - Quantify expression of mutations
  - Rank 25mer vaccine peptides by: *MHC affinity * RNA abundance*



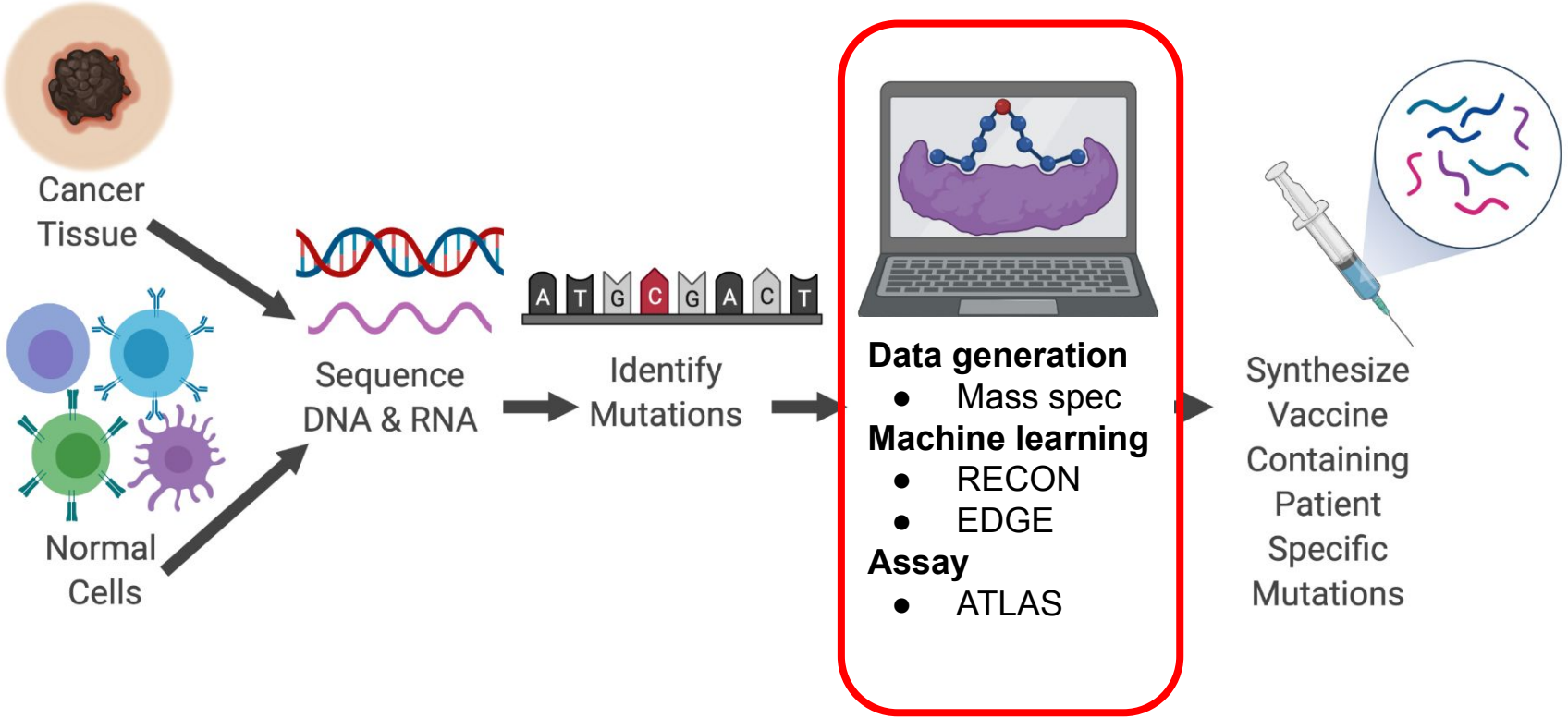
Neoantigen vaccines: secret sauce?



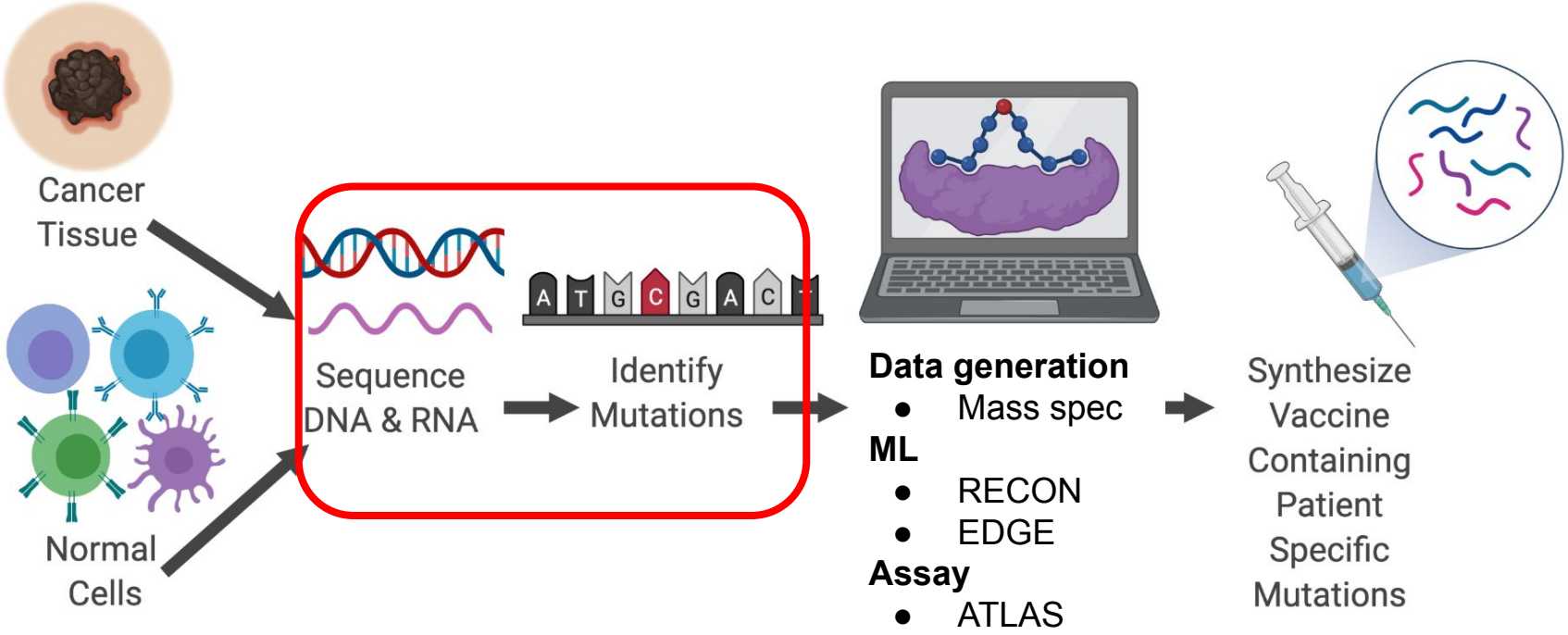
Secret sauce: vaccine platform



Secret sauce: immuno-informatics

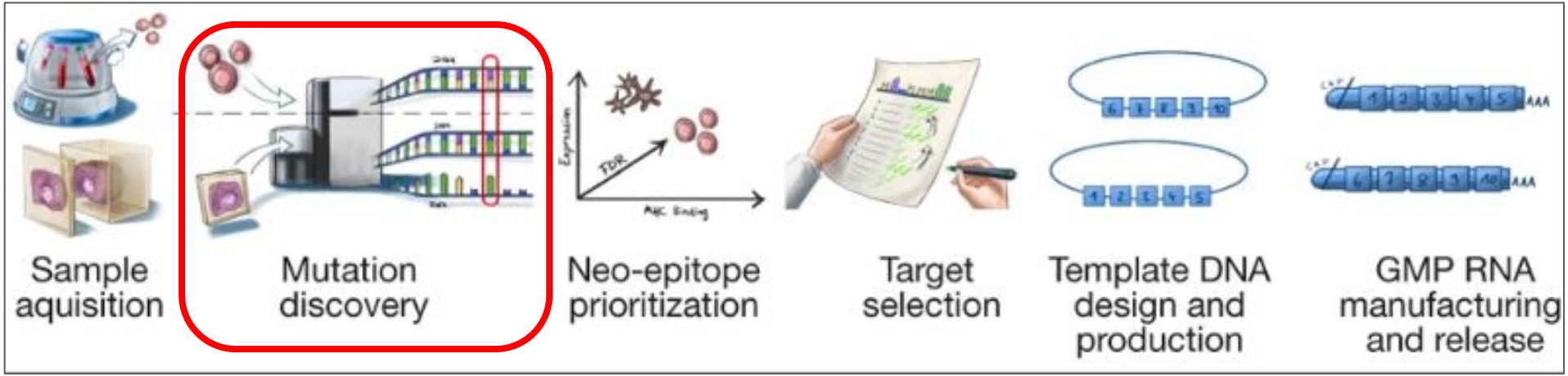


Secret sauce: genomics???



The neoantigen field is addicted to short read exome sequencing

BioNTech (Sahin, …, Türeci 2017)

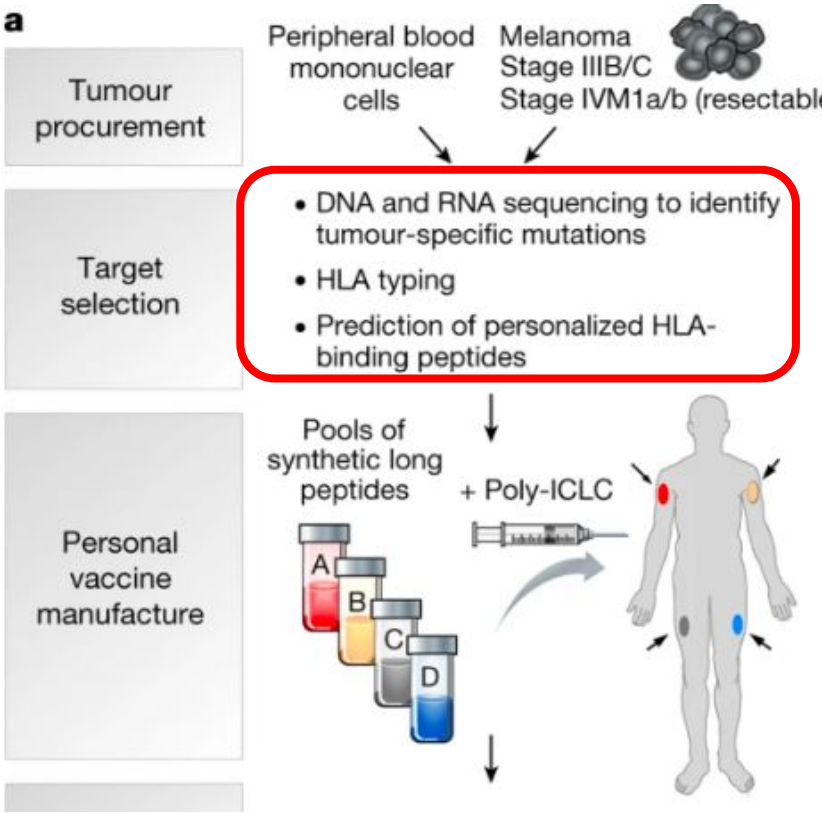


Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer

- Tumor/normal WES
- SNVs only

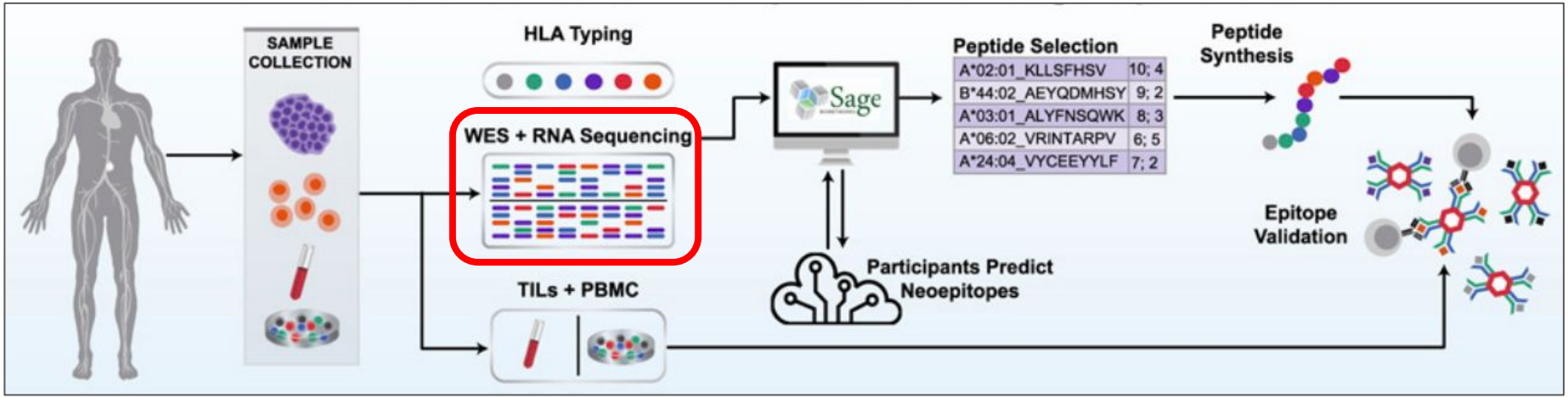
DFCI (Ott, …, Wu 2017)

- Tumor/normal WES
- SNVs and small indels
  - Mutect
  - Indelocator
  - Strelka



An immunogenic personal neoantigen vaccine for patients with melanoma

TESLA (Wells, …, Defranoux 2020)

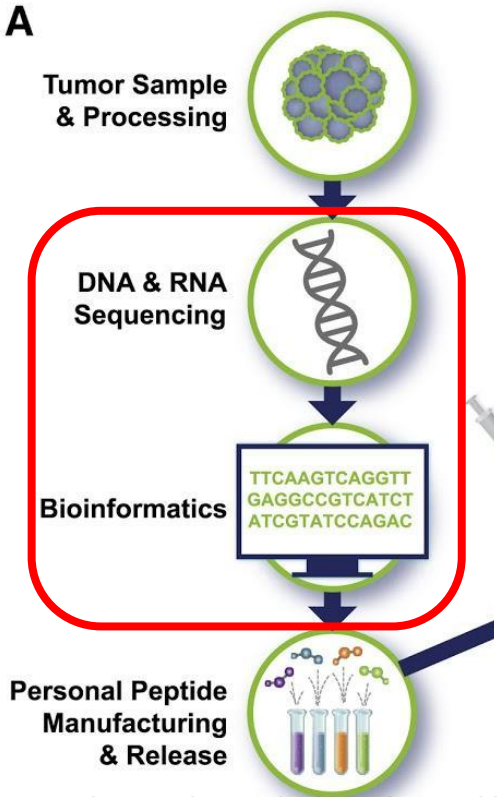


Key Parameters of Tumor Epitope Immunogenicity Revealed Through a Consortium Approach Improve Neoantigen Prediction

- Tumor/normal WES
  - Variant calling: SNVs and small indels
- Tumor mRNA-seq
- *Task*: prioritize variants, some T-cell response validation

Neon (Ott, …, Srinivasan 2020)

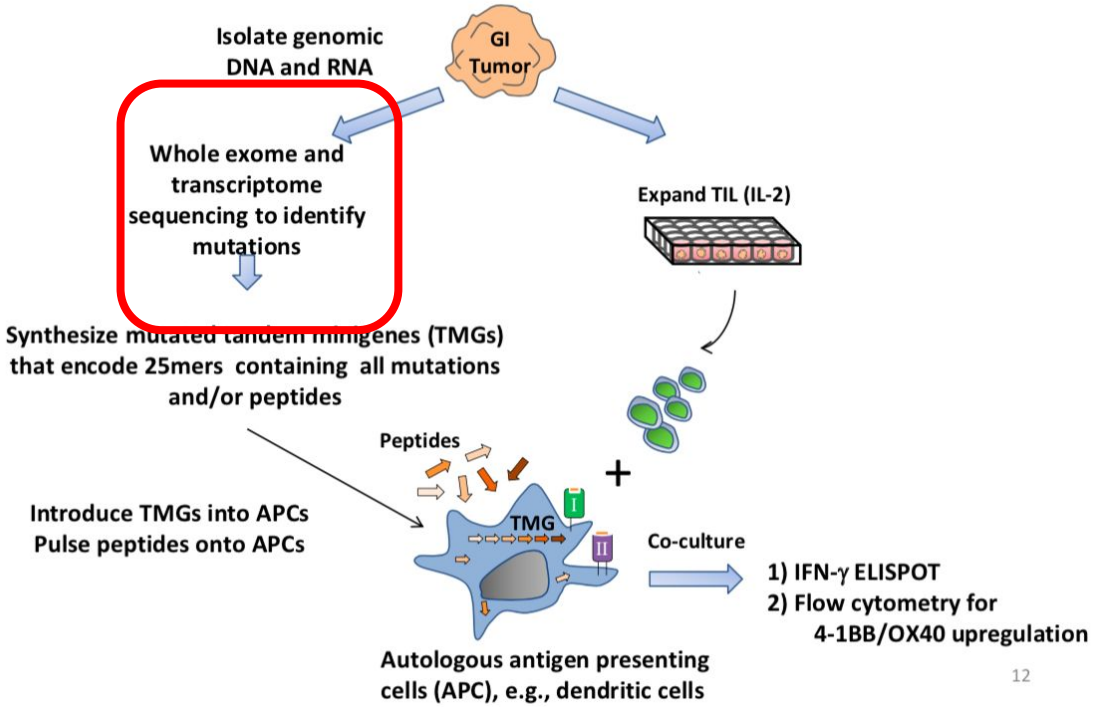
- Tumor/normal WES
- Variant calling: SNVs and small indels
  - VarDict
  - Strelka
  - Mutect2
  - VarScan2
  - Atlas Indel2
  - Seurat
  - Platypus



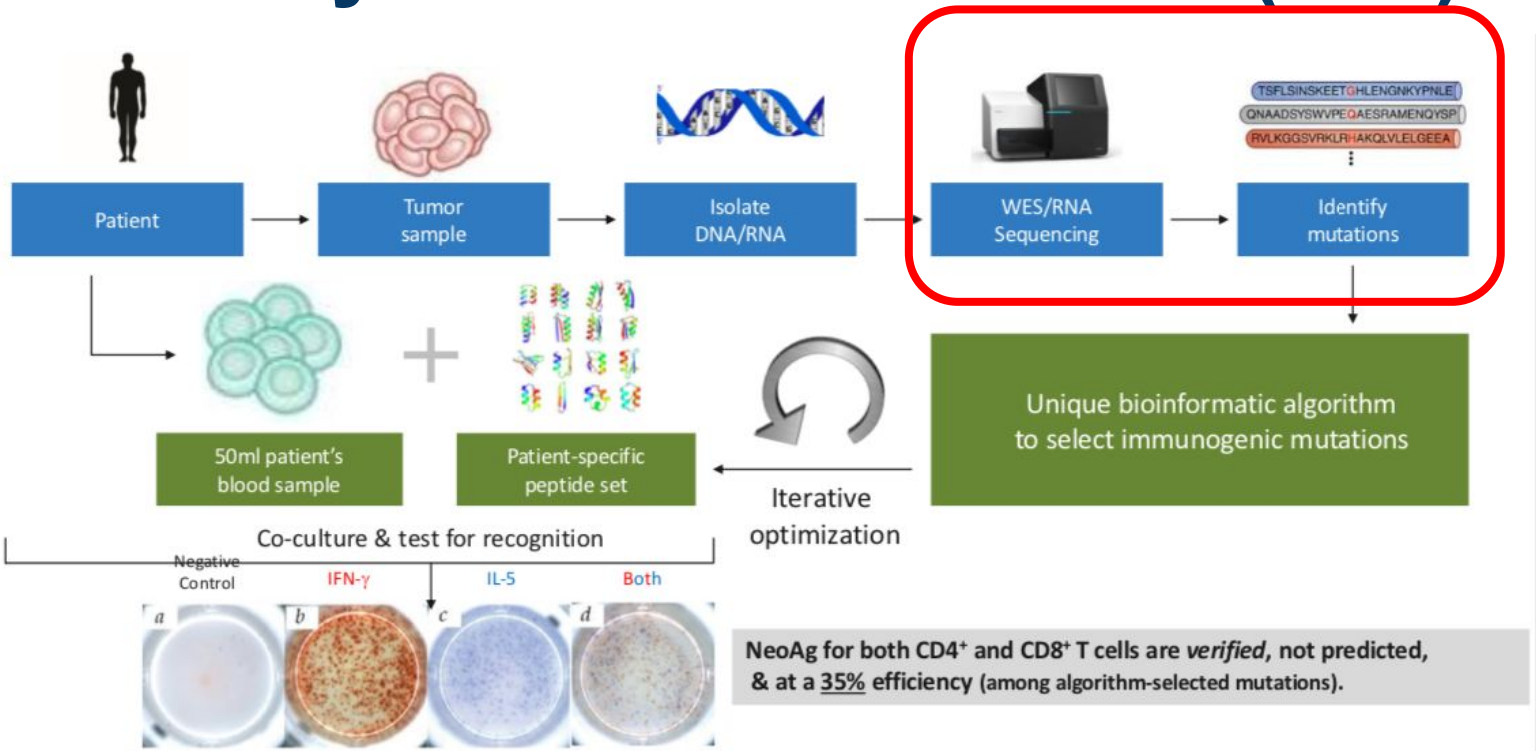
A personal neoantigen vaccine, NEO-PV-01, with anti-PD1 induces broad de novo anti-tumor immunity in patients with metastatic melanoma, NSCLC, and bladder cancer

Cell therapy: NCI's Rosenberg lab

- Preliminary screening of "all" coding mutations using tandem minigenes
  - WES
  - SNVs + small indels
- Identify specific reactive neoantigens using peptides

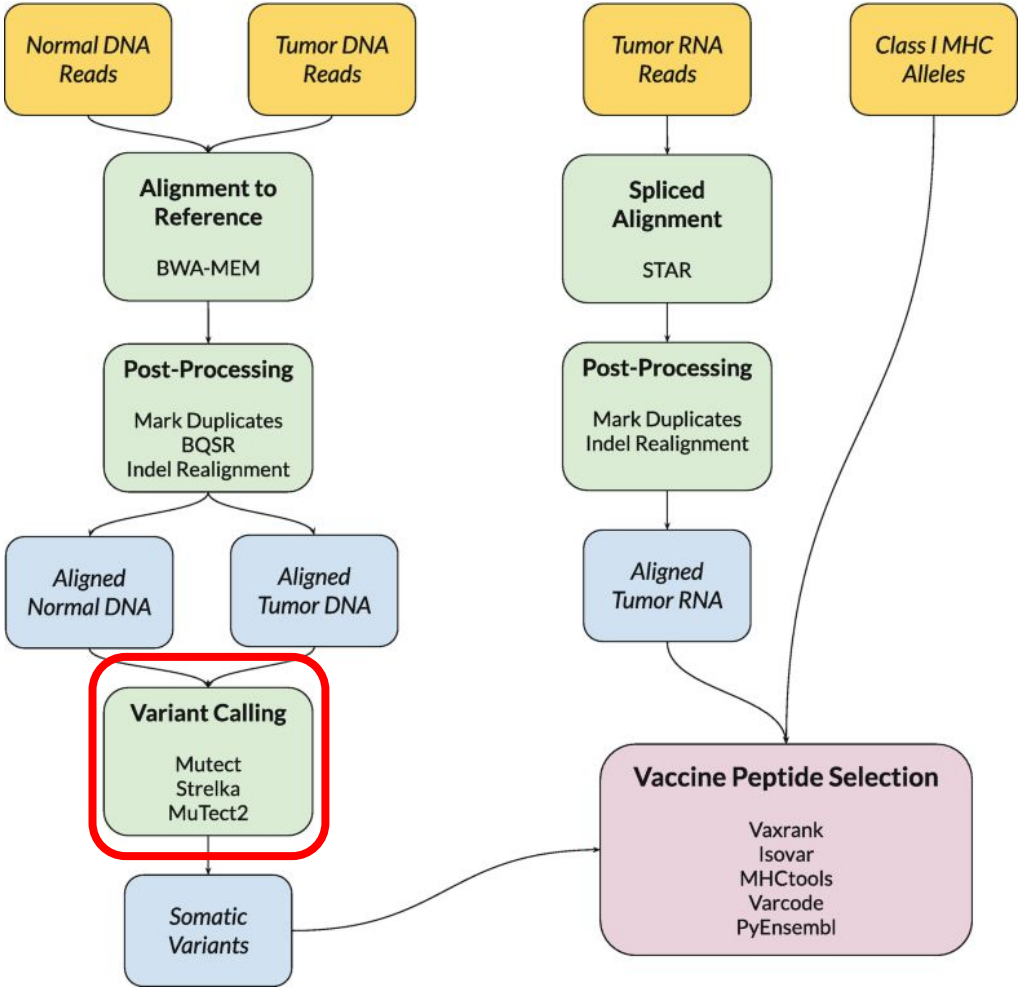


Cell therapy: LJI's Identify-Prioritize-Validate (IPV)



OpenVax (Mount Sinai)

- **SNVs & small indels**
- Inputs:
  - Tumor & normal exome
  - RNA-seq
- Outputs:
  - Vaccine peptides w/ multiple predicted neoantigens
- Special features: variant phasing & *some* splice variants

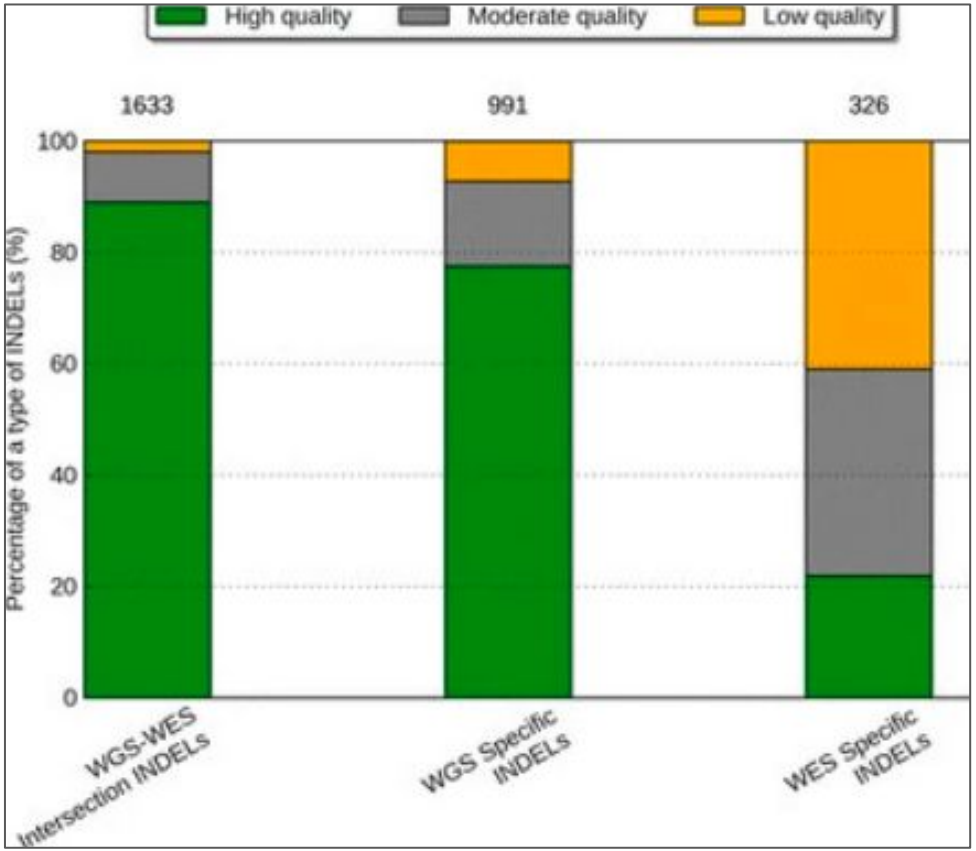


WES isn’t great even for small mutations

WGS better than WES for exonic indels

- Comparison of 110x WES vs. 30x WGS
- WGS detects more exonic indels
- WES-only indels are likely false positives

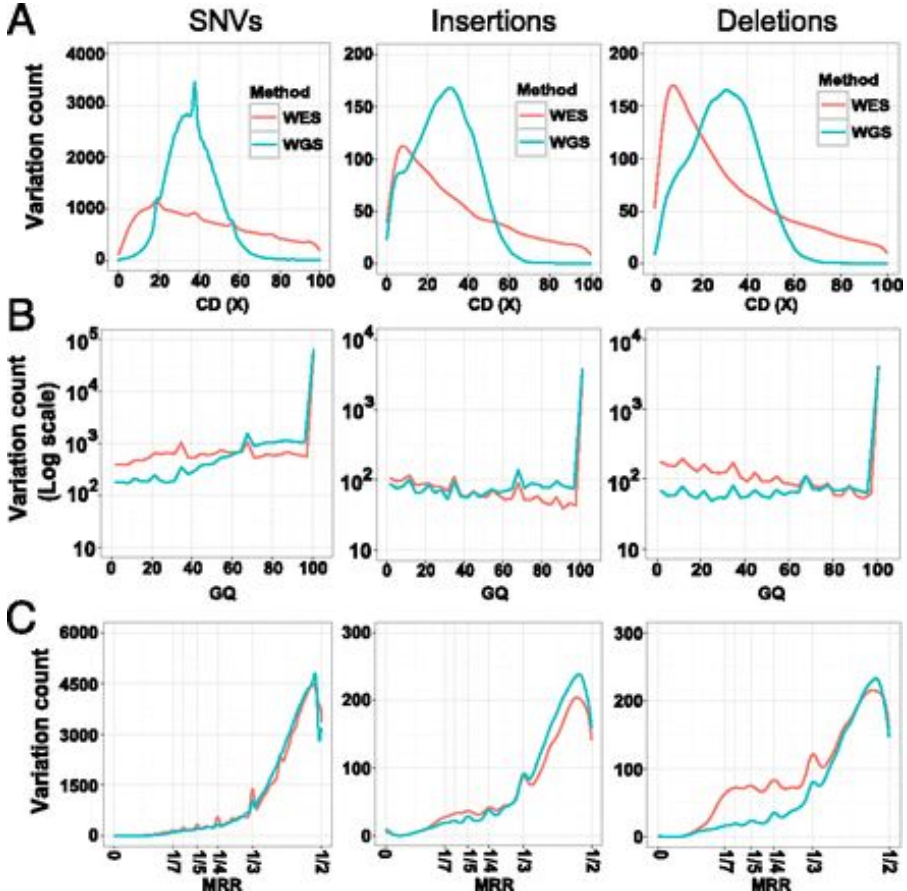
Reducing INDEL calling errors in whole genome and exome sequencing data



WGS better than WES for all exonic variants

- Comparison of 73x WES vs. 39x WGS
- Higher quality exonic variant calls in WGS data
- ~3% more high quality SNVs in exons from WGS

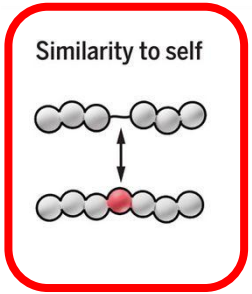
Whole-genome sequencing is more powerful than whole-exome sequencing for detecting exome variants

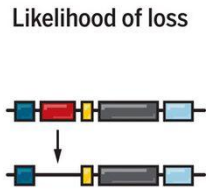


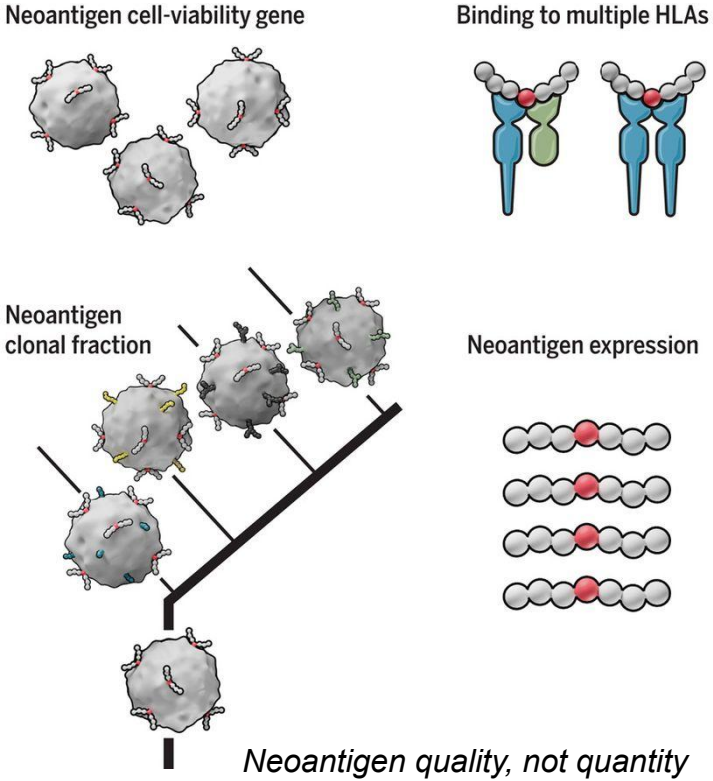
Beyond SNVs
(and a tiny number of small indels)

What makes a good neoantigen?

- Vaccine peptides containing substitutions derived from SNVs are almost entirely self
- You can (sometimes) get vaccine induced recognition of a single mutant amino acid, but why make things hard?



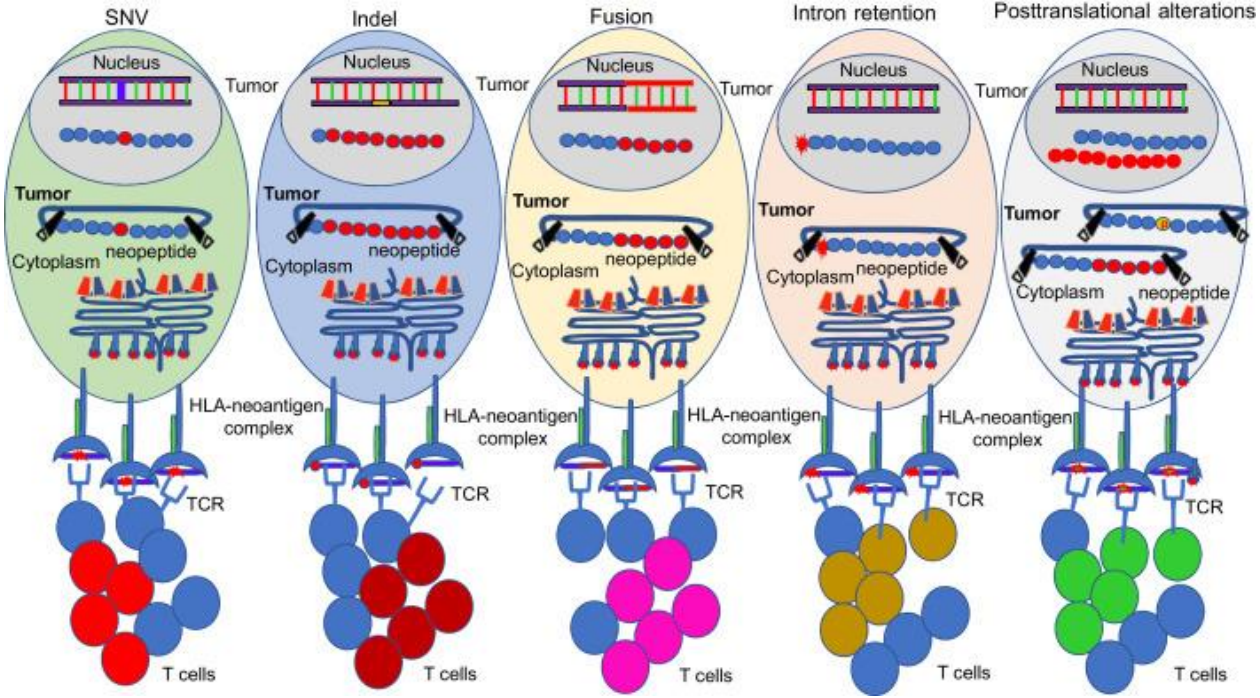




Neoantigen quality, not quantity

Neoantigens from “large” mutations

- Indels
  - Of all sizes!
- Structural variants
  - Fusions
  - Duplications
  - Inversions
- Splicing
  - Exon skipping
  - Intron retention
- Viral integration

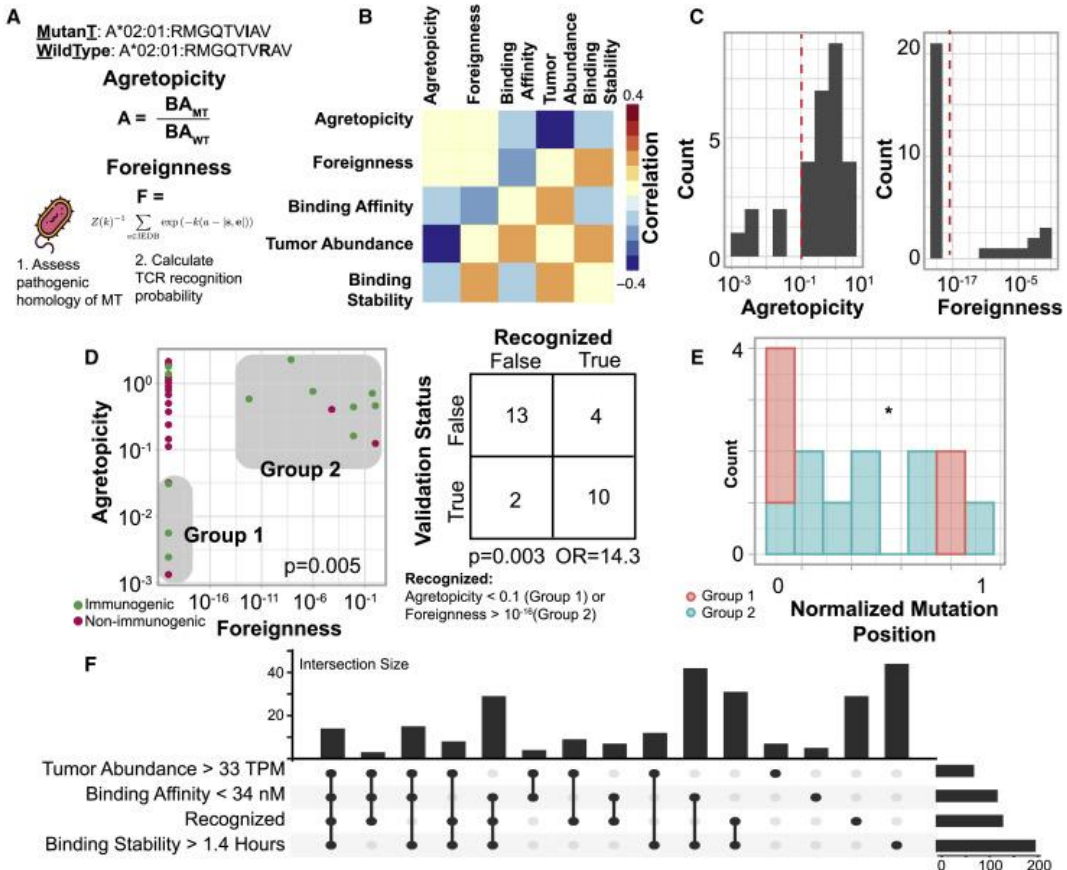


Diverse Neoantigens and the Development of Cancer Therapies

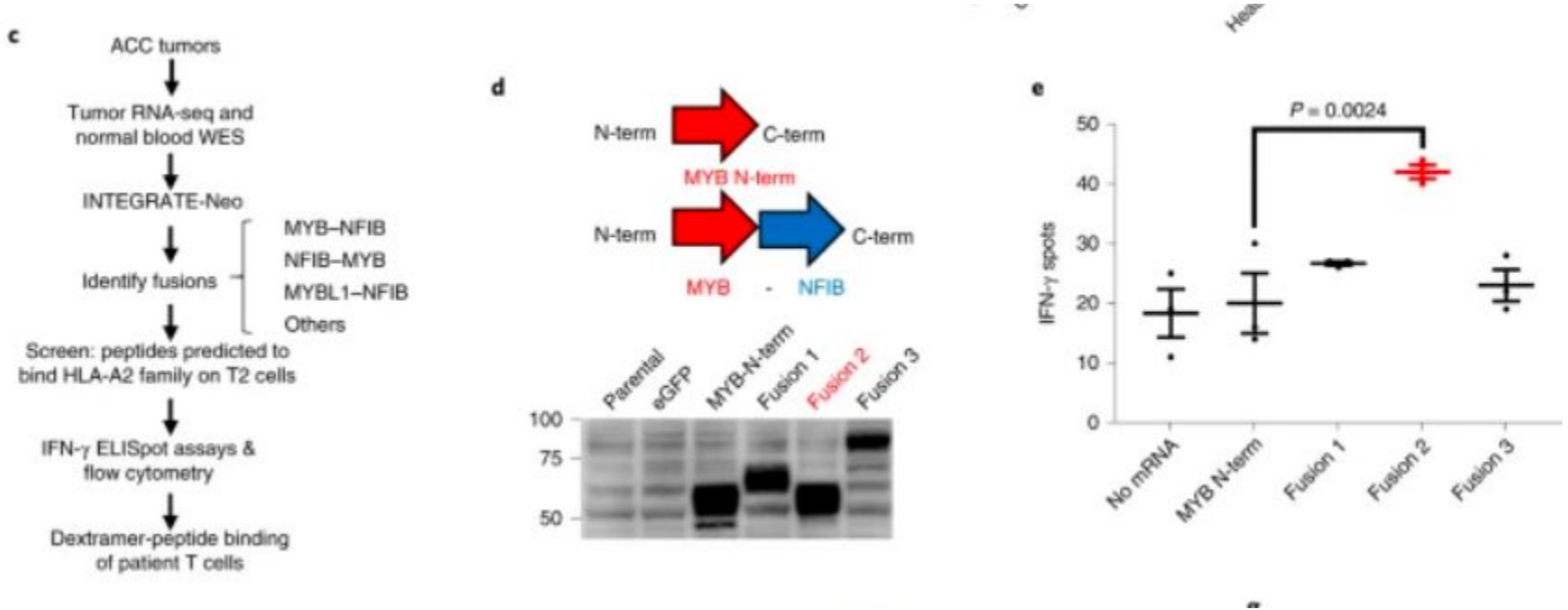
Quality of mutations: major blind spot

- Evaluated many different neoantigen predictive factors
- Starting set of mutations is impoverished due to short read WES!

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



Fusion neoantigens from RNA-seq

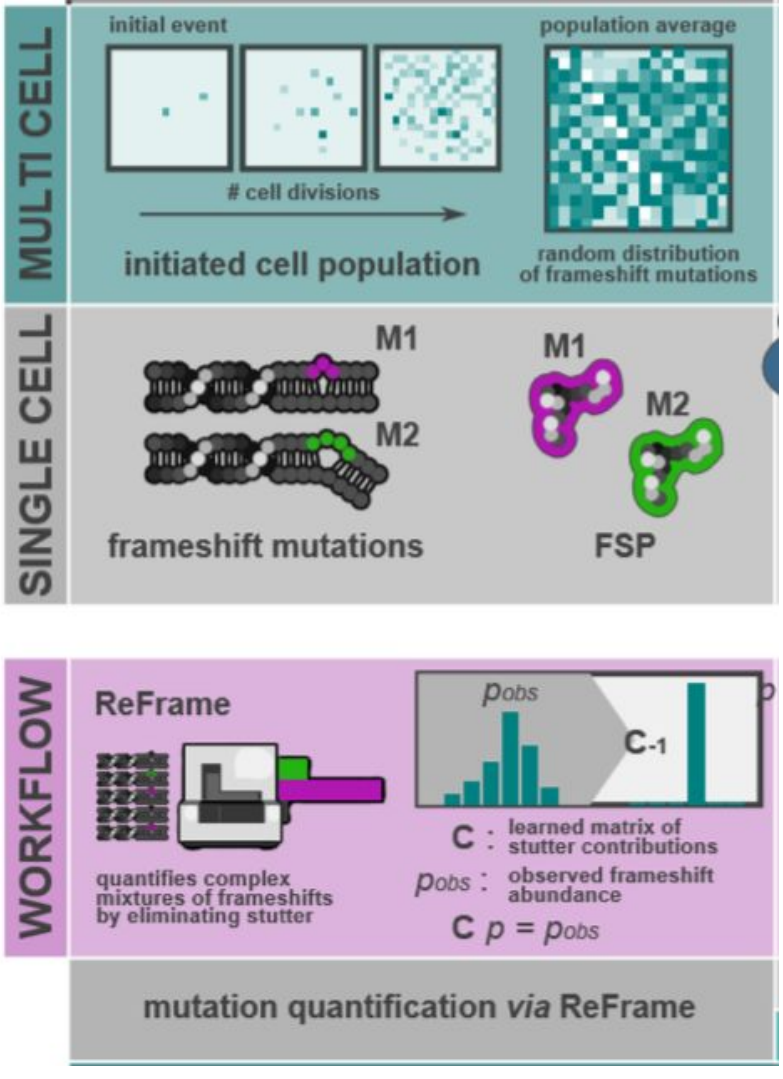


Immunogenic neoantigens derived from gene fusions stimulate T cell responses

Identifying MSI frameshifts from short read sequencing

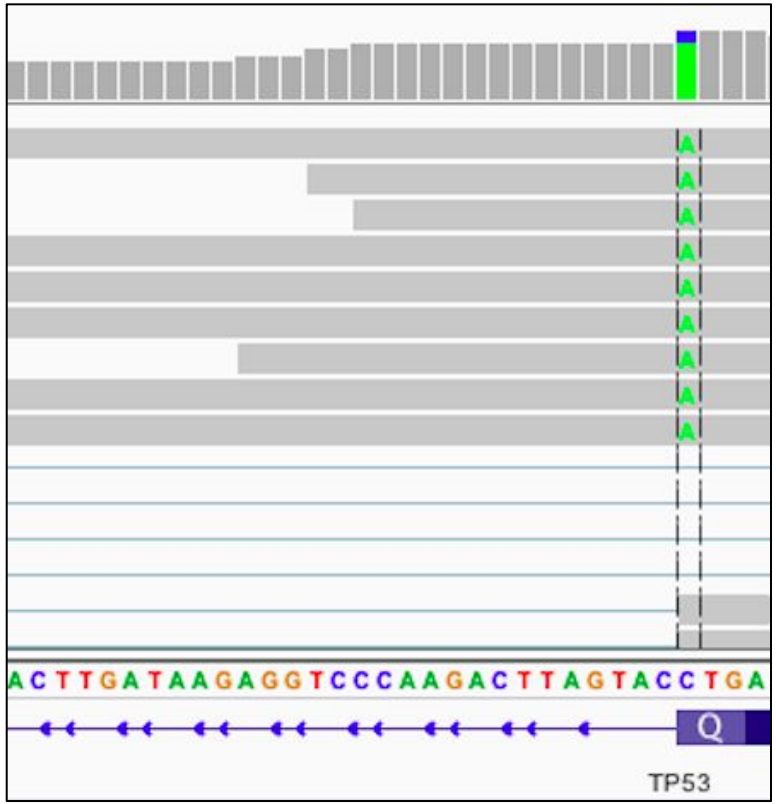
- Deconvolution of coding microsatellite frameshifts
- *“(NGS) approaches have a limited sensitivity for the detection of indel mutations at homopolymer sequences such as neoantigen-related cMS”*

The shared neoantigen landscape of MSI cancers reflects immunoediting during tumor evolution



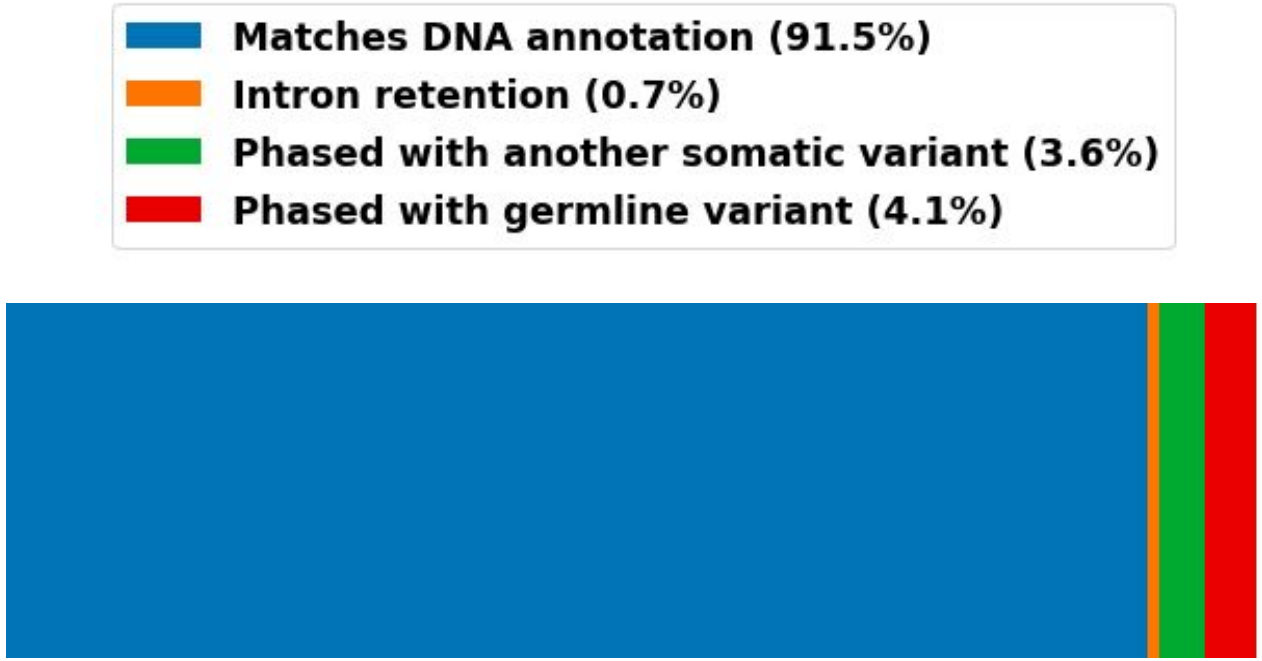
PGV: splice-site variant example

- Example in TP53 from PGV001 patient
- C>A genomic variant at last base of exon (G>U mRNA change)
- Consensus 5’ splice signal is GG|GU
- With mutation, this exon of TP53 ends with “AU” instead of “GG”
- All RNA reads with mutation retain intronic sequence!

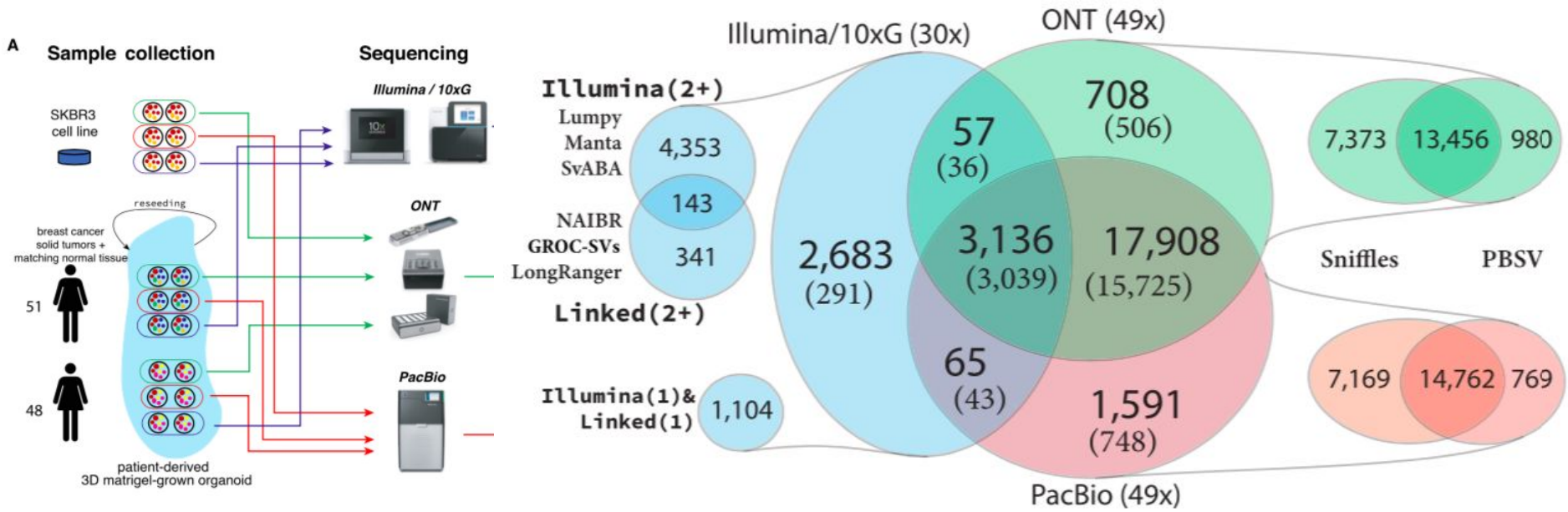


PGV: phasing + splice site variants

- Mount Sinai PGV patient mutations, DNA-only annotation is correct sequence for ~91.5% of variants
- Co-occurrence with germline and other somatic variants



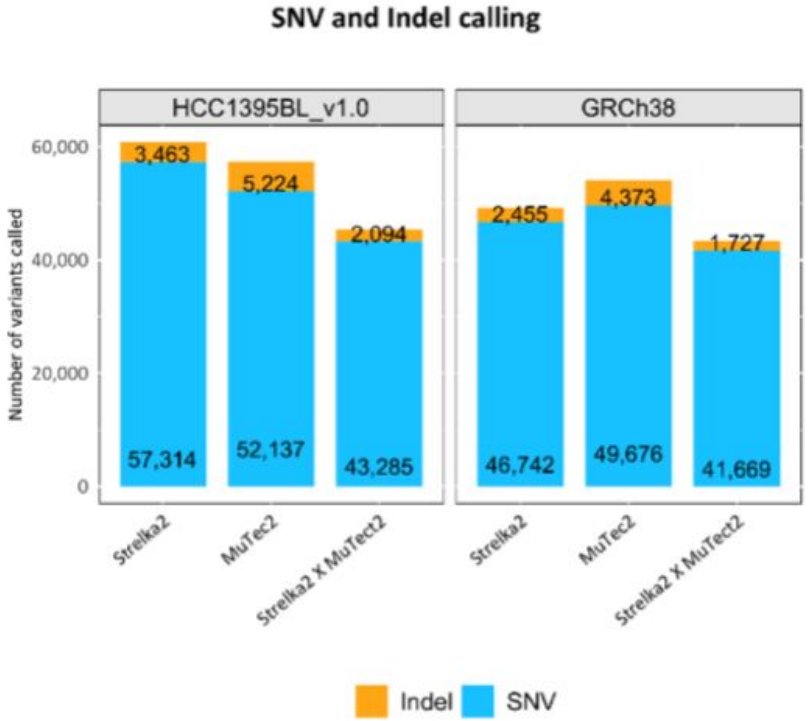
Short read sequencing misses most cancer mutations



Comprehensive analysis of structural variants in breast cancer genomes using single-molecule sequencing

Long read somatic variant calling with personalized genome assembly

- *“we identified 3,498 large deletions, 2,239 large insertions and 101 Duplications (DUP) as somatic SVs”*

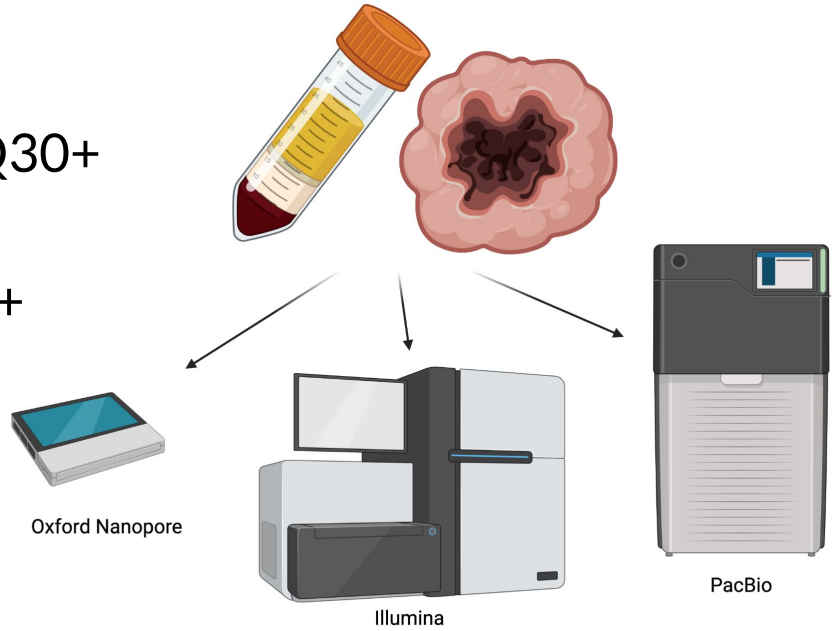


Personalized genome assembly for accurate cancer somatic mutation discovery using cancer-normal paired reference samples

Pilot study

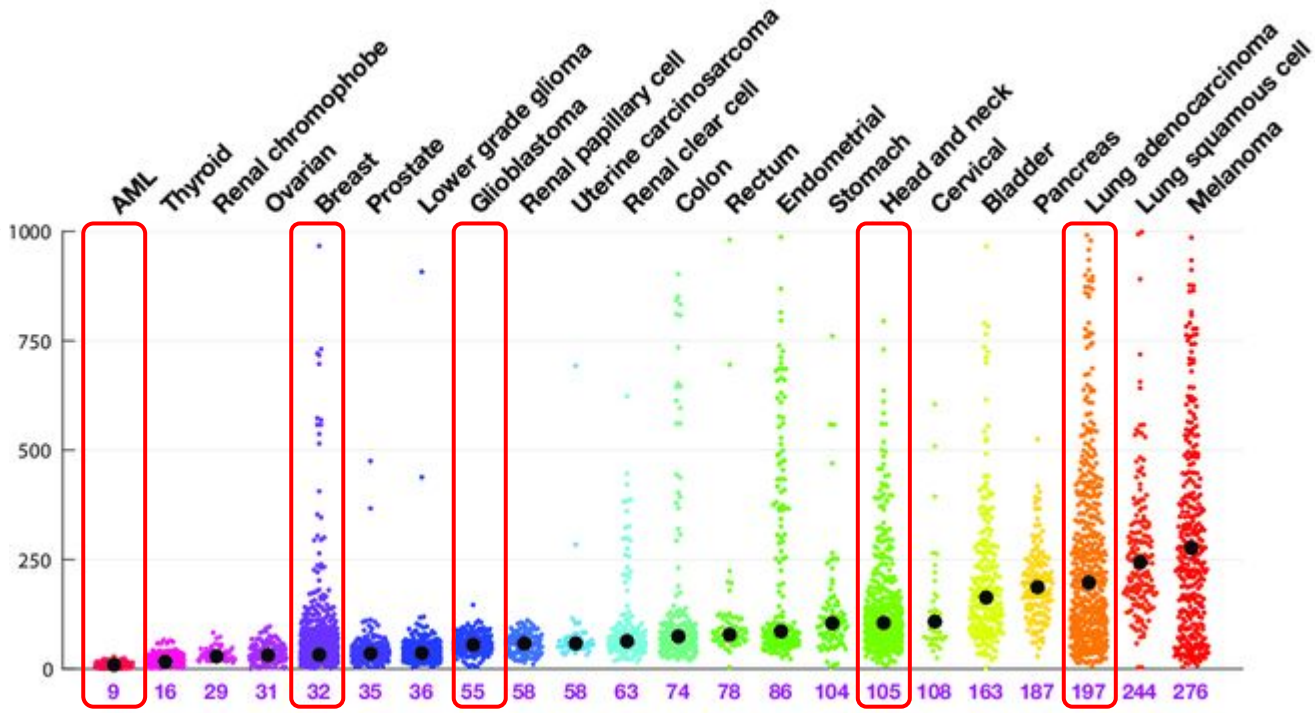
Sequencing technology bake-off

- **Tumor/Normal DNA**
  - Illumina WES
    - 150bp, variable coverage, Q30+
  - Illumina WGS
    - 150bp, even coverage, Q30+
  - Oxford Nanopore WGS
    - 10kb to 1Mb reads, <Q20
  - PacBio HiFi WGS
    - ~30kb reads, >Q40
- **Tumor mRNA**
  - Illumina vs. Oxford Nanopore vs. PacBio IsoSeq



Sample Types

- **Cell lines**
  - U87
  - U937
- **Clinical samples**
  - AML
  - GBM
  - TNBC
  - LUAD
  - HPV+ HNSCC



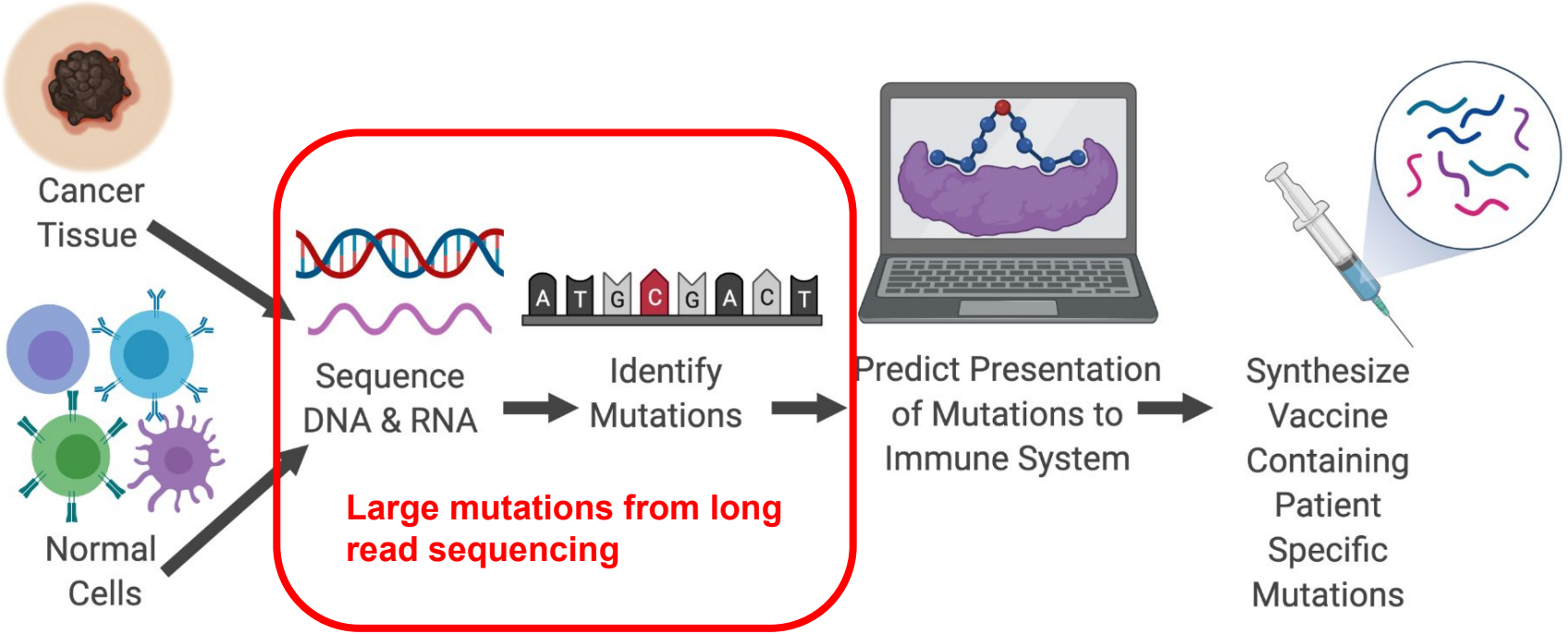
Mutation-Derived Neoantigens for Cancer Immunotherapy

Analysis

- **Basic principles**
  - discovery in DNA
  - validation and quantification in RNA
  - SNV-free zone (look for everything else)
- **Algorithmic Approaches**
  - Reference-guided
    - Difference of high sensitivity normal, high specificity tumor
  - Personalized genome assembly
  - Graph genome
- **Evaluation**
  - Validation in RNA (only care about expressed variants)

Big picture: better neoantigen vaccine

PANDA-VAC trial(s) with Ben Vincent and Jared Weiss @ UNC



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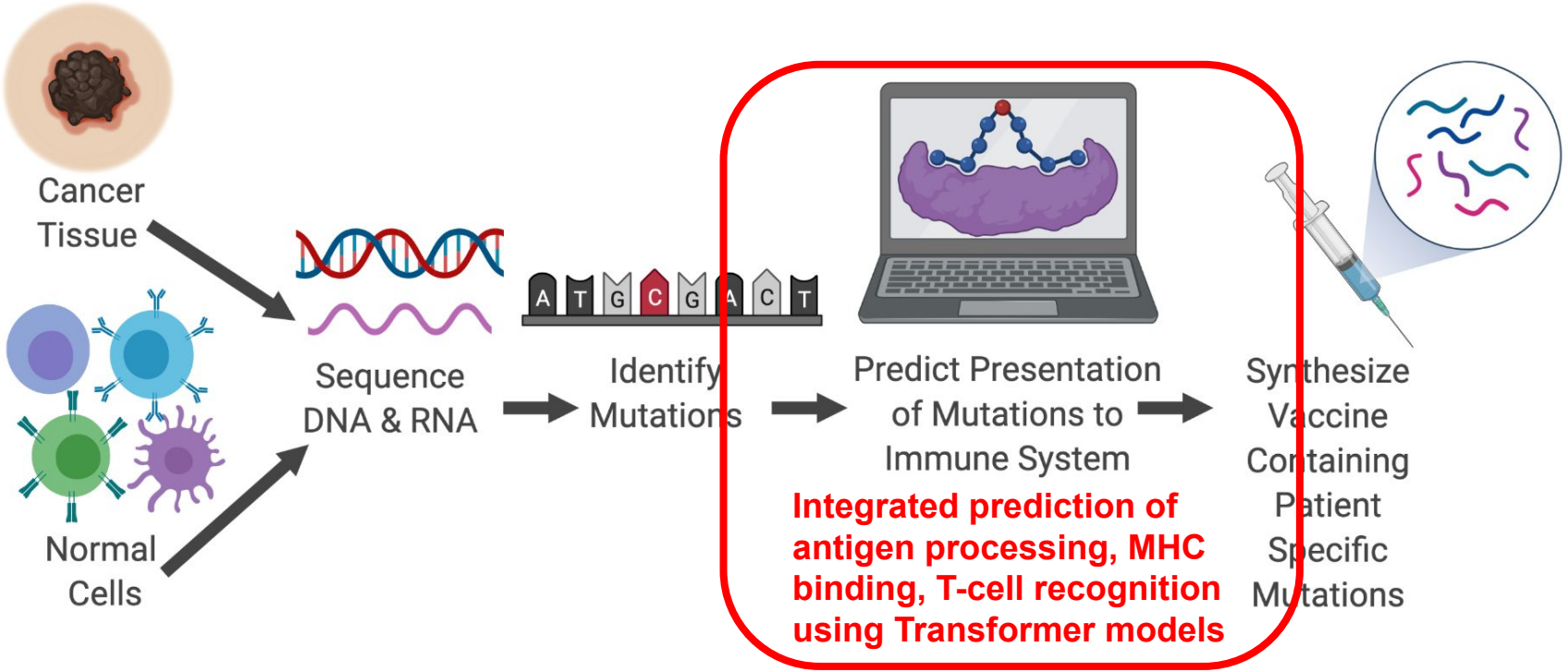

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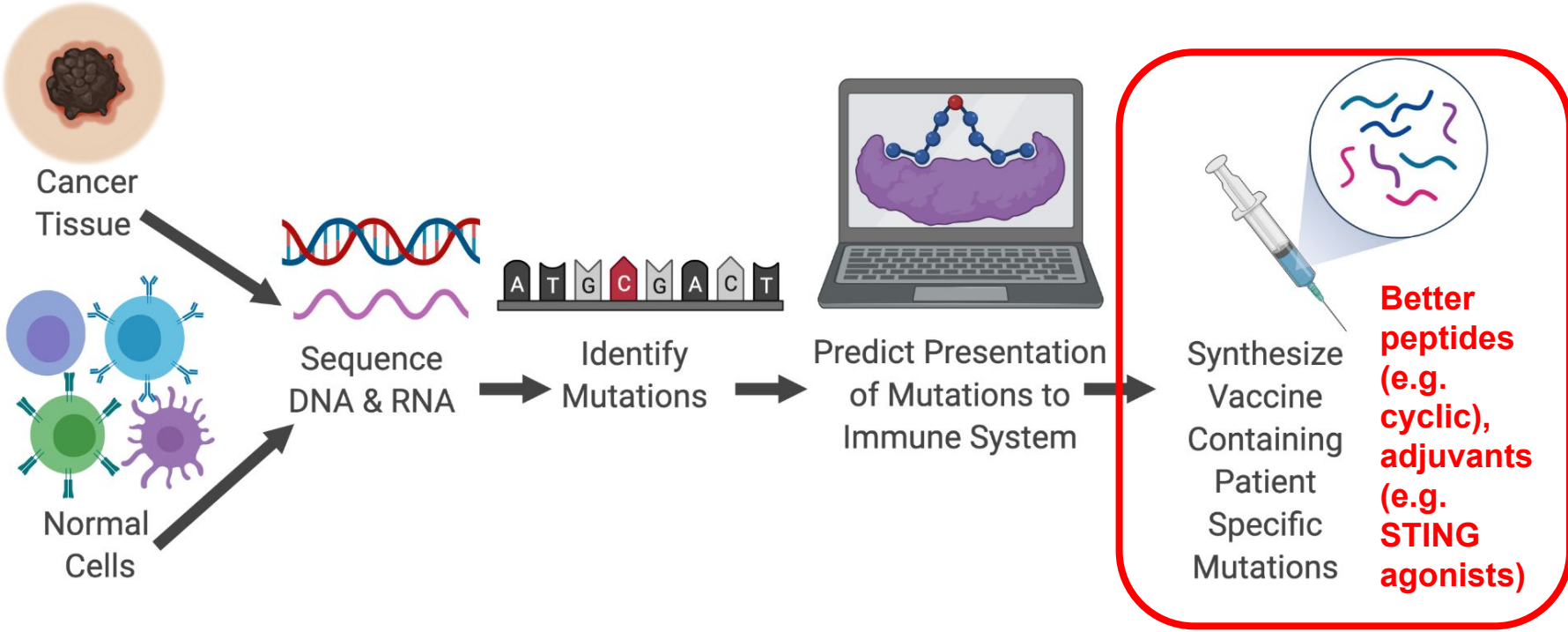


Fin

Big picture: better neoantigen vaccine

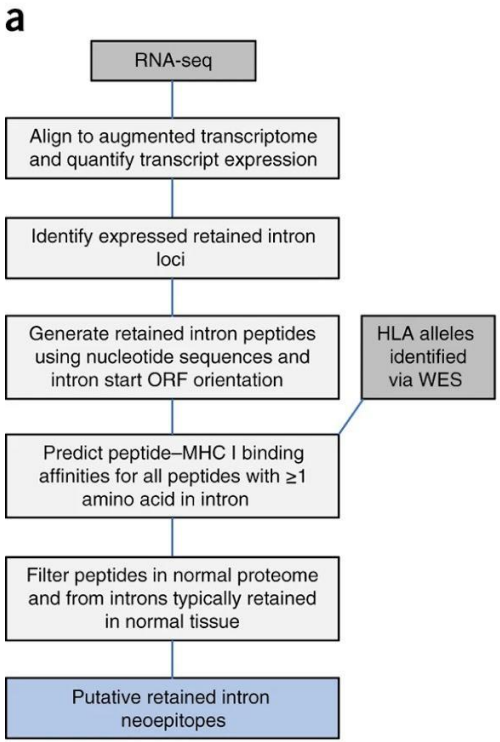
- **PANDA-VAC trial with Ben Vincent and Jared Weiss**
- **Rich initial set of “large” mutations**
  - High accuracy long read sequencing of tumors
  - Ignore SNVs, only target fusions, indels, and other “large” mutations such as viral integration sites, repeat expansions, &c
- **Immunogenic vaccine formulation**
  - Antigen and adjuvant based on SARS-CoV-2 experiments
- **Improved T-cell epitope prediction**
  - Multi-output Transformer model which predicts T-cell response assays as well as peptide-MHC affinity, antigen processing &c

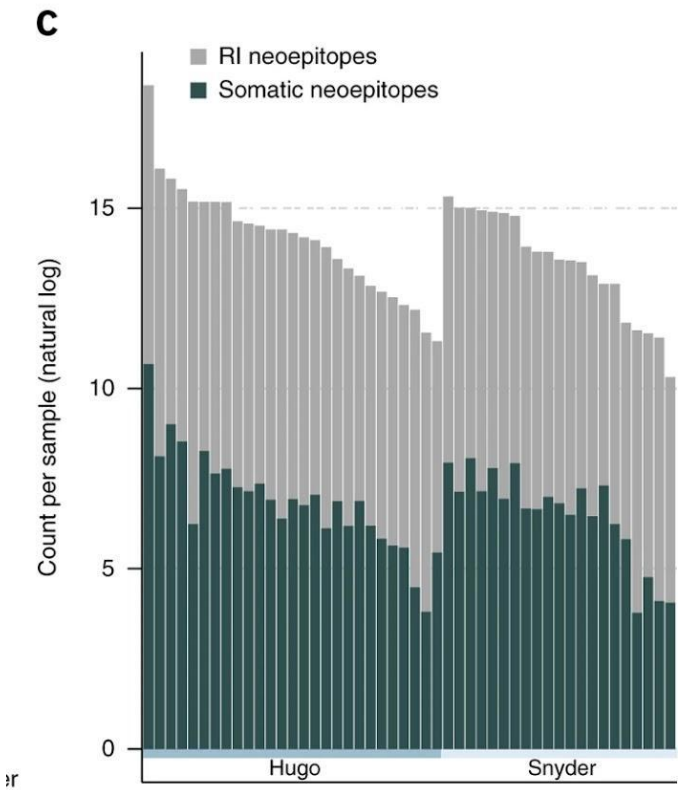
Intron retention in cancer

Smart, …, Van Allen 2018

- Widespread intron retention in clinical and cell line samples
- Generates much larger “novel” protein sequence than small variants

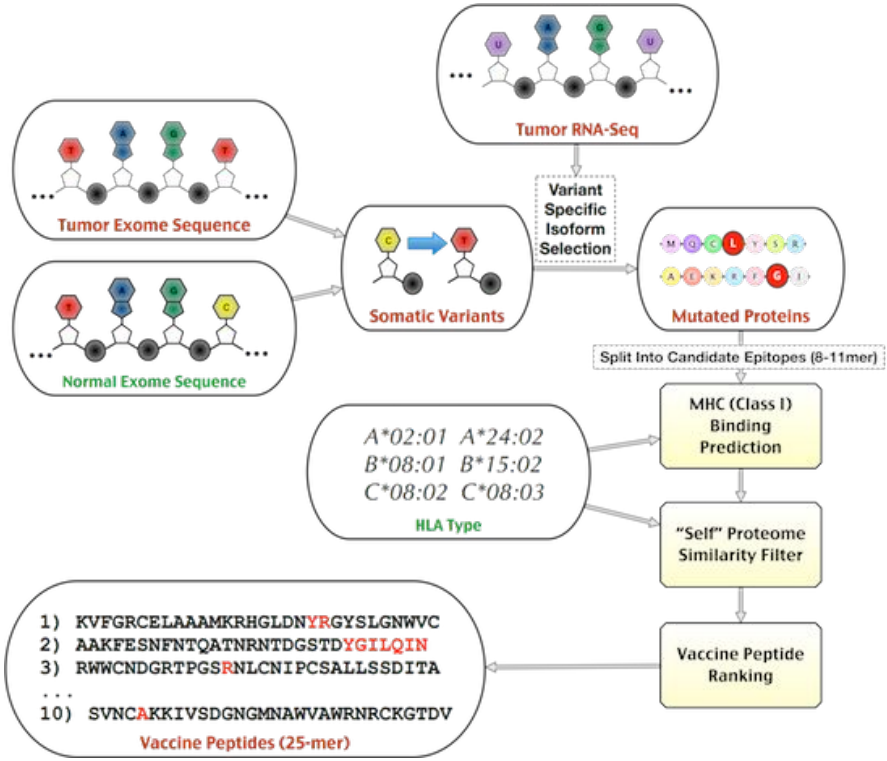
Intron retention is a source of neoepitopes in cancer





PGV sequencing & informatics

- **Sequencing**
  - 2x125bp on HiSeq 2500
  - Tumor/normal WES
  - Tumor mRNA-seq
- **OpenVax pipeline**
  - Identify tumor-specific mutations
  - Predict patient HLA binding of mutant peptides
  - Quantify expression of mutations
  - Rank 25mer vaccine peptides by: *MHC affinity * RNA abundance*

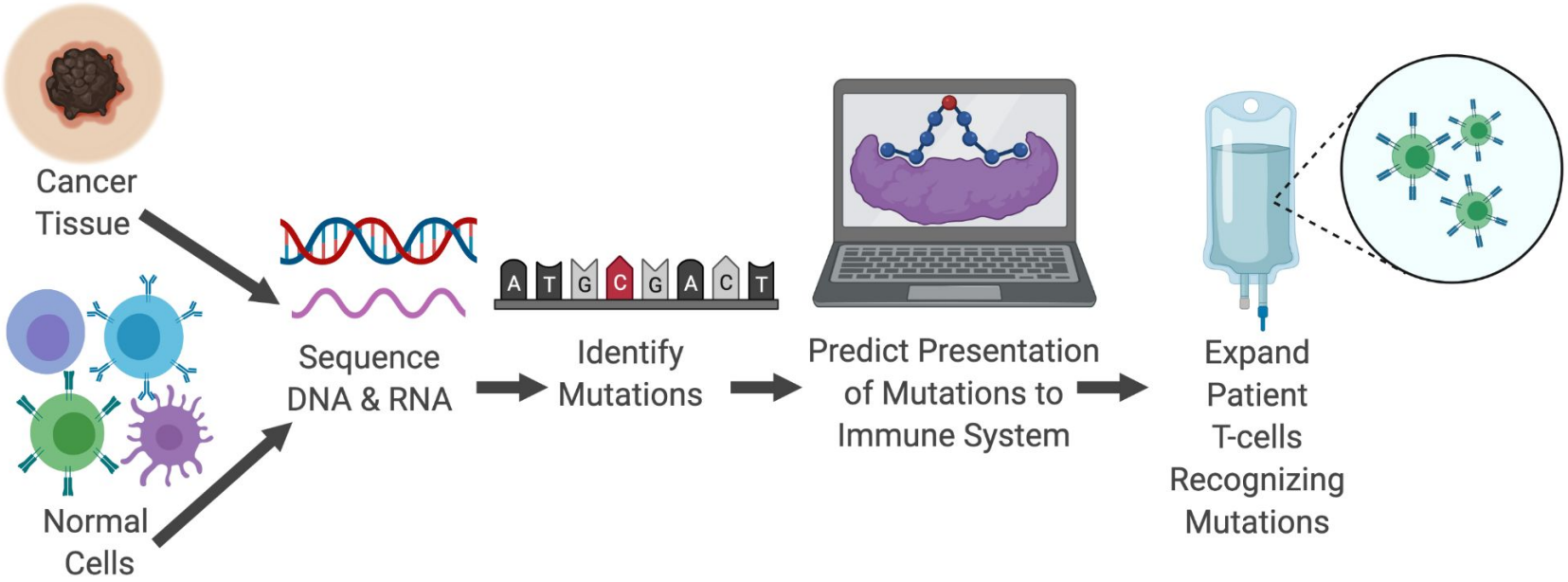


Shared antigen vaccines

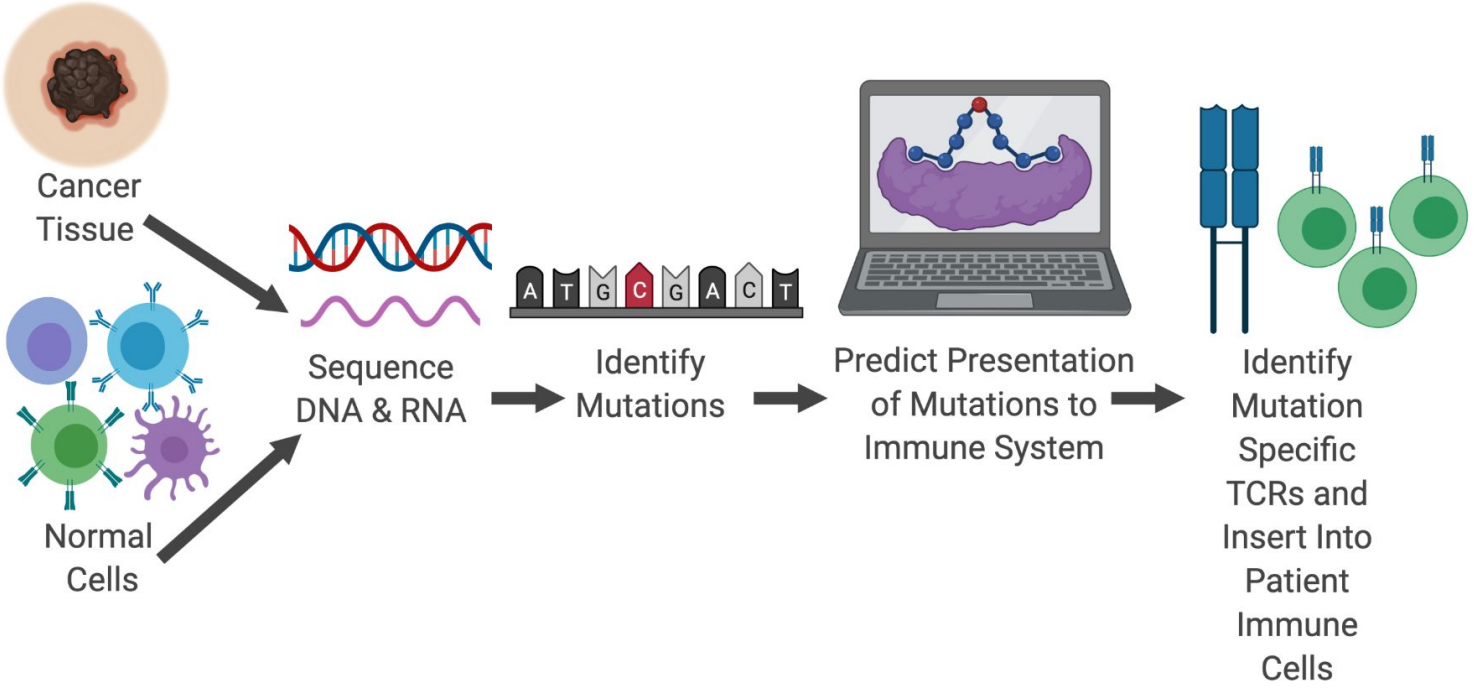
Cancer type	Vaccine	Total patients	Patients responding
Melanoma	Tyrosinase + GMCSF	16	0
Melanoma	Peptides in IFA or on DC	26	3
Melanoma	MART-1 + IL-12	28	2
Prostate	Peptides	10	0
Melanoma	Peptides on PBMC + IL-12	20	2
Breast and prostate	Telomerase	7	0
Cervix	HPV16 E7	17	0
Colorectal	Peptides in IFA	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

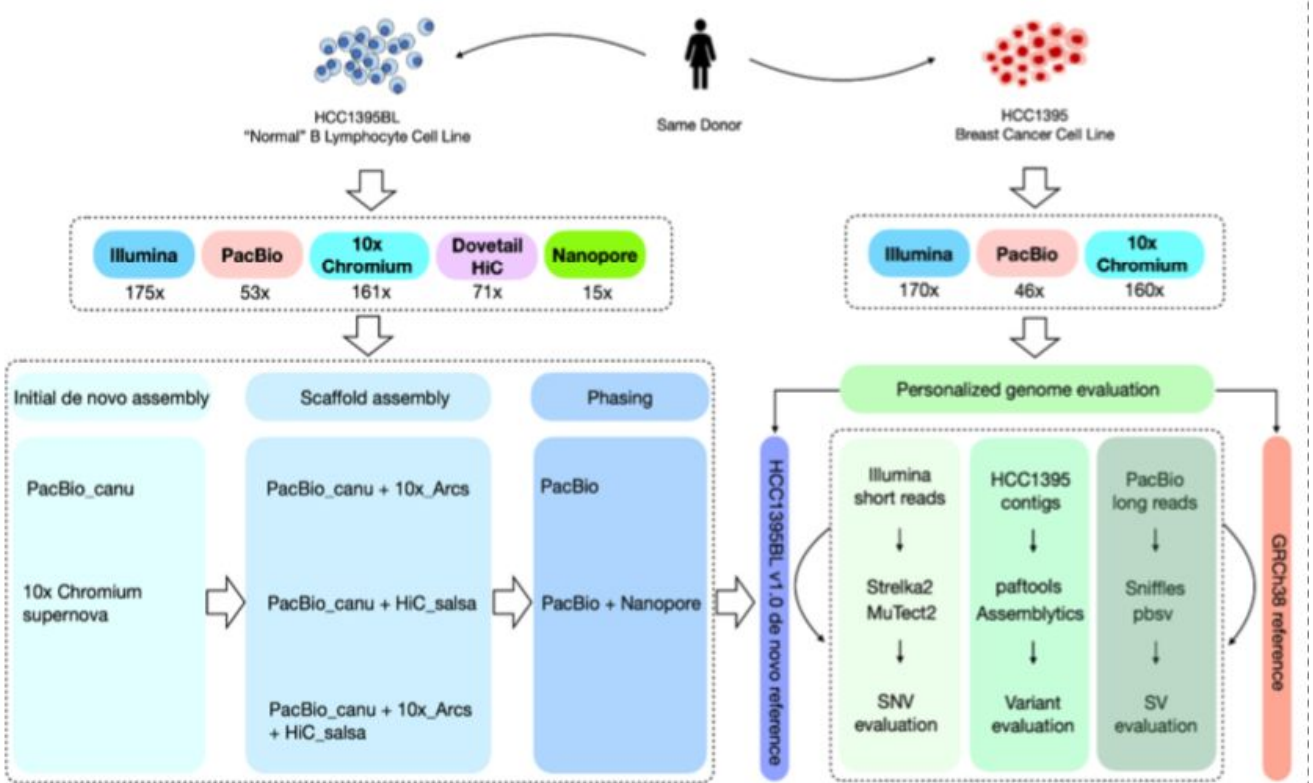
Not just vaccines: cellular therapy



Not just vaccines: TCR therapy



Long read somatic variant calling with personalized genome assembly



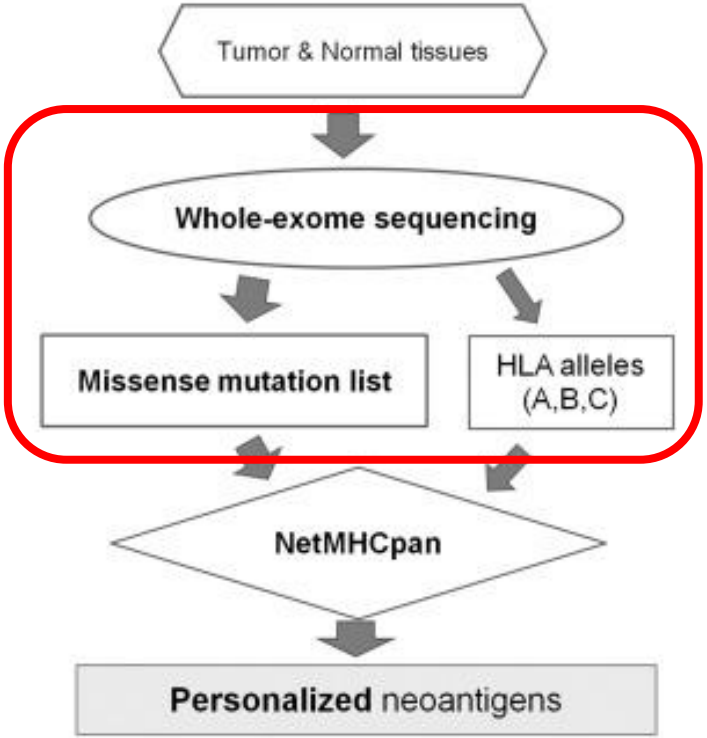
Personalized genome assembly for accurate cancer somatic mutation discovery using cancer-normal paired reference samples

Status

- Cell line DNA+RNA submitted for Illumina + ONT sequencing
- Sending cell line DNA+RNA to DHMRI (Kannapolis)
- AML sample going to be sorted into tumor+normal soon
- Ongoing optimization of HMW DNA extraction kits for low input volume surgical samples
- Working with Variant Graph (vg) team on graph based long+short read somatic variant calling

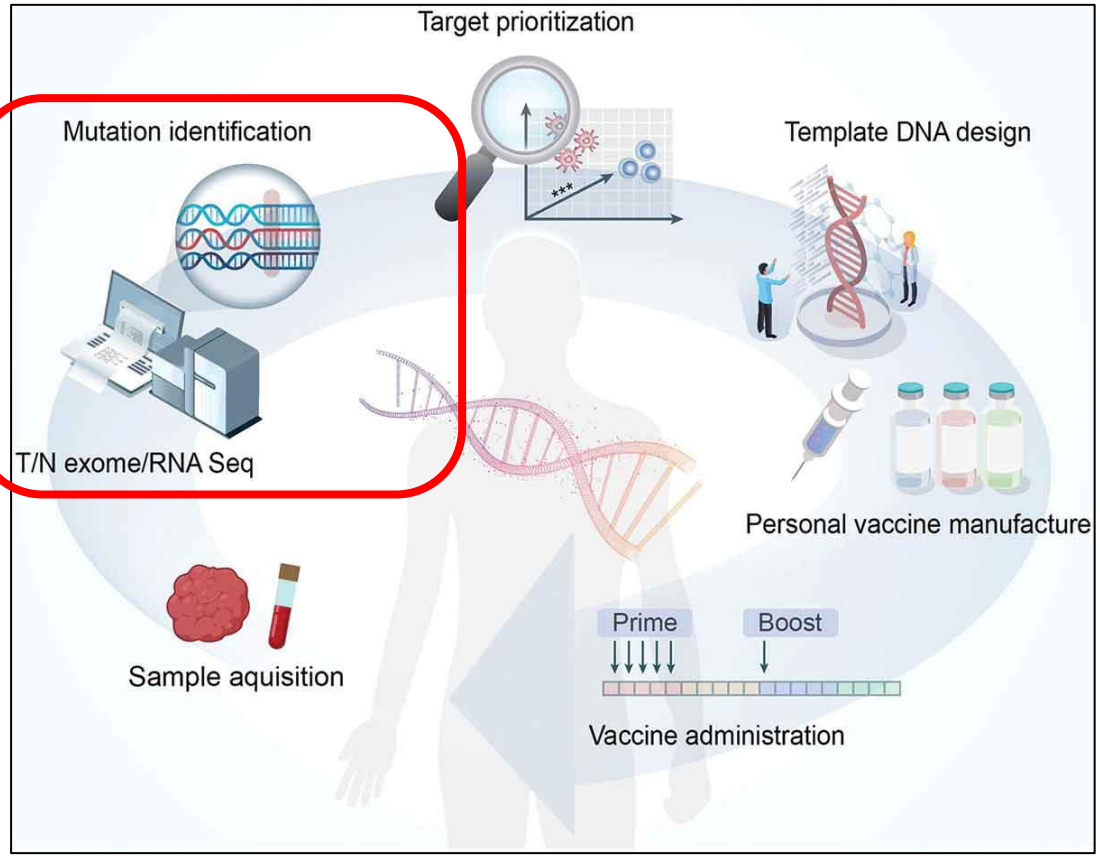
Karasaki, …, Nakajima 2016

Identification of Individual Cancer-Specific Somatic Mutations for Neoantigen-Based Immunotherapy of Lung Cancer



Xu, …, Fan 2020

Towards customized cancer vaccines: a promising field in personalized cancer medicine

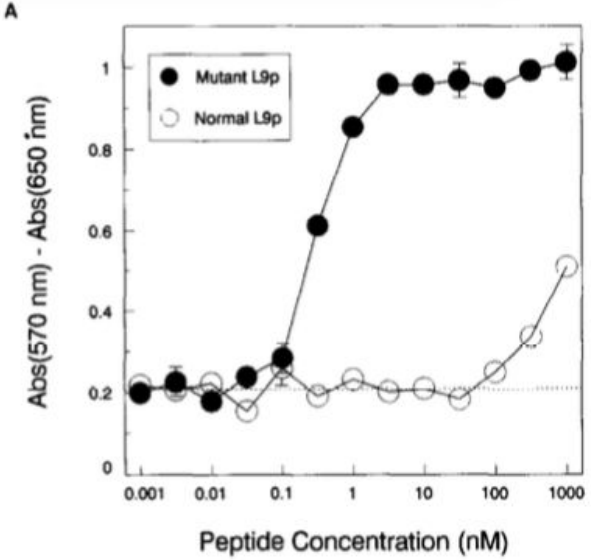


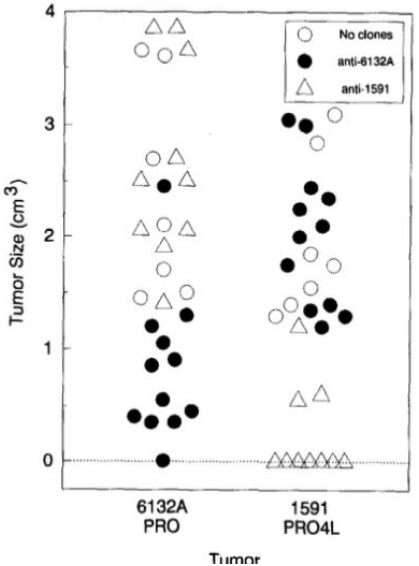
Neoantigen history

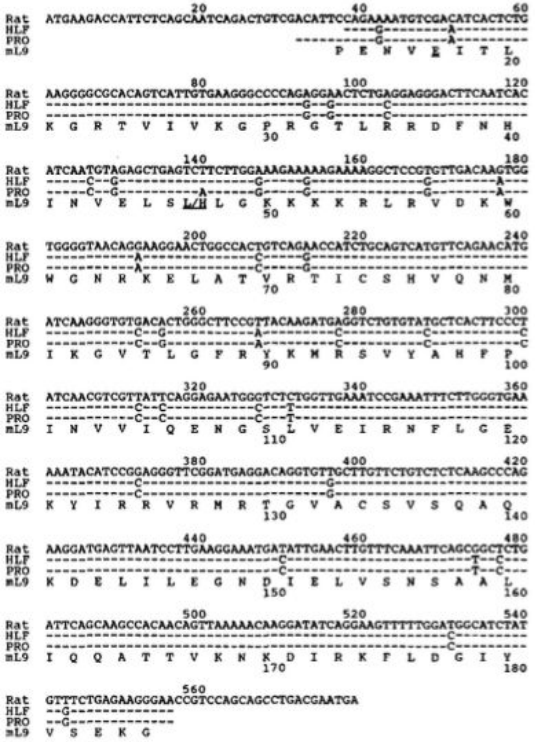
The first neoantigen paper

(Monarch, …, Schreiber 1995)

A Unique Tumor Antigen Produced by a Single Amino Acid Substitution

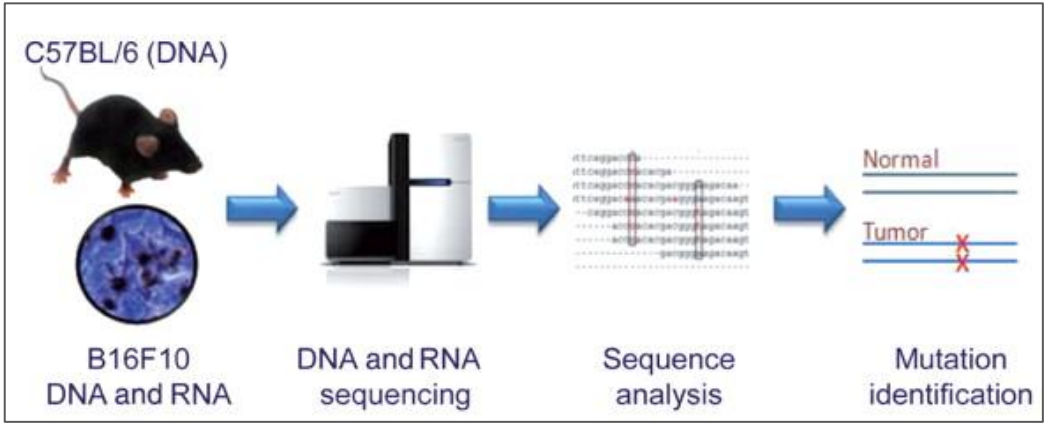


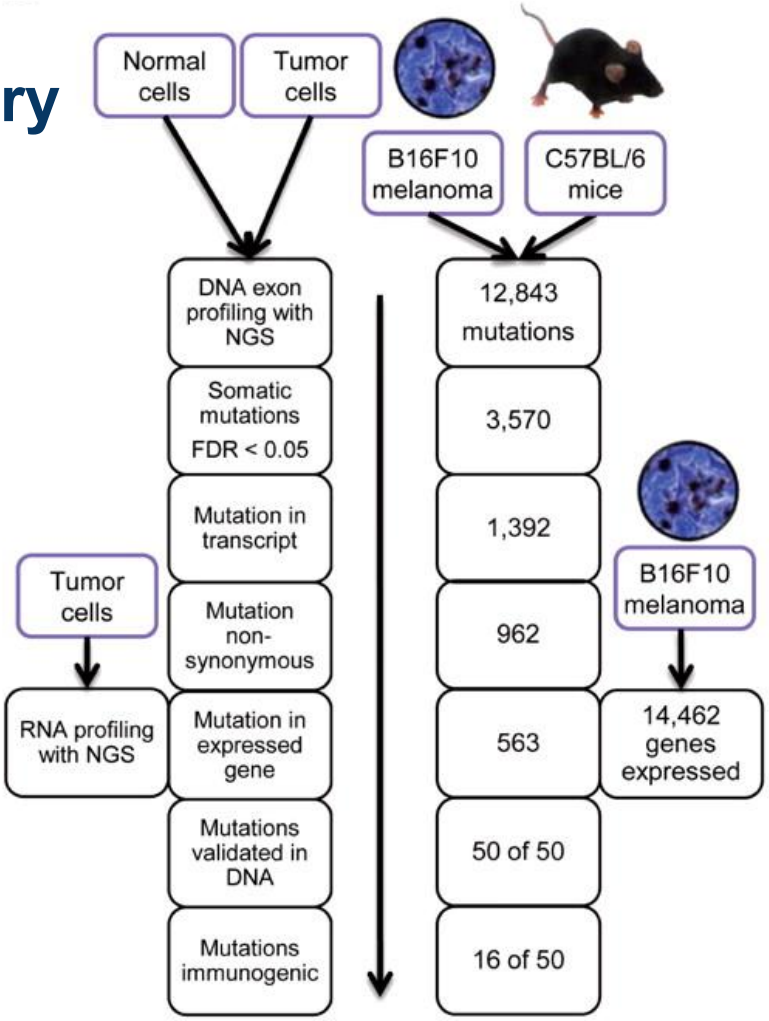




NGS scales up neoantigen discovery *(Castle, …, Sahin 2012)*

Exploiting the Mutanome for Tumor Vaccination





- Tumor/normal WES
- Tumor mRNA-seq
- *Variant calling*: SNVs only
- *Immune prediction*: MHC binding

The other original neoantigen pipeline

(Matsushita, …, Schreiber 2012)

Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting

- Looking for evidence of immunoediting
  - Schreiber Lab later applied same pipeline to vaccination
- Tumor/normal WES
- Tumor mRNA-seq
- *Variant calling*: SNVs only
- *Immune prediction*: MHC binding

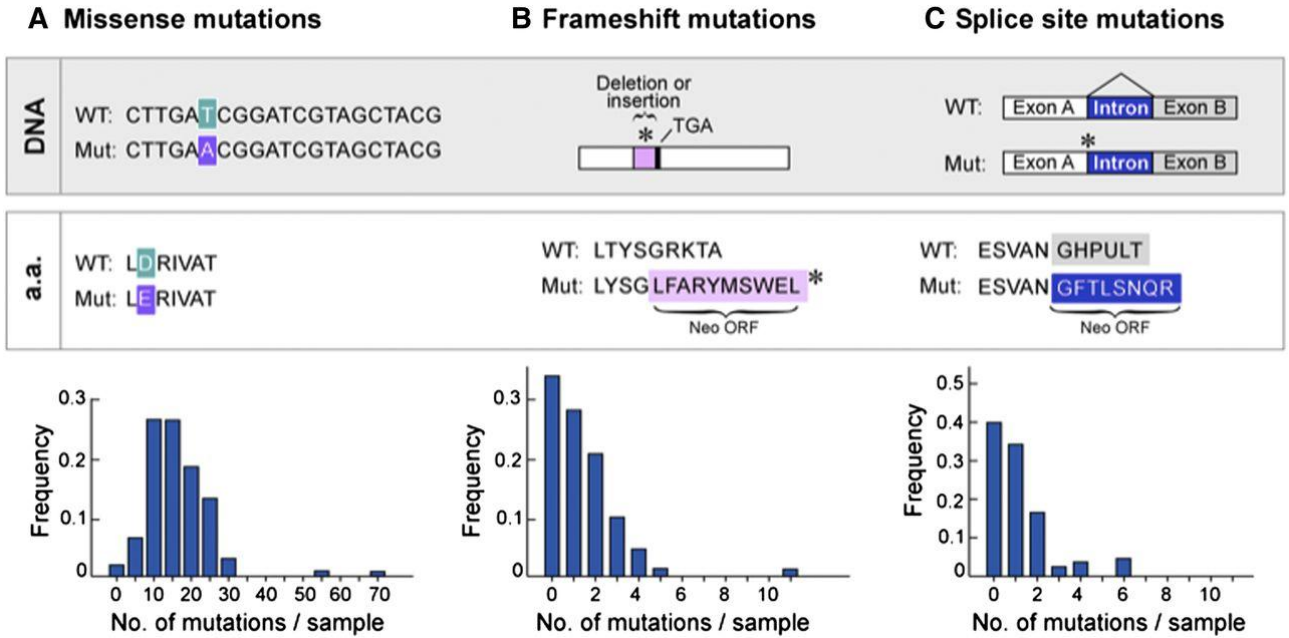
H-2D^b:

	Peptides		IC_{50} (nM)
Olfr195 (L14M)	YSVTNEFIL	(wild-type)	5
	YSVTNEFI**M**	(mutant)	5
Spnb2 (R913L)	VAVVNQIAR	(wild-type)	5305
	VAVVNQIA**L**	(mutant)	7
Tbk1 (G722S)	GGLRNVDCL	(wild-type)	36
	G**S**LRNVDCL	(mutant)	8
Fam38a (M134I)	MAGINTDHL	(wild-type)	23
	IAGINTDHL	(mutant)	14
Cxx1b (D54N)	YMLVDDRTF	(wild-type)	4303
	YMLV**N**DRTF	(mutant)	14
H2-Q6 (R142L)	FAYEGRDYI	(wild-type)	27
	FAYEG**L**DYI	(mutant)	14
A2m (G777V)	FCLSNDTGL	(wild-type)	502
	FCLSNDT**V**L	(mutant)	15
Ubqln4 (R262L)	RALSNLESV	(wild-type)	10
	LALSNLESV	(mutant)	24

Moving a little beyond SNVs

(Rajasagi, …, Wu 2014)

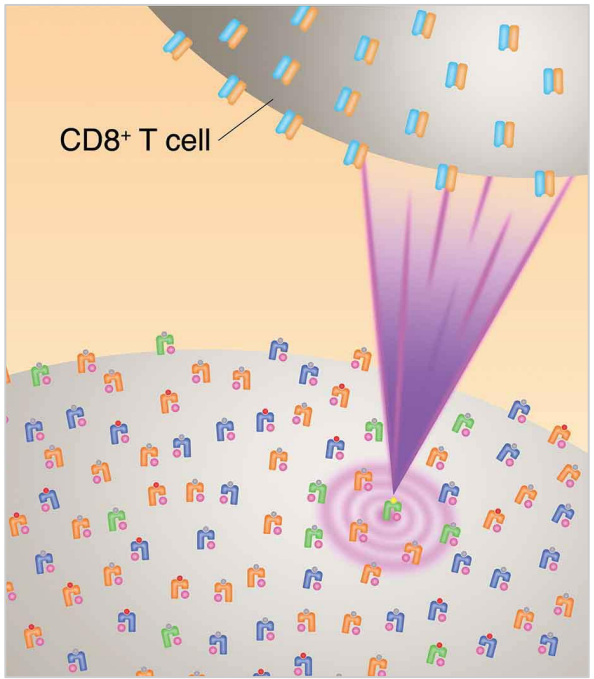
- Mostly WES (some WGS)
- Variant calling with Mutect
- SNVs > indels > splice site variants



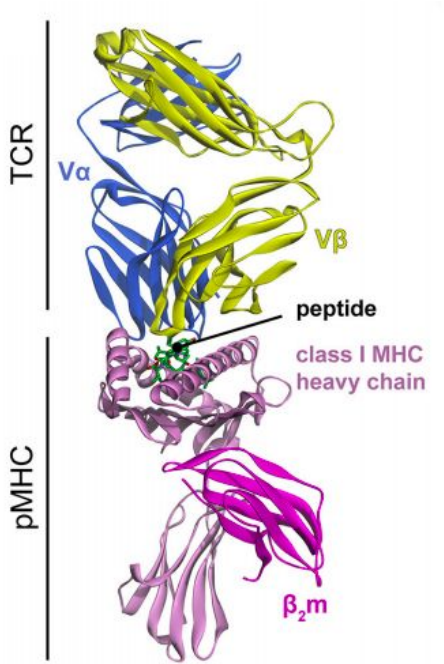
Systematic identification of personal tumor-specific neoantigens in chronic lymphocytic leukemia

How do cytotoxic T-cells recognize neoantigens?

- Cells present peptide fragments of their protein contents bound to MHC-I
- CD8+ T-cells selected recognize non-self, then “licensed” to kill by APCs
- TCR recognition of peptide-MHC: kill!

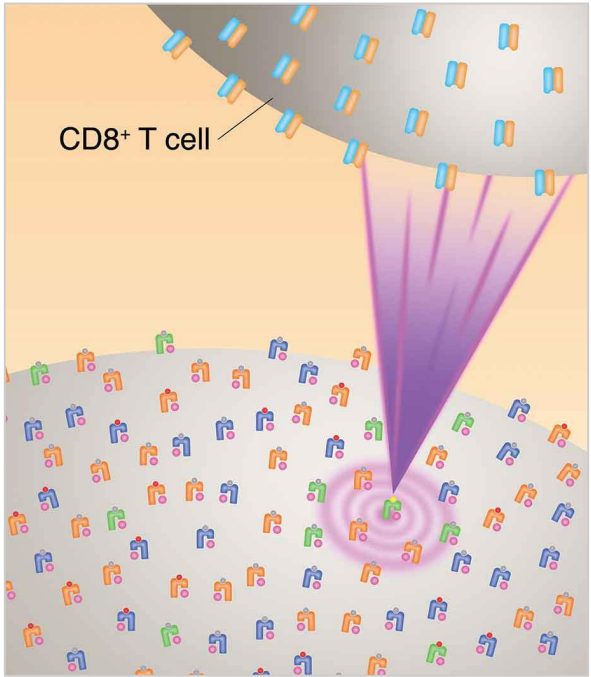


Lost in the crowd: identifying targetable MHC class I neoepitopes for cancer immunotherapy

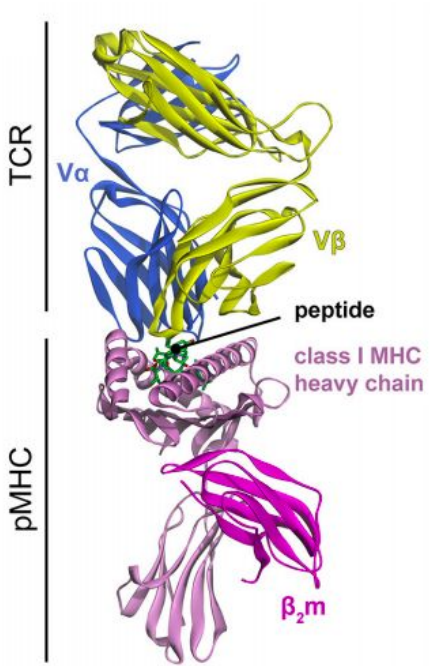


Using Global Analysis to Extend the Accuracy and Precision of Binding Measurements with T cell Receptors and Their Peptide/MHC Ligands

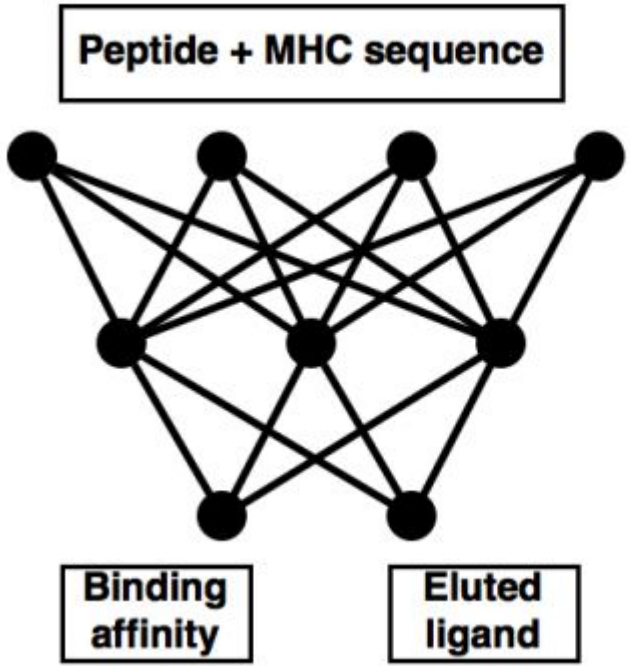
MHC binding prediction



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

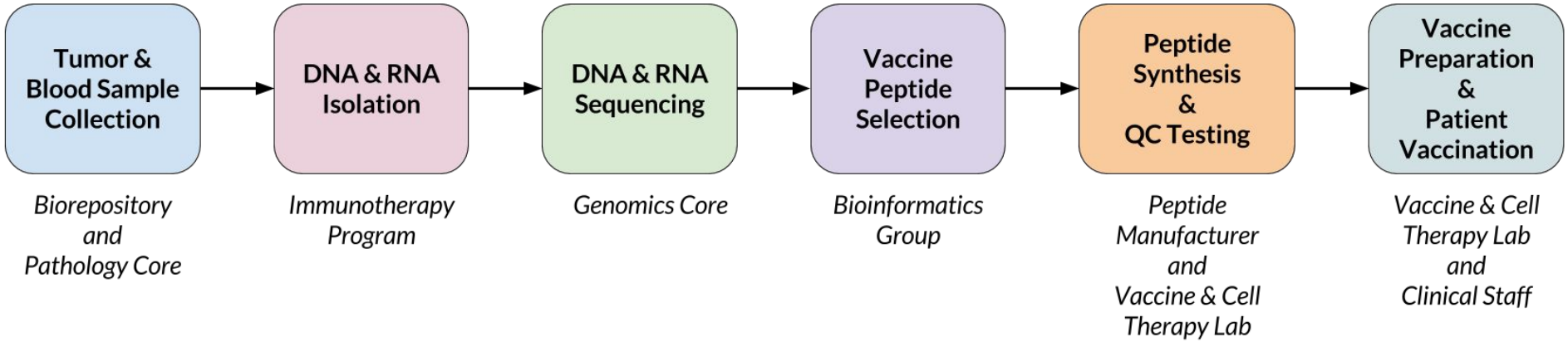


Using Global Analysis to Extend the Accuracy and Precision of Binding Measurements with T cell Receptors and Their Peptide/MHC Ligands



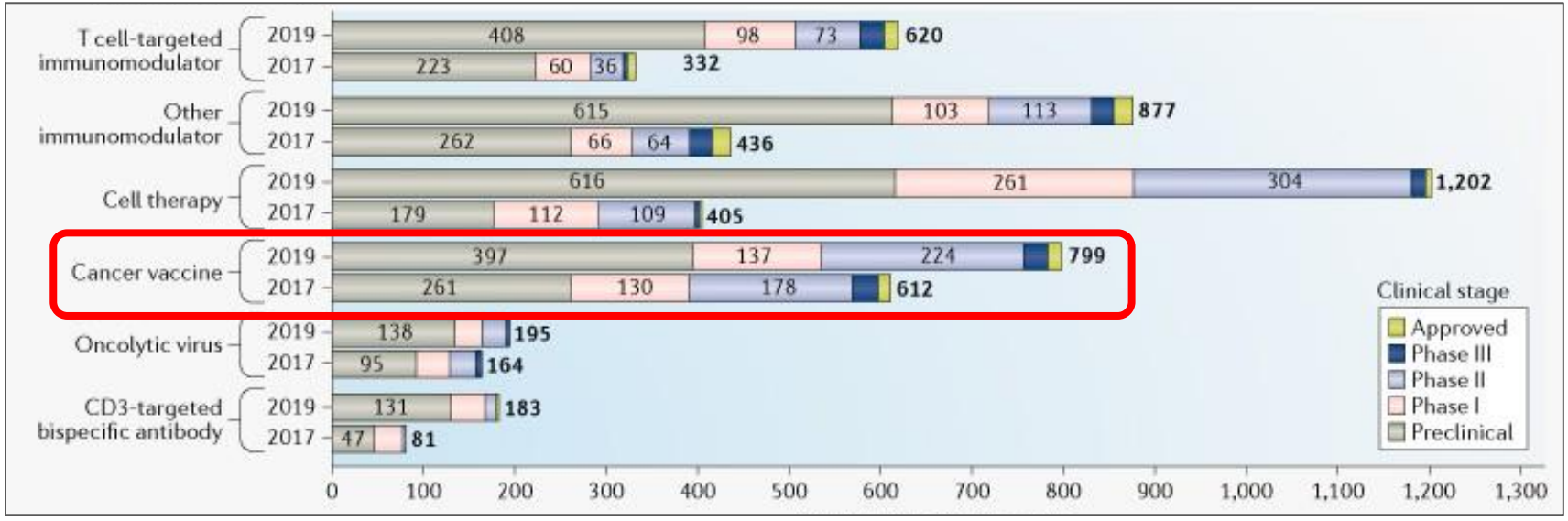
NetMHCpan 4.0

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

Neoantigens driving cancer vaccine resurgence

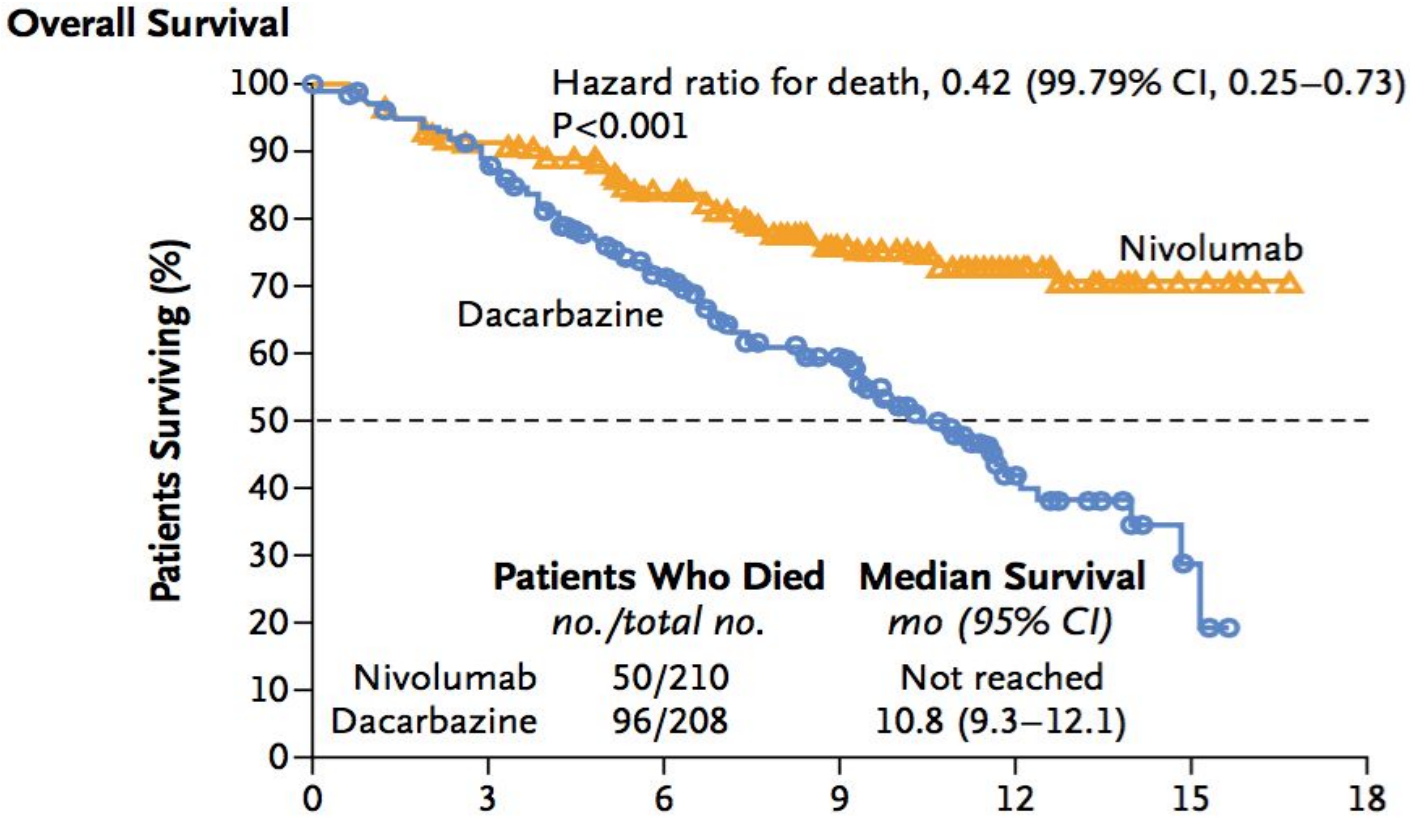


- >$1B investments in startups
  - **BioNTech** (bought Neon)
  - **Moderna** (reabsorbed Caperna)
  - Gritstone, Genocea, EpiVax Oncology, &c

Immuno-oncology drug development goes global

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

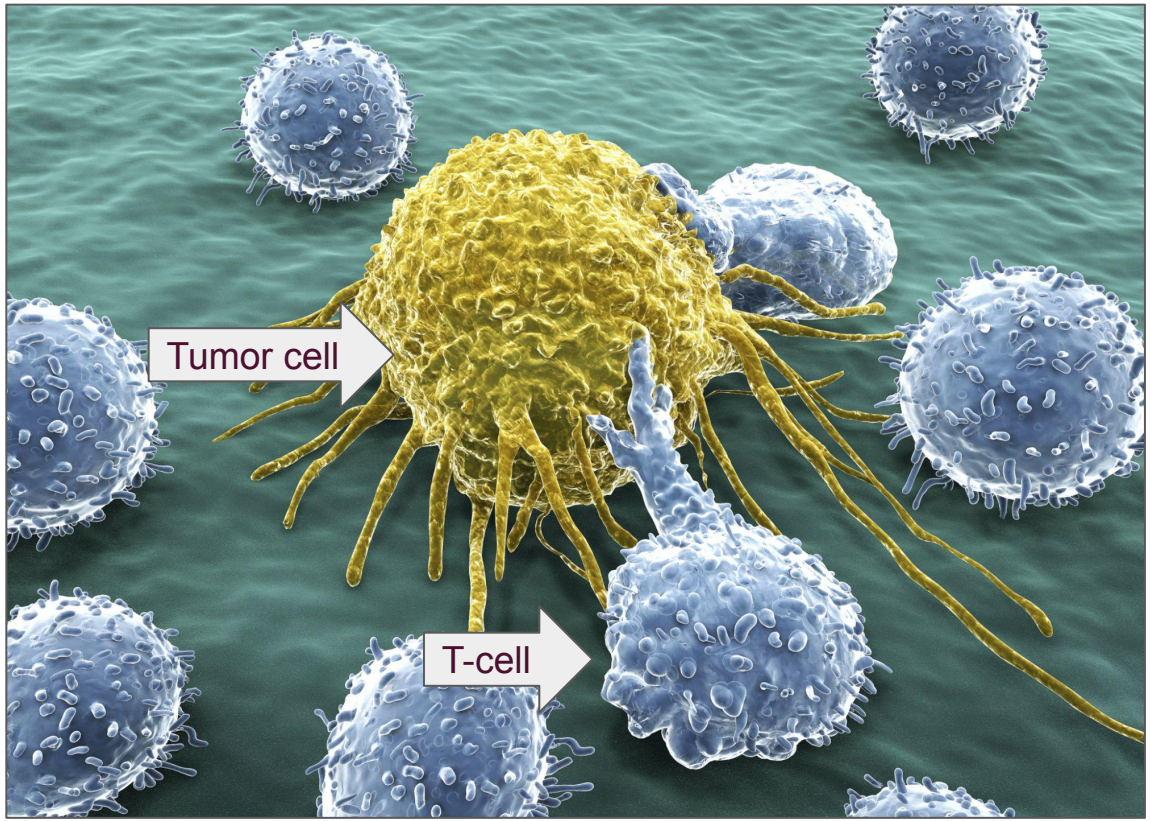
Immunotherapy vs. chemotherapy



Nivolumab in Previously Untreated Melanoma… (Robert NEJM 2015)

What is a T-cell?

- Patrols the body, looks for cells making “unexpected” proteins
  - Viruses and cancer
- T-cells have very diverse receptors to recognize different patterns
- When a (cytotoxic) T-cell finds its target: kill! kill!



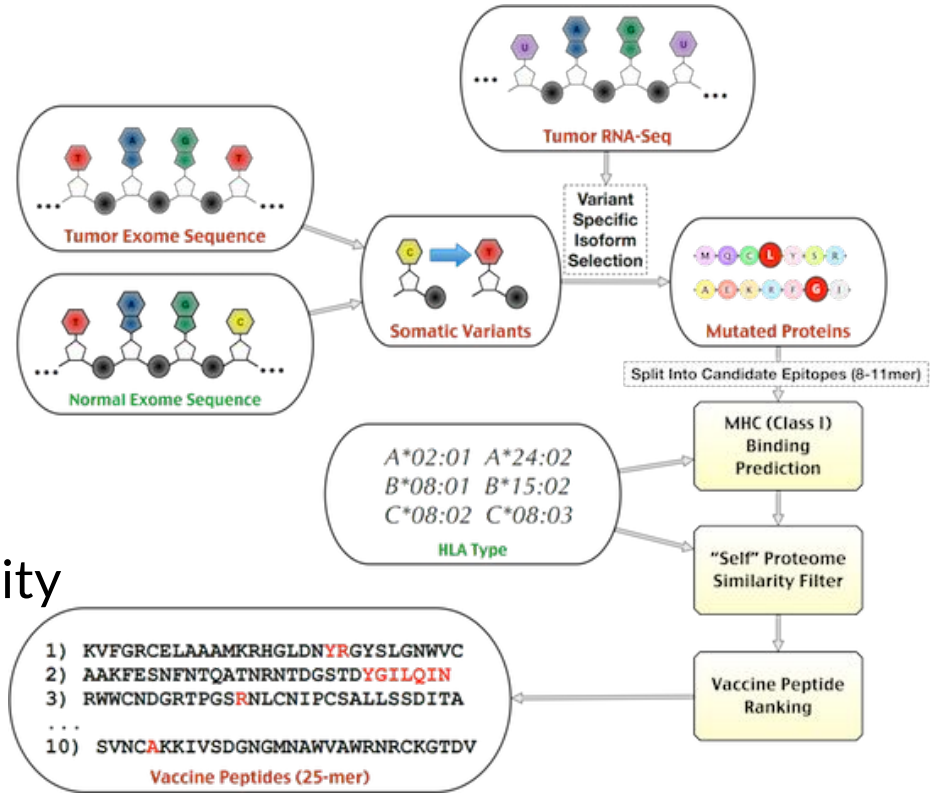
Juan Gartner / Getty Images

Neoantigens to the rescue

- No overlap with normal cells
  - genomic mutations
  - abnormal splicing
  - abnormal post-translational modifications
- Tumor specific + no immune tolerance

OpenVax Pipeline overview

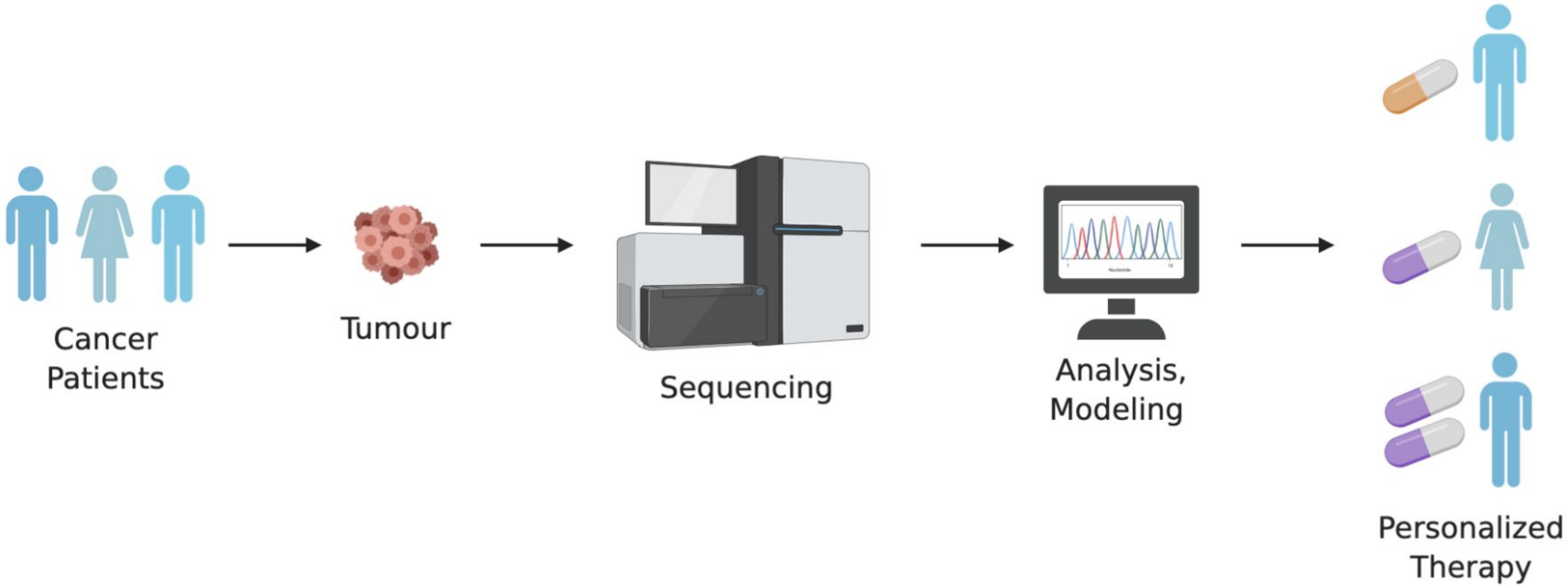
- Tumor + normal DNA
  - Somatic variant calling
- Tumor RNA
  - Phase co-expressed variants
  - Mutant protein sequence
  - Quantify mut. allele expression
- Rank by expression and MHC-I affinity
- Select manufacturable peptides
- www.github.com/openvax/



Flavors of cancer immunotherapy

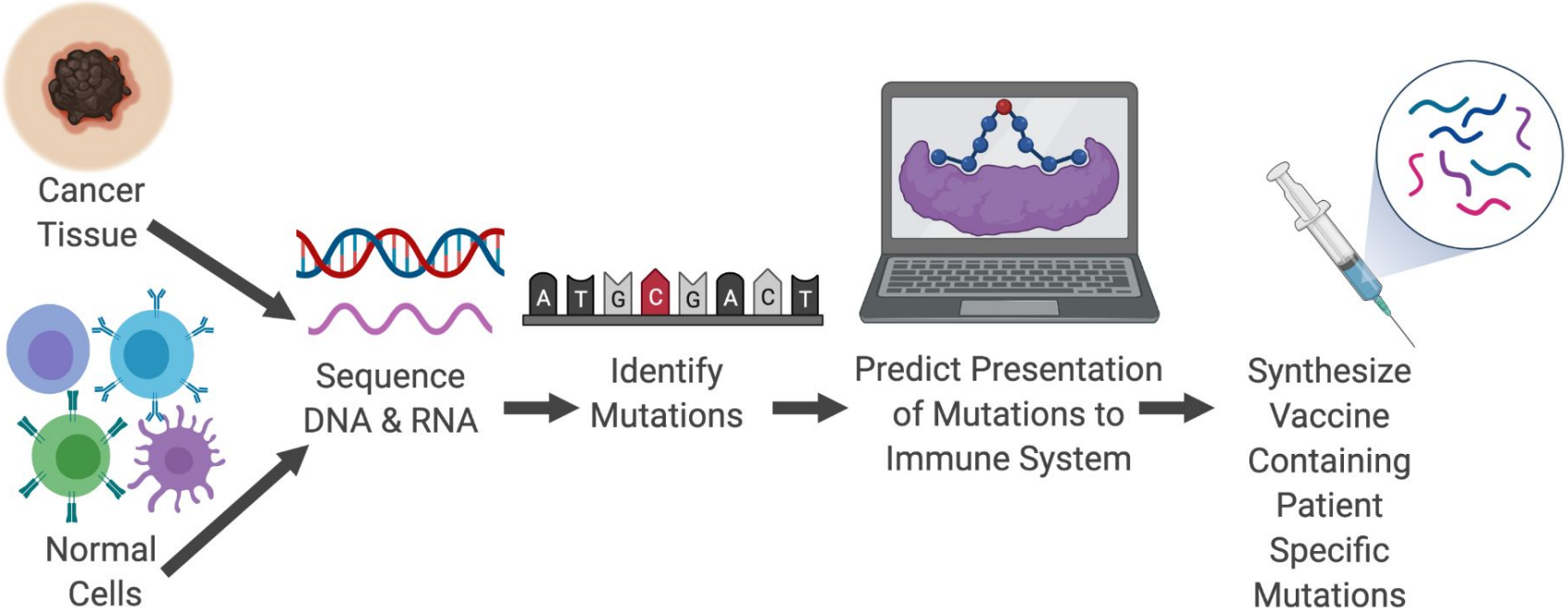
Checkpoint blockade	Cellular therapies	Vaccines
Disinhibit T-cells. Antigens responsible for tumor clearance typically unknown. <u>Success stories:</u> • αCTLA-4 (ipi) • αPD-1 (pembro, nivo, cemi) • αPD-L1 (atezo, ave, durva)	Expand patient T-cells after receptor engineering and/or selection. <u>Success 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> • ???

“Traditional” personalized medicine



https://en.wikipedia.org/wiki/Personalized_medicine

Neoantigen vaccines: extremely personalized medicine



Personalized cellular therapies

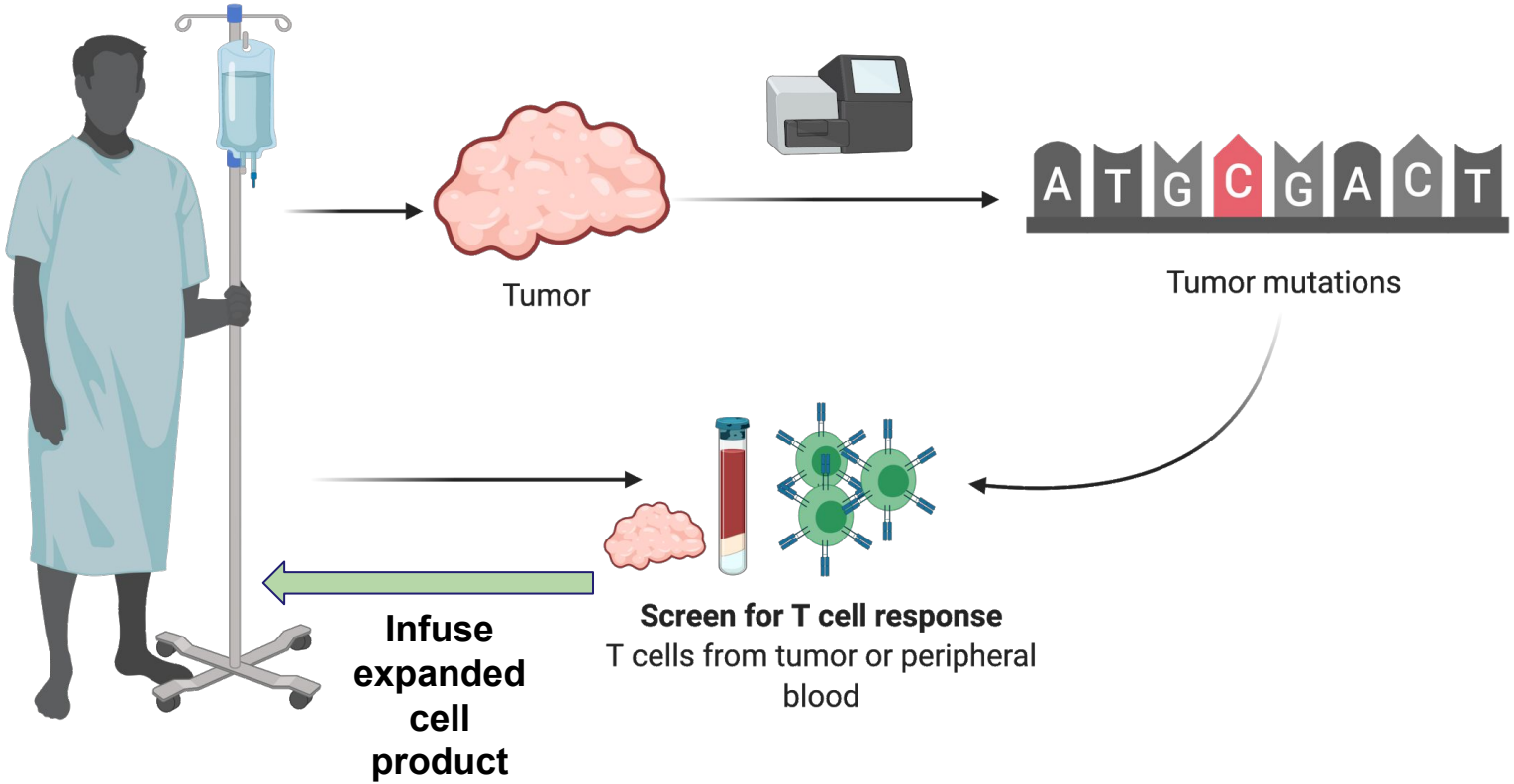
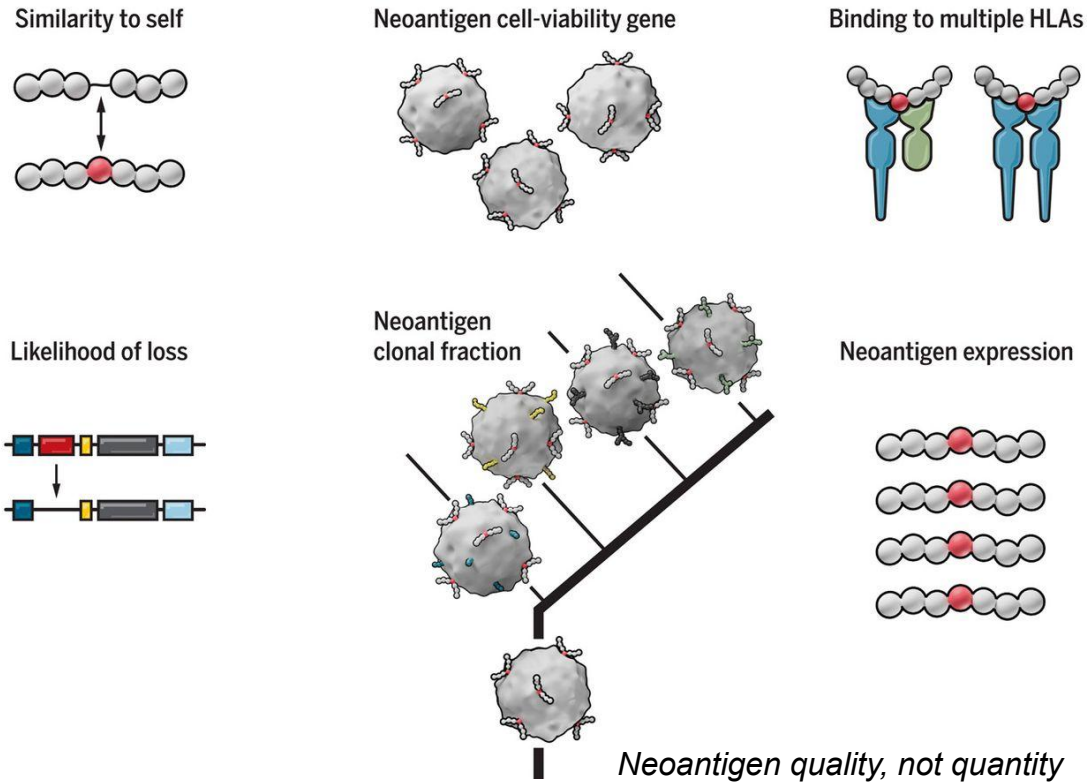


Figure adapted from Tim O'Donnell's PhD defense

What makes a good neoantigen?

- Dissimilarity from self
  - neoORFs >>> SNVs
- Abundance
- Clonality
- Mutant peptide binds patient MHC
  - Antigen processing
  - HLA allele loss
- Prefer driver and homozygous mutations



Neoantigen quality, not quantity

Clinical trials at Mount Sinai

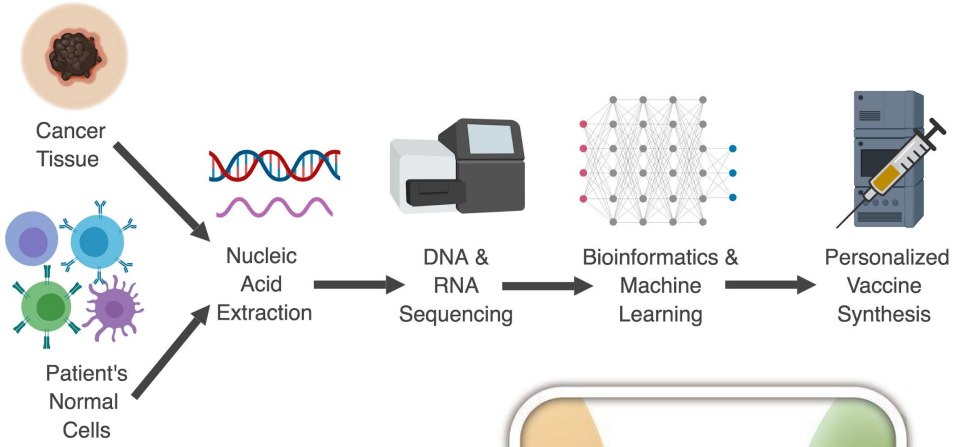
- **PGV001** (*Nina Bhardwaj*)
  - Solid cancers, multiple myeloma
  - Long peptides + poly-ICLC
  - 13 vaccinated
- **PGV for GBM** (*Adilia Hormigo*)
  - + TMZ, Tumor Treating Fields
  - 8 vaccinated
- **PGV for Bladder Cancer** (*Matt Galsky*)
  - + Atezolizumab (anti-PD-L1)
  - 3 vaccinated

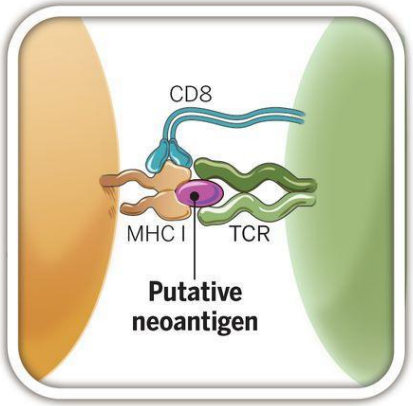
Shared design:

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

Mount Sinai PGV pipeline

- **Inputs**
  - Tumor + Normal DNA
  - Tumor RNA
- **Selection**
  - Identify coding mutations
  - Quantify expression
  - Predict MHC binding of mutant peptides
  - Rank by MHC affinity * RNA abundance
- **Vaccine**
  - Peptides + adjuvant (poly-ICLC)
  - *Alternatives: mRNA, DNA, viral vector, &c*





Schumacher & Schreiber 2015

Vaccine peptide ranking

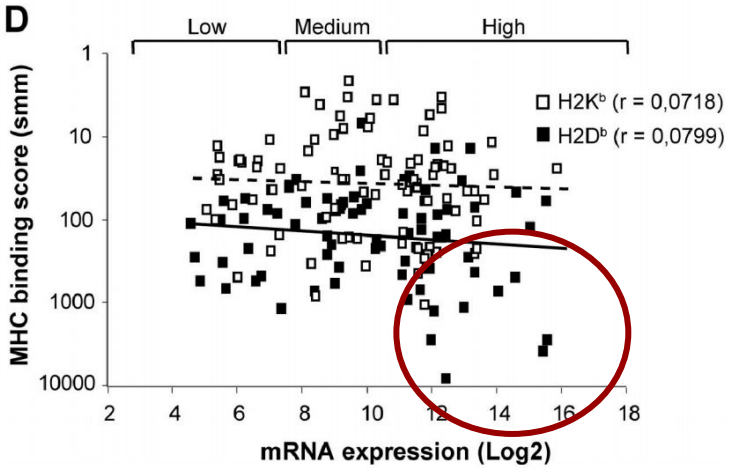
- Multiplicative ranking inspired by T cell epitopes which have low MHC affinity but high abundance

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

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

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

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



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

Concordance of neoantigen pipelines

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

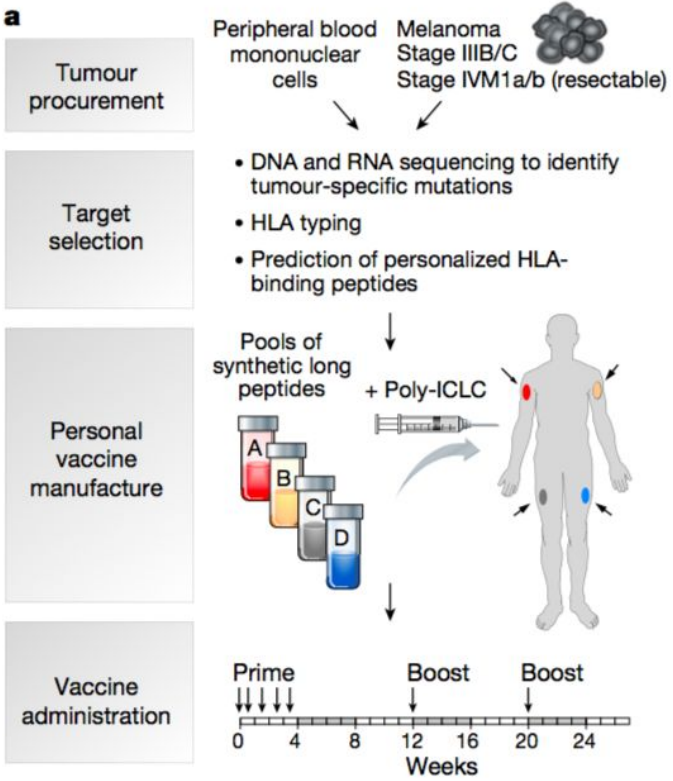
Peptides + poly-ICLC @ DFCI (2017)

An immunogenic personal neoantigen vaccine for patients with melanoma

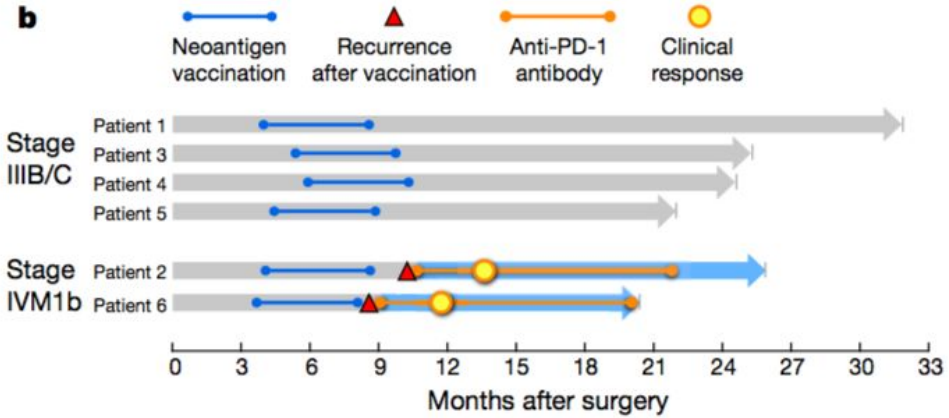
Patrick A. Ott[1,2,3]*, Zhuting Hu[1]*, Derin B. Keskin[1,3,4], Sachet A. Shukla[1,4], Jing Sun[1], David J. Bozym[1], Wandi Zhang[1], Adrienne Luoma[5], Anita Giobbie-Hurder[6], Lauren Peter[7,8], Christina Chen[1], Oriol Olive[1], Todd A. Carter[4], Shuqiang Li[4], David J. Lieb[4], Thomas Eisenhaure[4], 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

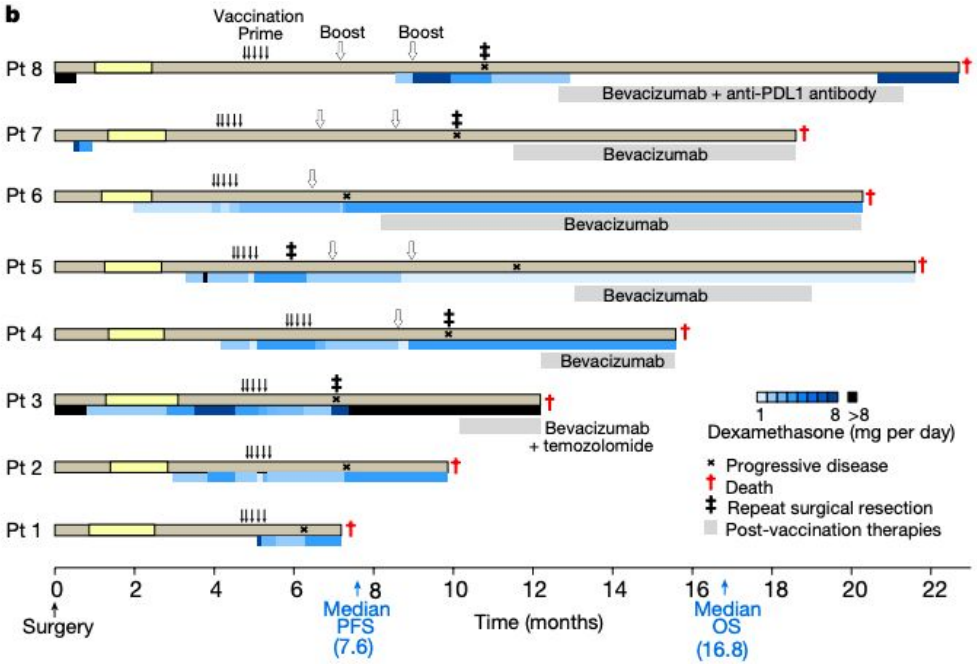
Peptides + poly-ICLC: Tumor control?

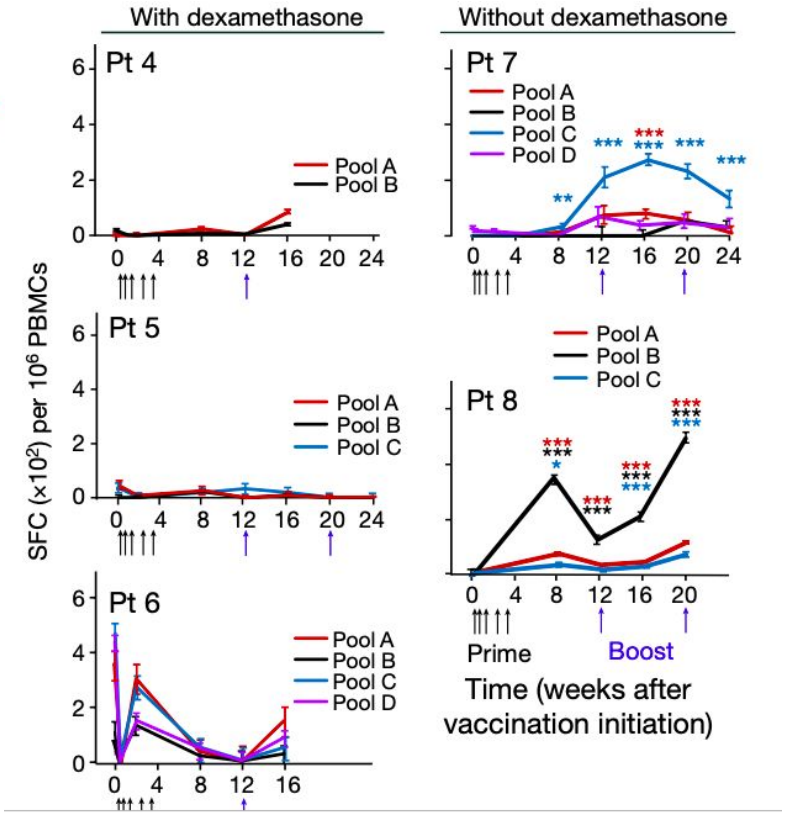


Of six vaccinated patients, four had no recurrence at 25 months after vaccination, while two with recurrent disease were subsequently treated with anti-PD-1 (anti-programmed cell death-1) therapy and experienced complete tumour regression, with expansion of the repertoire of neoantigen-specific T cells.



GBM 2018: steroids during priming = bad





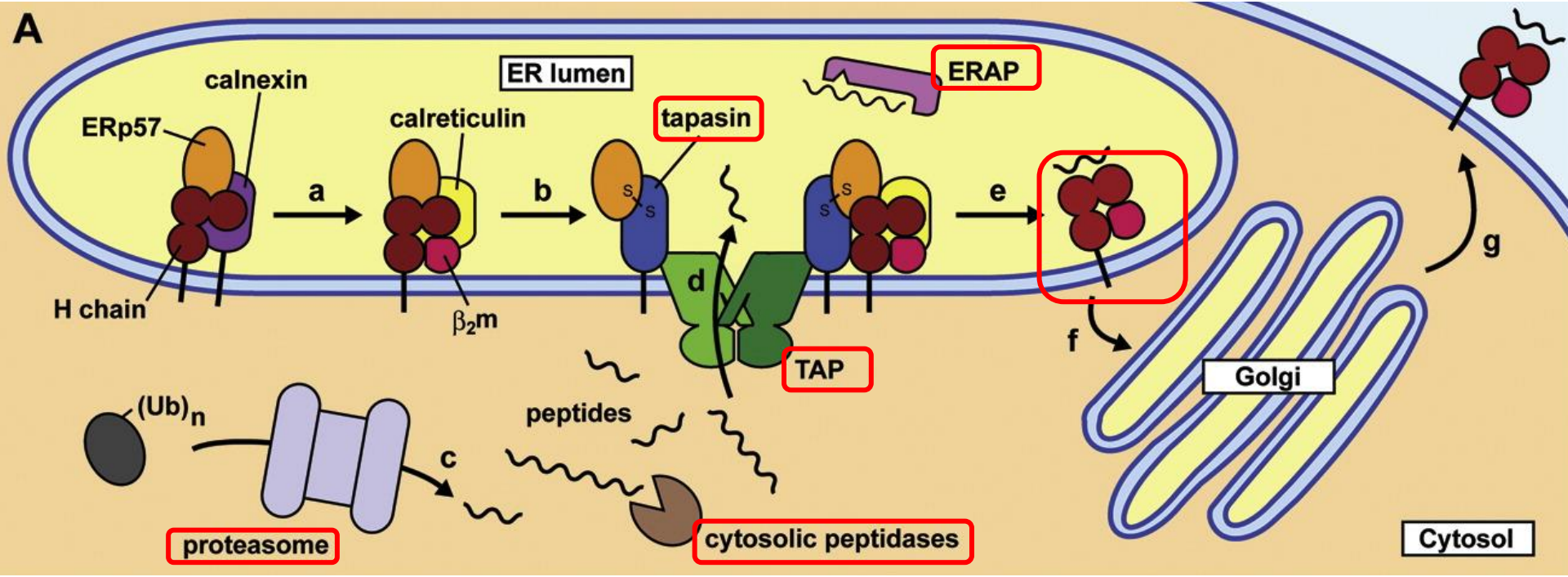
mRNA vaccine: Tumor control

- 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

Antigen Processing

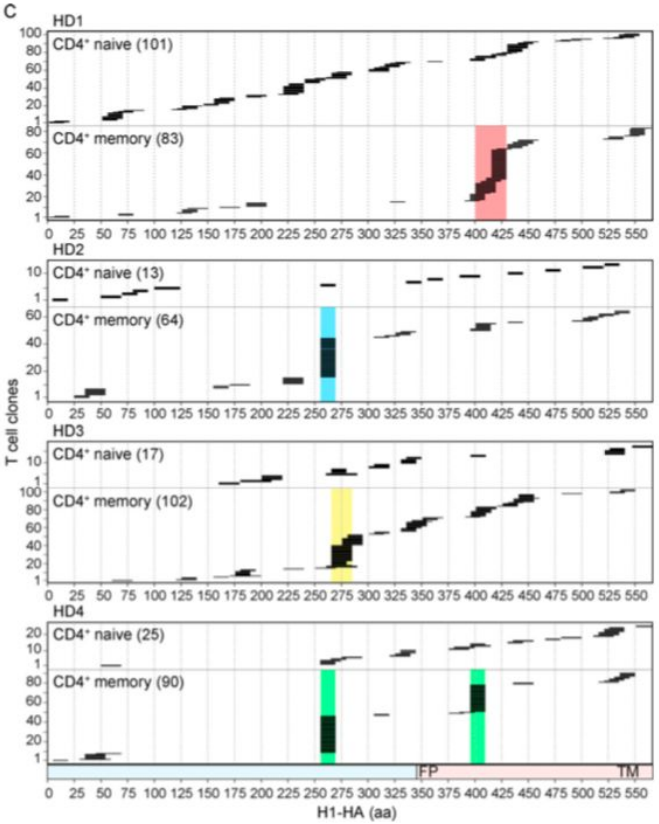
Components which influence which peptides seen by T-cells:

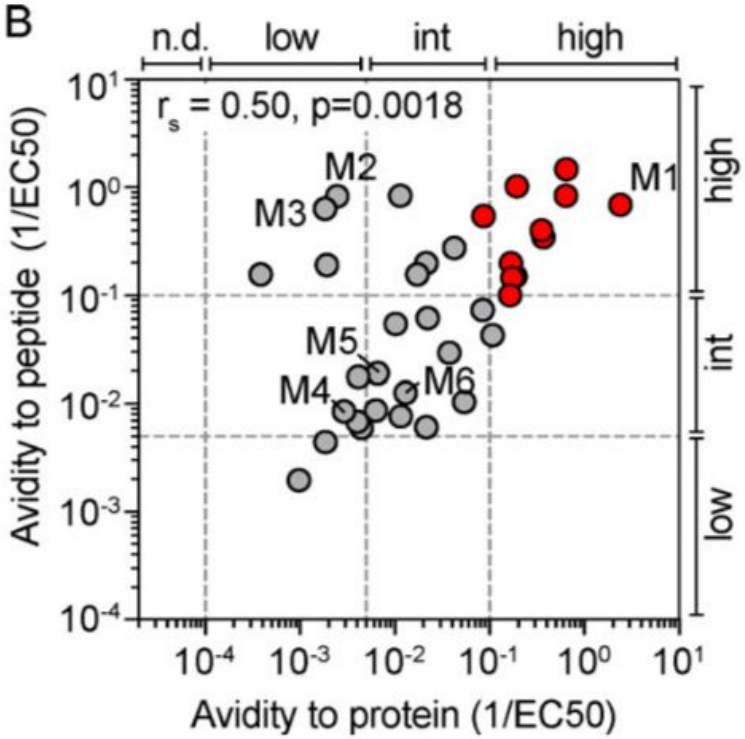
proteasome, cytosolic peptidases, TAP, ERAP, tapasin, MHC



Antigen processing and presentation: TAPping into ABC transporters

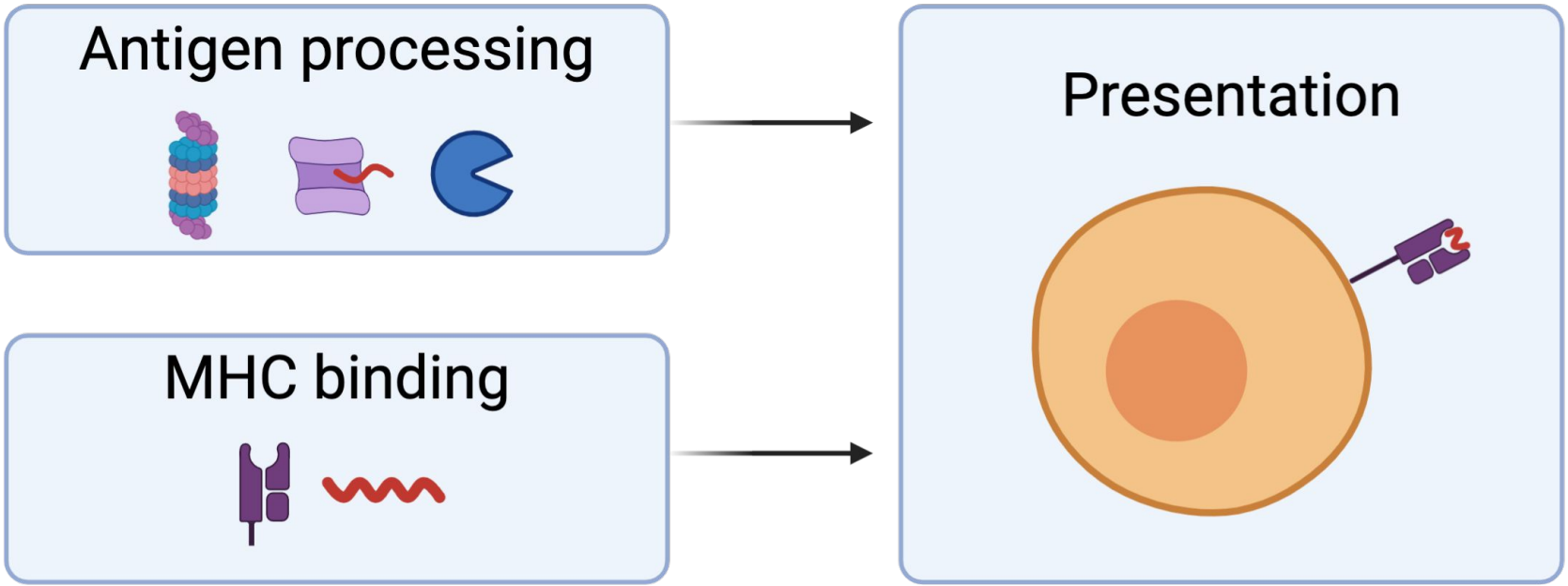
Does antigen processing matter?





Deciphering and predicting CD4+ T cell immunodominance of influenza virus hemagglutinin

MHCflurry 2.0: Binding + Processing



MHCflurry 2.0 binding affinity (BA)

MHCflurry 2.0 presentation score (PS)

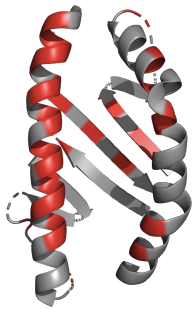
Adapted from Tim O'Donnell's PhD defense

MHCflurry 2.0: Data Sources

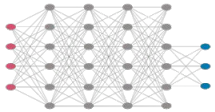
MHC I ligands identified by MS
~500,000 peptides
> 100 publications

Peptide / MHC binding affinities
~220,000 measurements
> 600 publications

MHC primary sequences
37 polymorphic positions



PEPTIDE SEQUENCE
MHC SEQUENCE



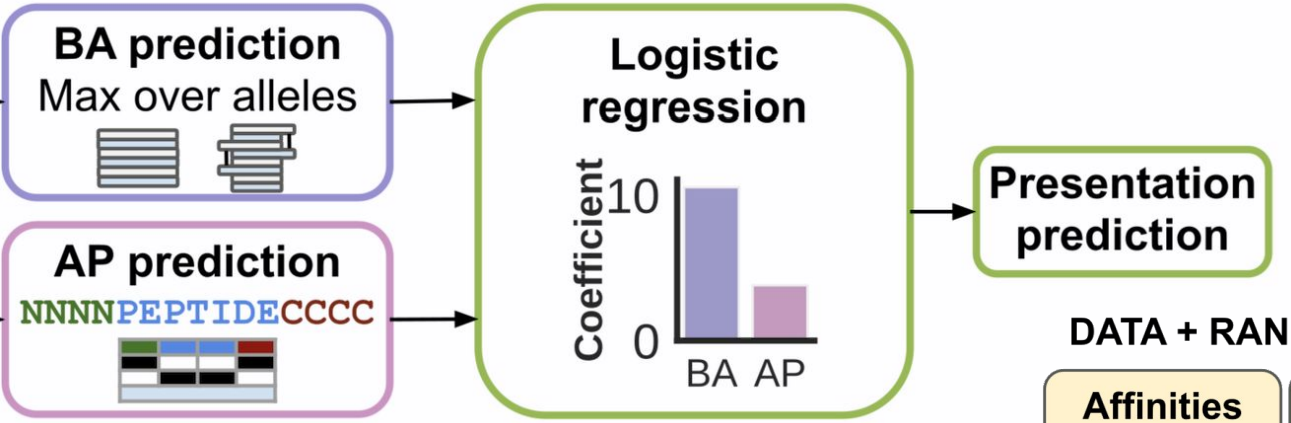
PREDICTED AFFINITY (nM)

Machine learning model
Neural networks

O'Donnell et al Cell Systems 2020

Adapted from Tim O'Donnell's PhD defense

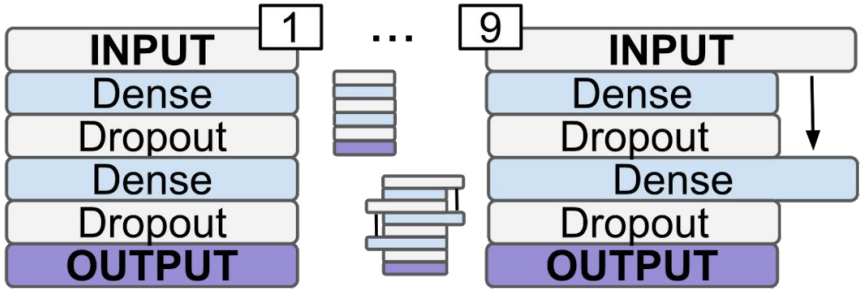
MHCflurry 2.0: Architecture



DATA + RANDOM NEGATIVE PEPTIDES

Affinities	Mass spec	Random
K_d = *x* nM	K_d < 100 nM	K_d > 30 μM

NEURAL NETWORK ENSEMBLE



INPUT REPRESENTATION

MHC I allele: selected positions

...HVDTLYGVRYDHYYTWAVL...

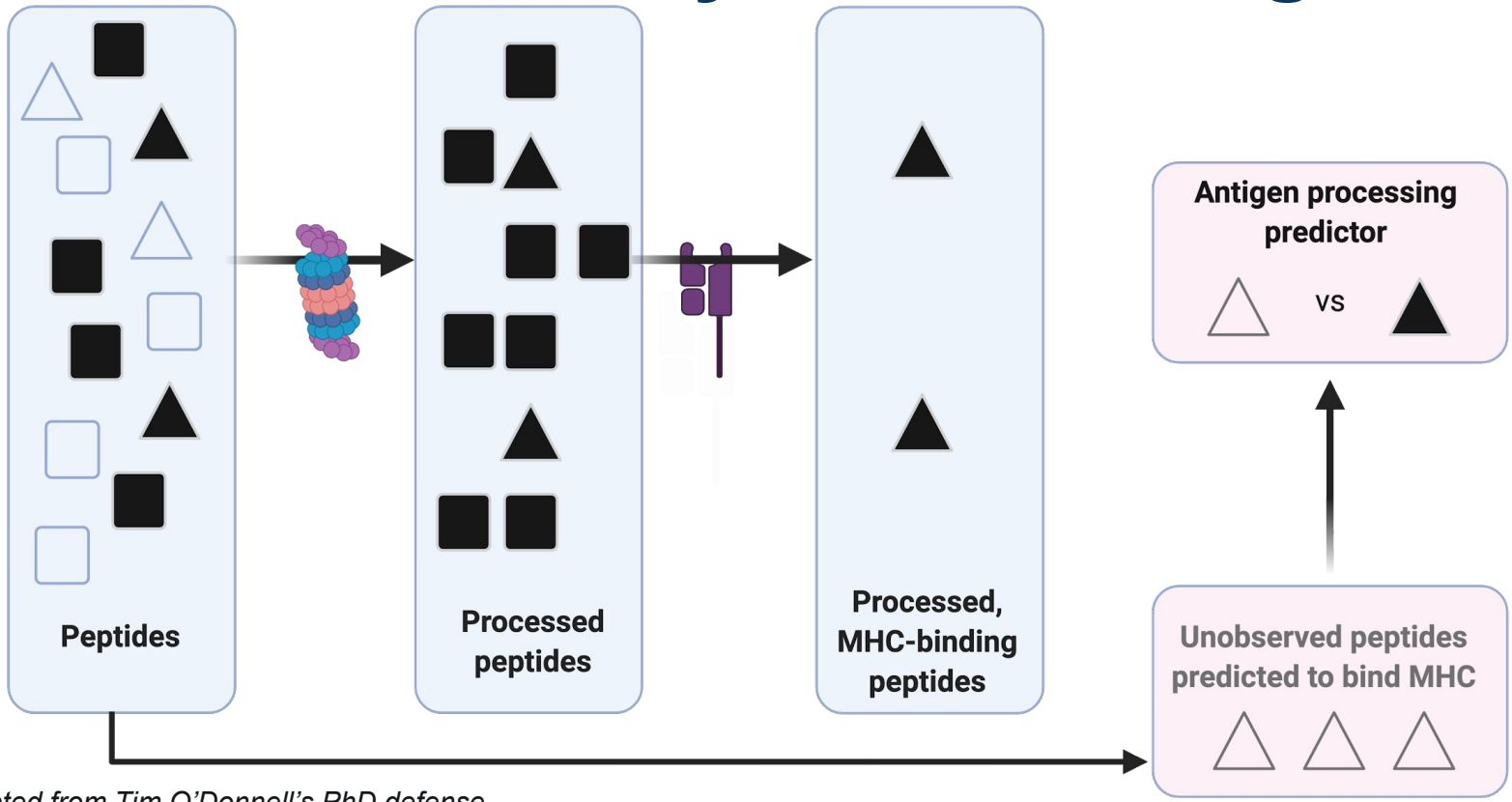
\+

Peptide: left, centered, right

PEPTIDEXXXPEPTIDEXXXPEPTIDE

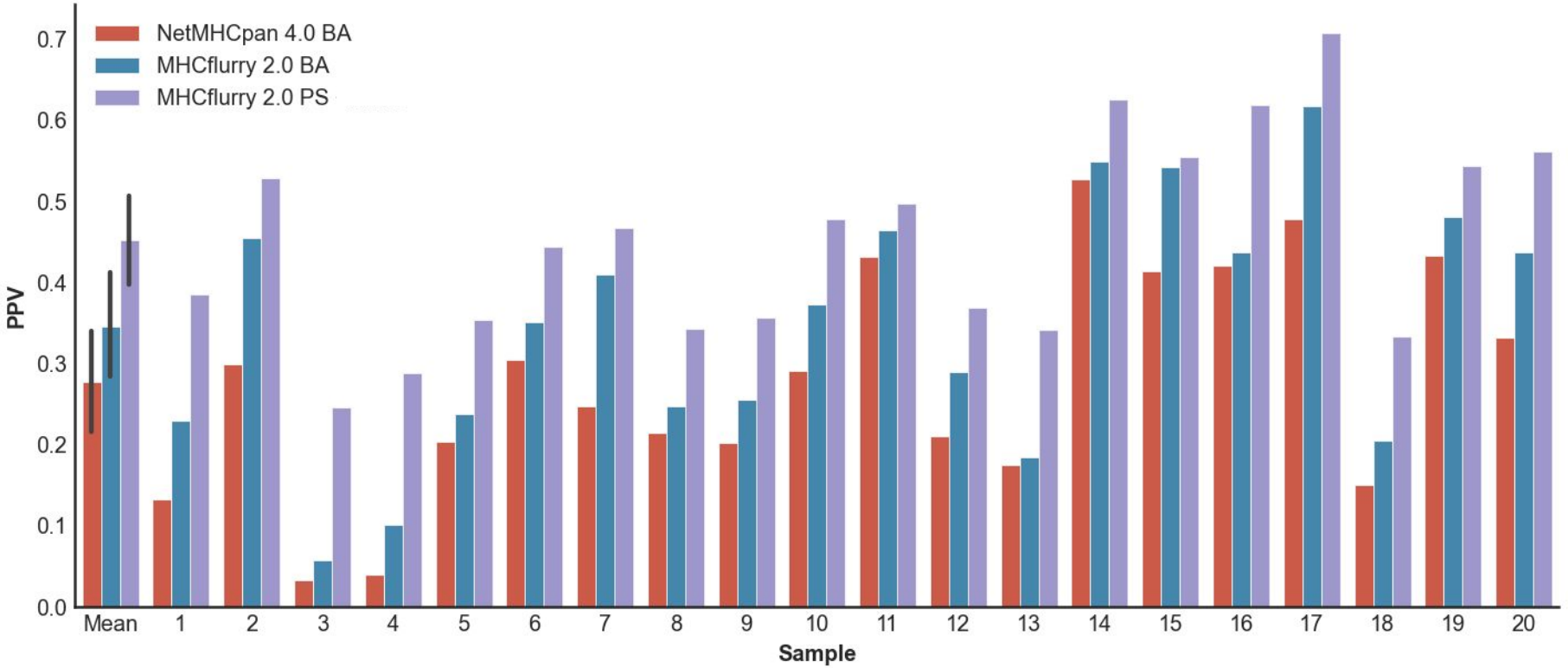
Adapted from Tim O'Donnell's PhD defense

MHCflurry 2.0: Training



Adapted from Tim O'Donnell's PhD defense

MHCflurry 2.0: Performance

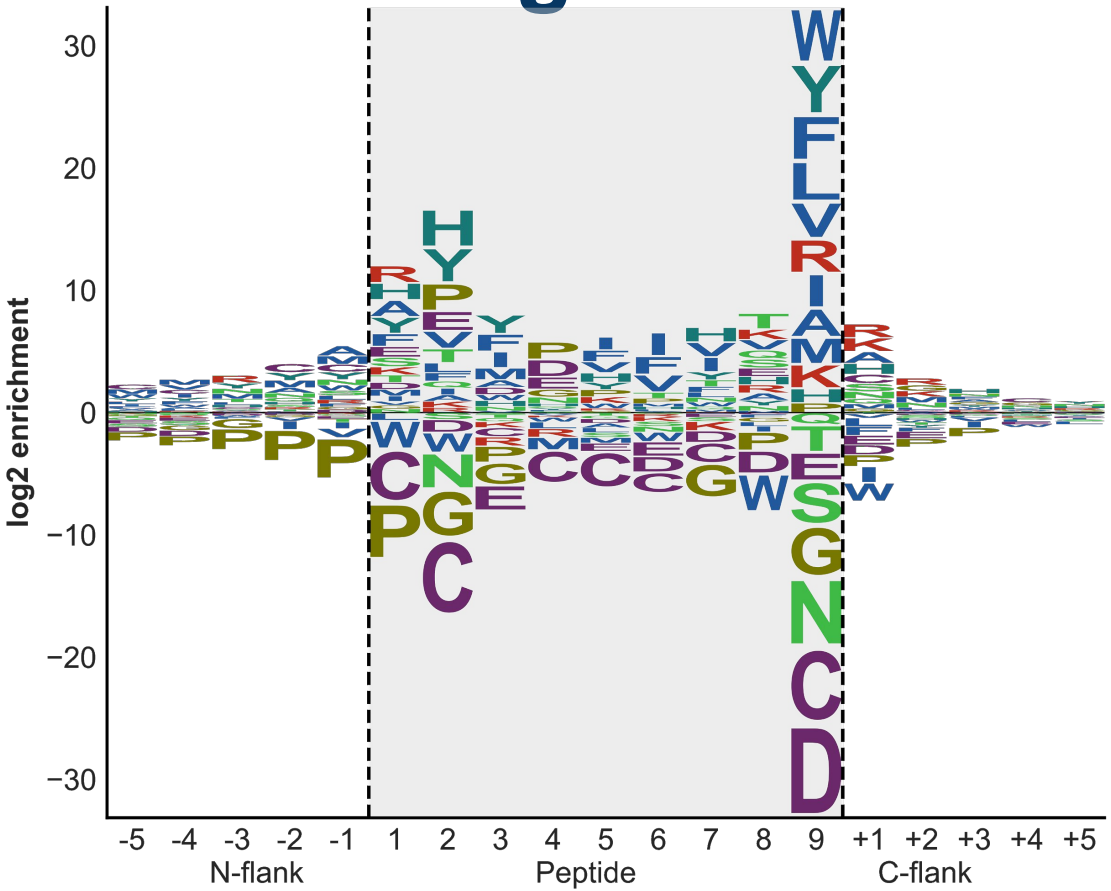


Glioblastoma CLL Glioblastoma Melanoma Ovarian

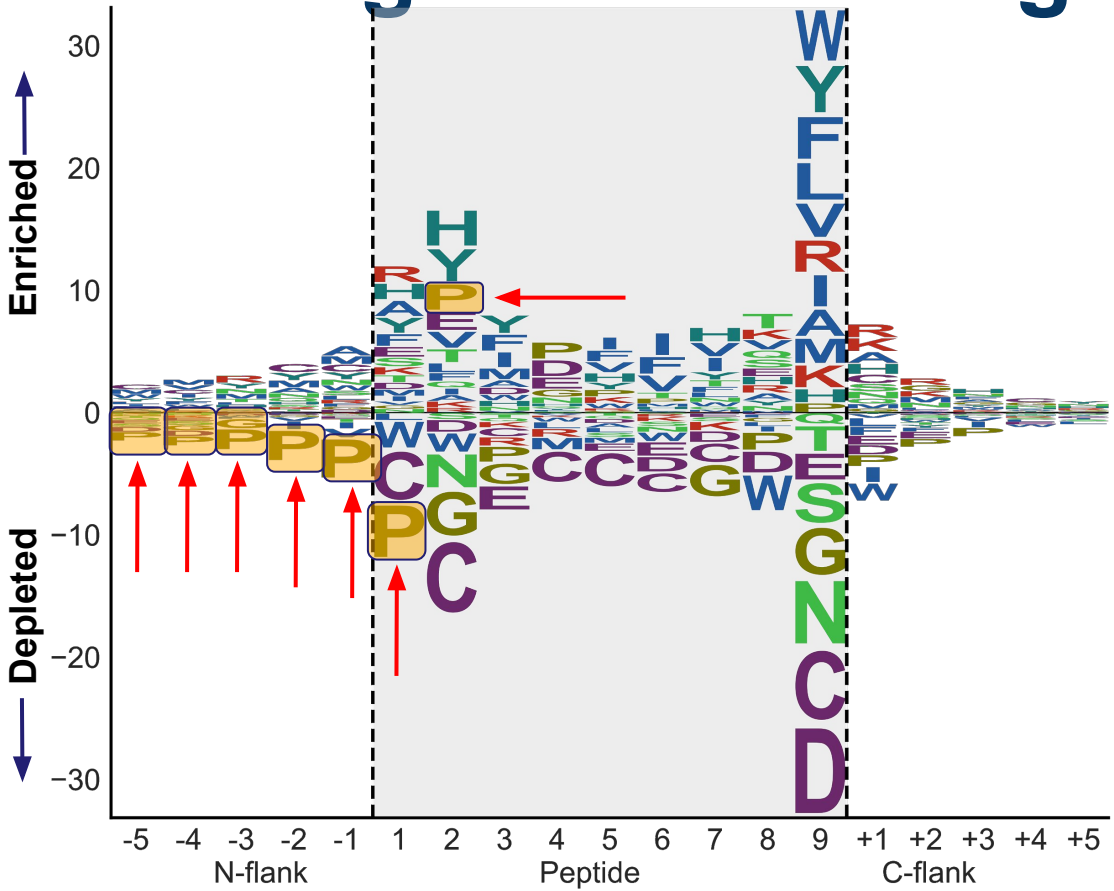
Shraibman et al. *Mol. Cell Proteomics* 2019

Sarkizova et al. *Nature Biotechnology* 2019

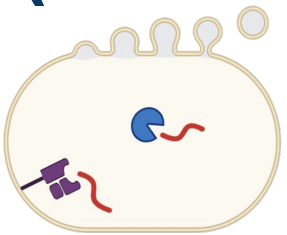
Antigen Processing Motif



Antigen Processing Motif (ERAP)

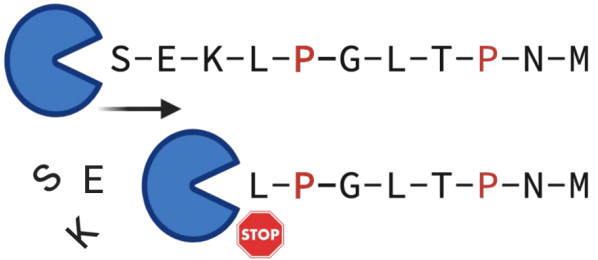


ERAP



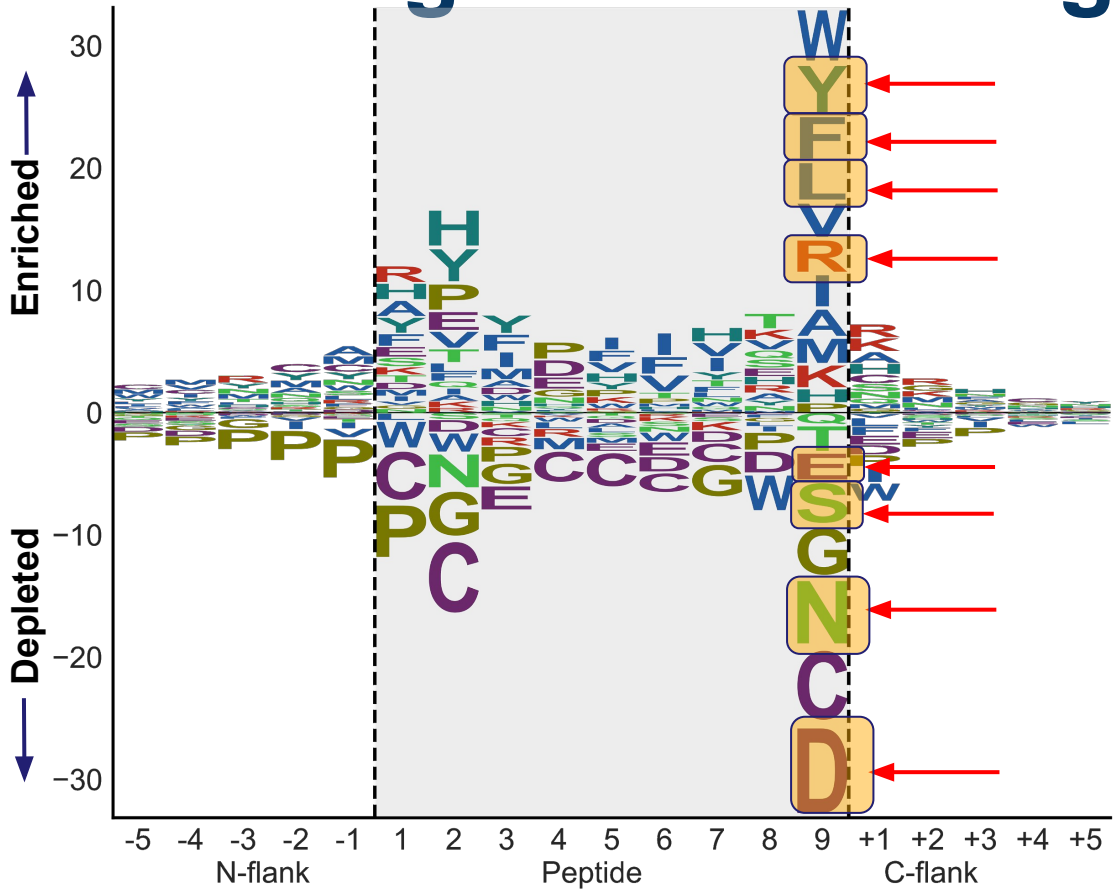
Known bias:

Unable to cleave the X-Proline bond. Can trim until there is a P at the second position

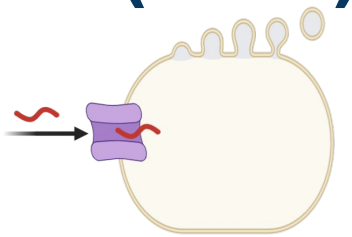


Serwold et al. *Nature* 2002

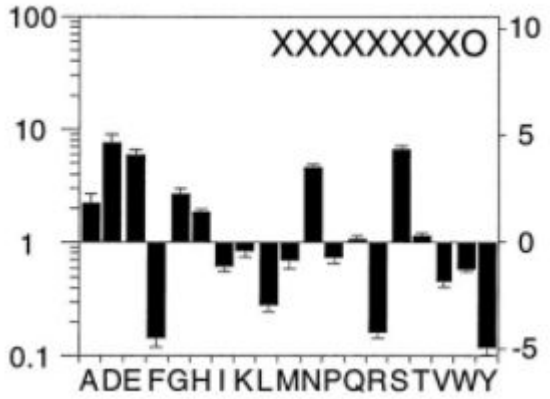
Antigen Processing Motif (TAP)



TAP



Known bias:
Prefers C-terminal Y, F, L, R
Disfavors C-terminal D, E, N, S



Uebel et al *PNAS* 1997

Next ML Frontier: T-cell Epitope Prediction

- Given:
  - Peptide sequence
  - Surrounding source protein sequence
  - Abundance of source protein
  - MHC alleles
- Predict:
  - What's the probability of a T-cell response?
  - All upstream steps (antigen processing, MHC binding) necessary but not sufficient

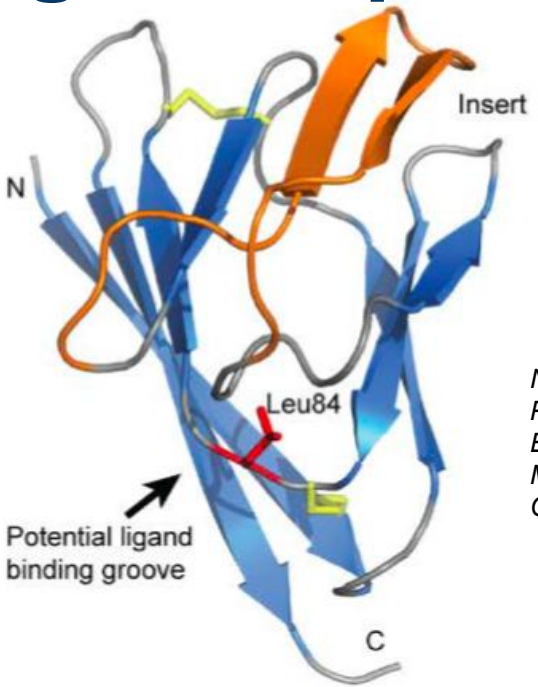
Big picture: better neoantigen vaccine

- **PANDA-VAC trial with Ben Vincent and Jared Weiss**
- **Rich initial set of “large” mutations**
  - High accuracy long read sequencing of tumors
    - PacBio HiFi reads or polished Oxford Nanopore
  - Ignore SNVs, only target fusions, indels, and other “large” mutations such as ERV integration sites, repeat expansions, &c
- **Immunogenic vaccine formulation**
  - Antigen and adjuvant based on SARS-CoV-2 experiments
- **Improved T-cell epitope prediction**
  - Multi-output Transformer model which predicts T-cell response assays as well as peptide-MHC affinity, antigen processing &c

Fin

Antigen Processing Example: Source Protein

- Example protein: SARS-CoV-2 ORF8
- Right: **structure**
- Below: **sequence**



Novel Immunoglobulin Domain Proteins Provide Insights into Evolution and Pathogenesis Mechanisms of SARS-Related Coronaviruses

MKFLVFLGIITTVAAFHQECSLQSCTQHQPYVVDDPCPIHFYSKWYIRVGARKSAPLIELCVDEA
GSKSPIQYIDIGNYTVSCLPFTINCQEPKLGSLVVRCSFYEDFLEYHDVRVVLDFI

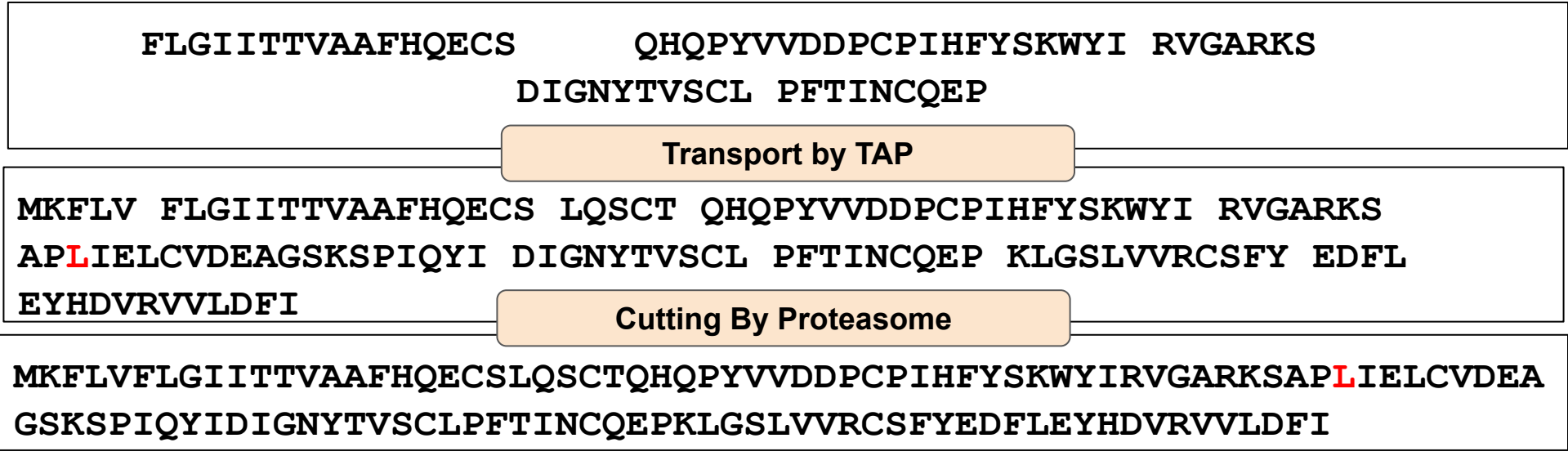
Antigen Processing Example: Initially Cutting By Proteasome

MKFLV FLGIITTVAAFHQECS LQSCT QHQPYVVDDPCPIHFYSKWYI RVGARKS
APLIELCVDEAGSKSPIQYI DIGNYTVSCL PFTINCQEP KLGSLVVRCSFY EDFL
EYHDVRVVLDFI

Cutting By Proteasome

MKFLVFLGIITTVAAFHQECSLQSCTQHQPYVVDDPCPIHFYSKWYIRVGARKSAPLIELCVDEA
GSKSPIQYIDIGNYTVSCLPFTINCQEPKLGSLVVRCSFYEDFLEYHDVRVVLDFI

Antigen Processing Example: Transport by TAP



Antigen Processing Example: Trimming by ERAP

ITTVAAFHQECS PYVVDDPCPIHFYSKWYI GARKS
IGNYTVSCL PFTINCQEP

Trimming by ERAP

FLGIITTVAAFHQECS QHQPYVVDDPCPIHFYSKWYI RVGARKS
DIGNYTVSCL PFTINCQEP

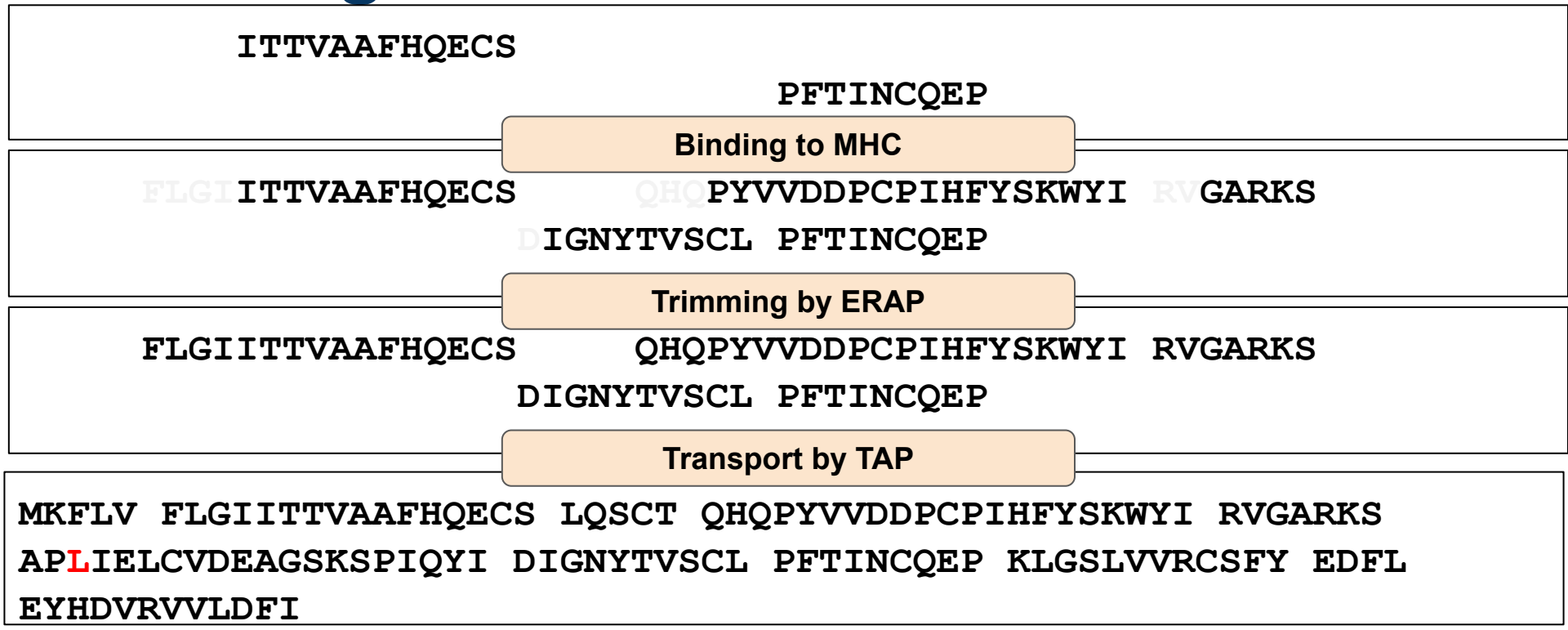
Transport by TAP

MKFLV FLGIITTVAAFHQECS LQSCT QHQPYVVDDPCPIHFYSKWYI RVGARKS
APLIELCVDEAGSKSPIQYI DIGNYTVSCL PFTINCQEP KLGSLVVRCSFY EDFL
EYHDVRVVLDFI

Cutting By Proteasome

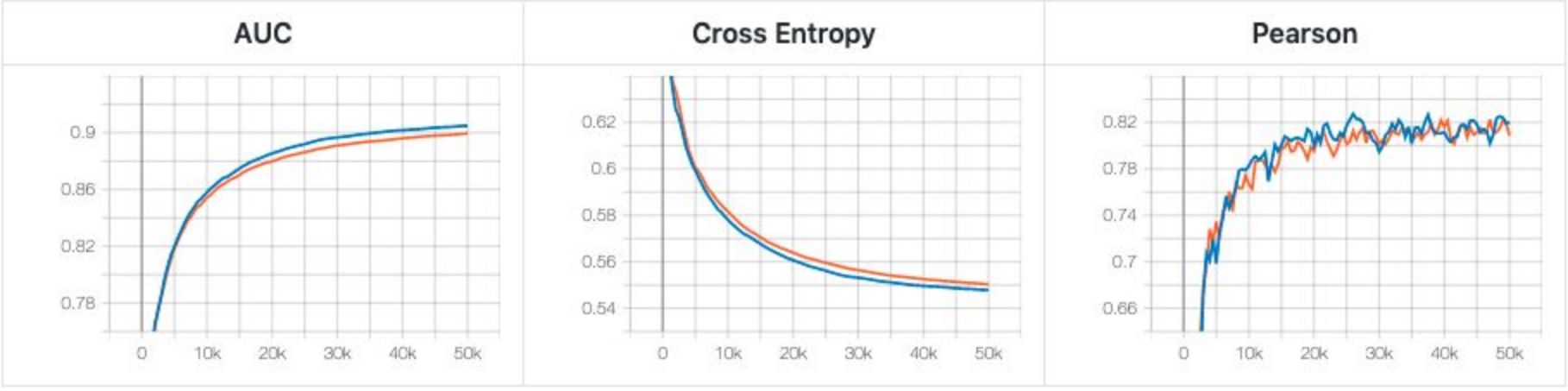
MKFLVFLGIITTVAAFHQECSLQSCTQHQPYVVDDPCPIHFYSKWYIRVGARKSAPLIELCVDEA
GSKSPIQYIDIGNYTVSCLPFTINCQEPKLGSLVVRCSFYEDFLEYHDVRVVLDFI

Antigen Processing Example: Binding to MHC

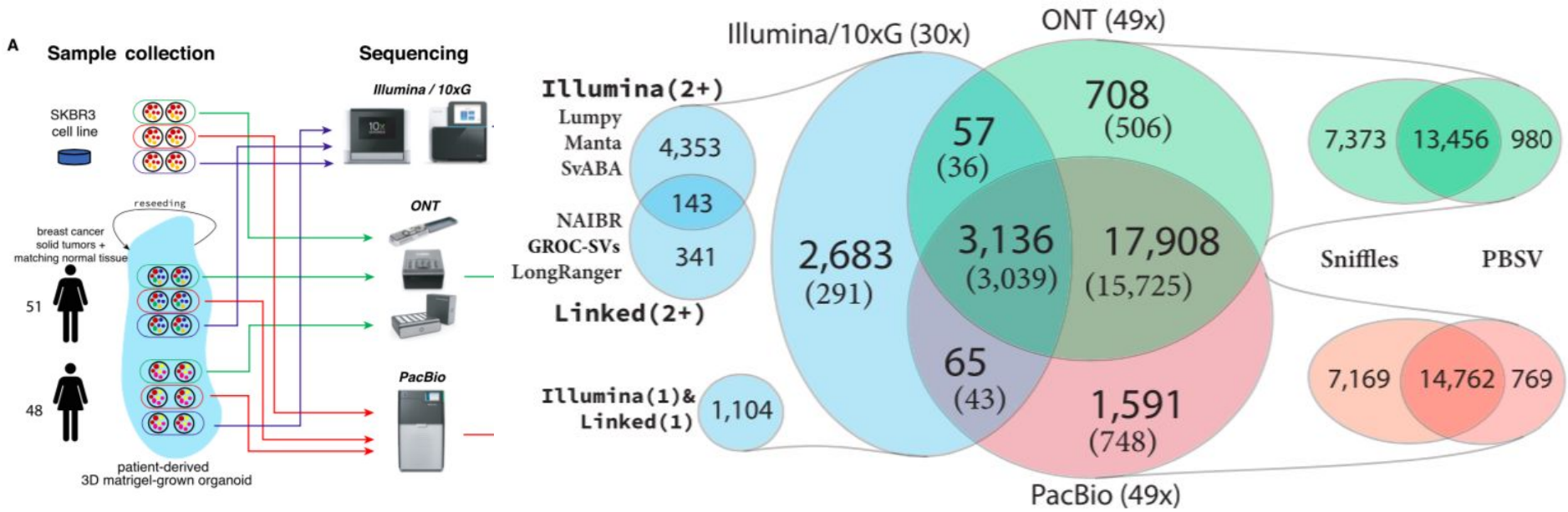


ML experiment: Transformers!

- Pretrained “Evolutionary Scale Modeling” Transformer (Facebook)
- Single sequence input: “**peptide#MHCsequence**”
- Fine tuning: Predict single scalar, peptide-MHC affinity

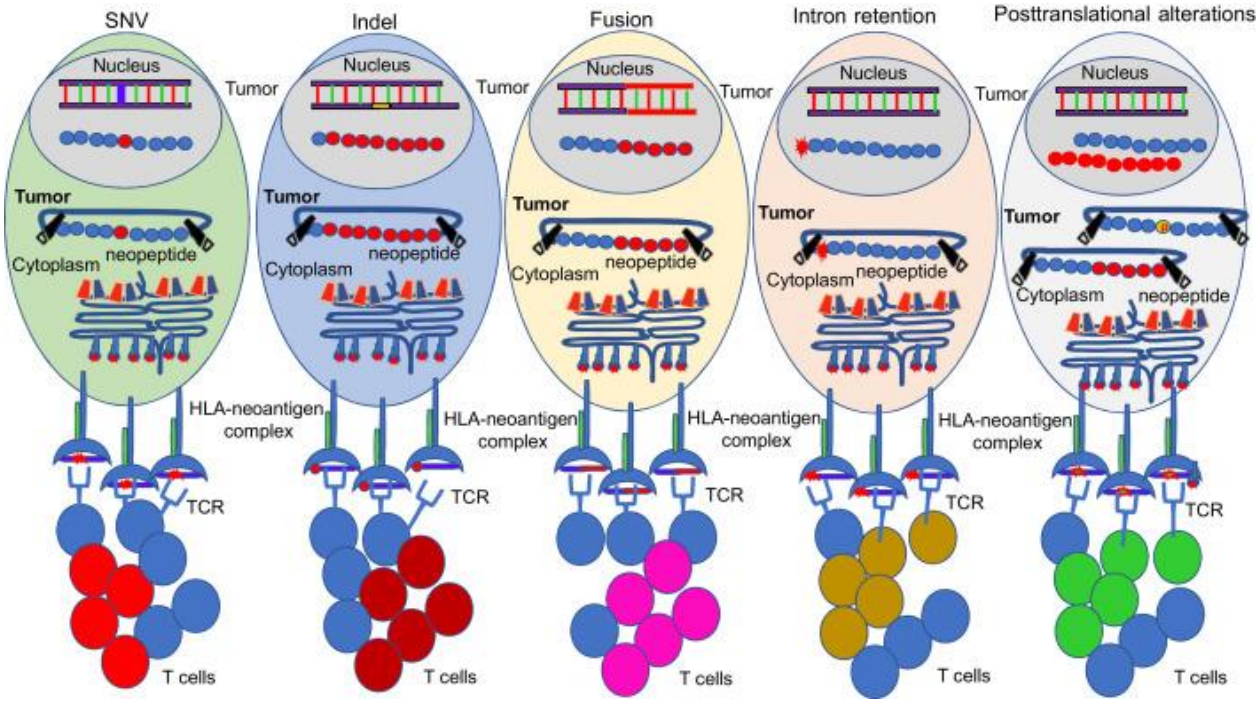


Which mutations?



Comprehensive analysis of structural variants in breast cancer genomes using single-molecule sequencing

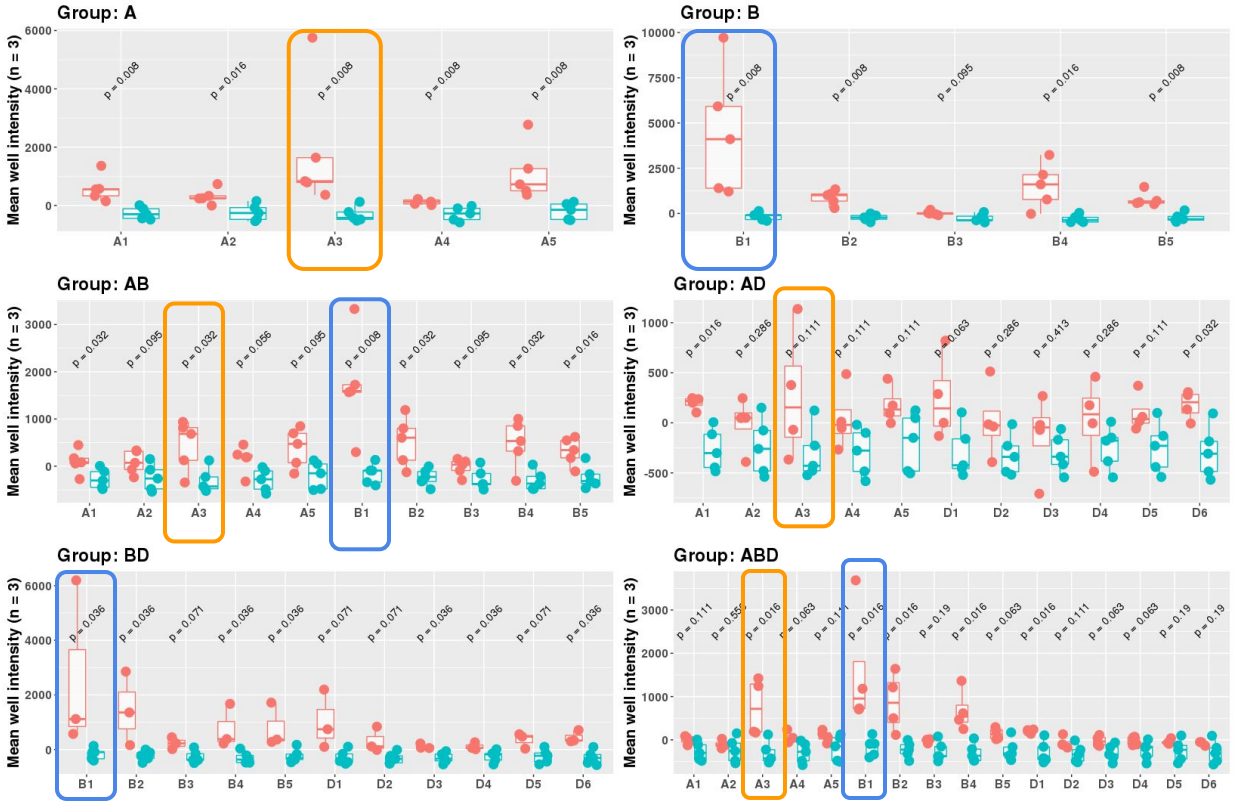
Beyond Mutations: Splicing (and PTMs)



Diverse Neoantigens and the Development of Cancer Therapies

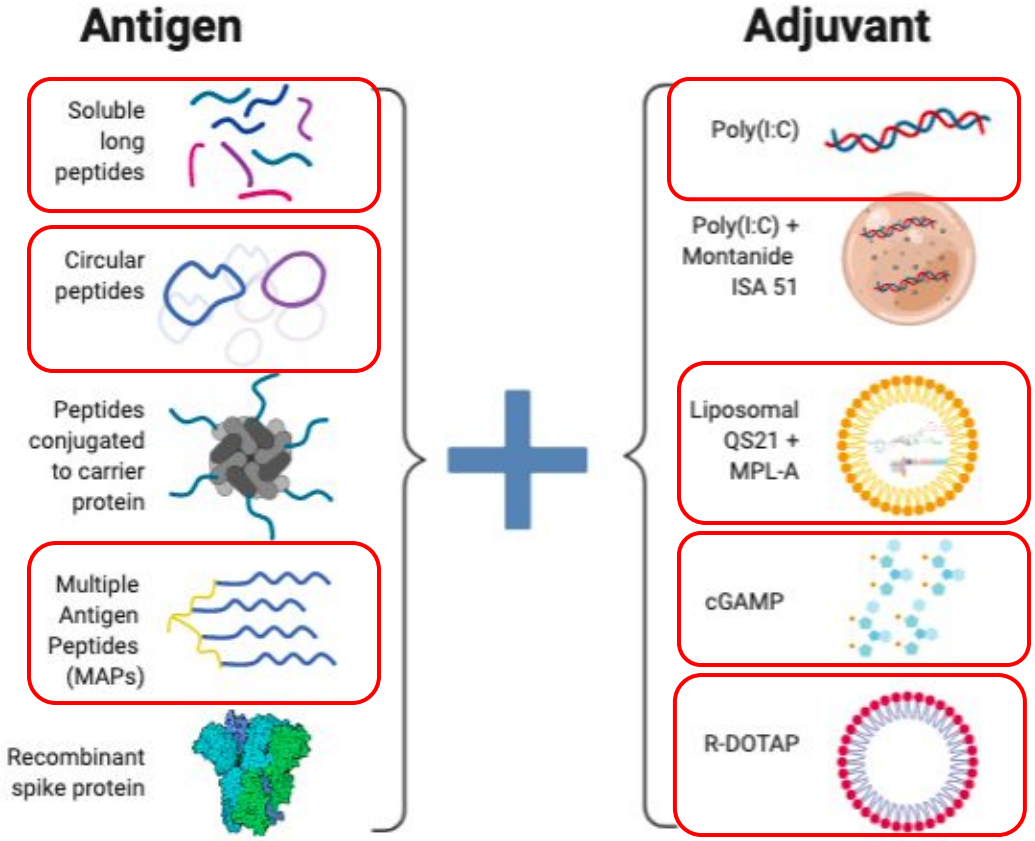
Preliminary ELISpot results

- 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



Ongoing & planned experiments

- Find best adjuvant for each antigen
  - small # of mice
- Compare antigens to each other + recombinant spike
- Circular peptides:
  - Hard to manufacture!
  - 9/16 successfully synthesized
- Branched peptides (MAPs):
  - Slow to manufacture!



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%	+	-	-	+	$\boxplus\boxplus^b\ \boxdot\boxdot^d$ $\boxplus$

How do T-cells recognize tumor as “non-self”?

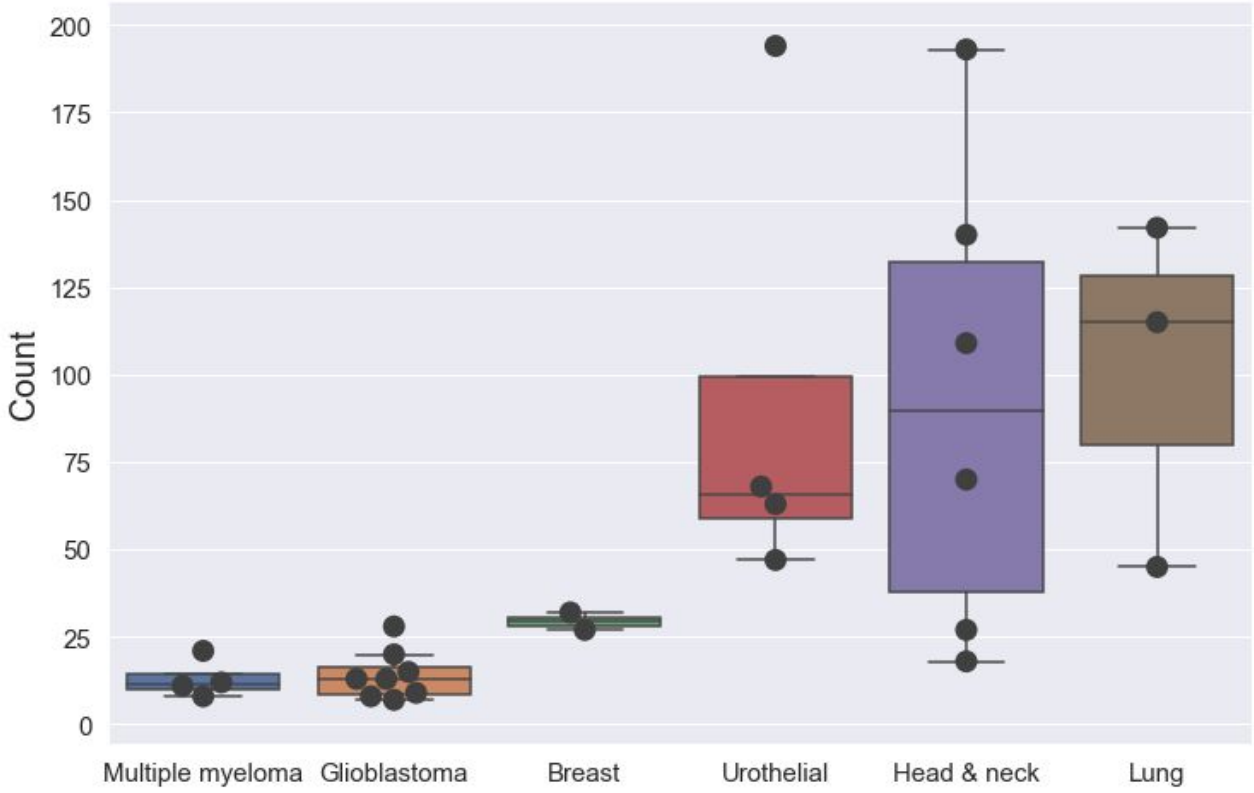
Intracellular

- **Generate “non-self”**
  - Abnormal expression
  - Mutant coding DNA
  - Abnormal splicing
  - Abnormal translation
  - PTMs
- **Antigen Processing**
  - Only a few peptides per protein make it to MHC
- **MHC Binding / Stability**

Immune

- **Germline bias of TCRs**
  - Some TCRs have limited junctional diversity, germline gene segments.
- **Thymic Selection**
  - “Self” ~= what peptides are presented to T-cells in the thymus
- **Pathogen exposure**
  - Cross-reactivity rare but expanded T-cell clones more likely to encounter tumor

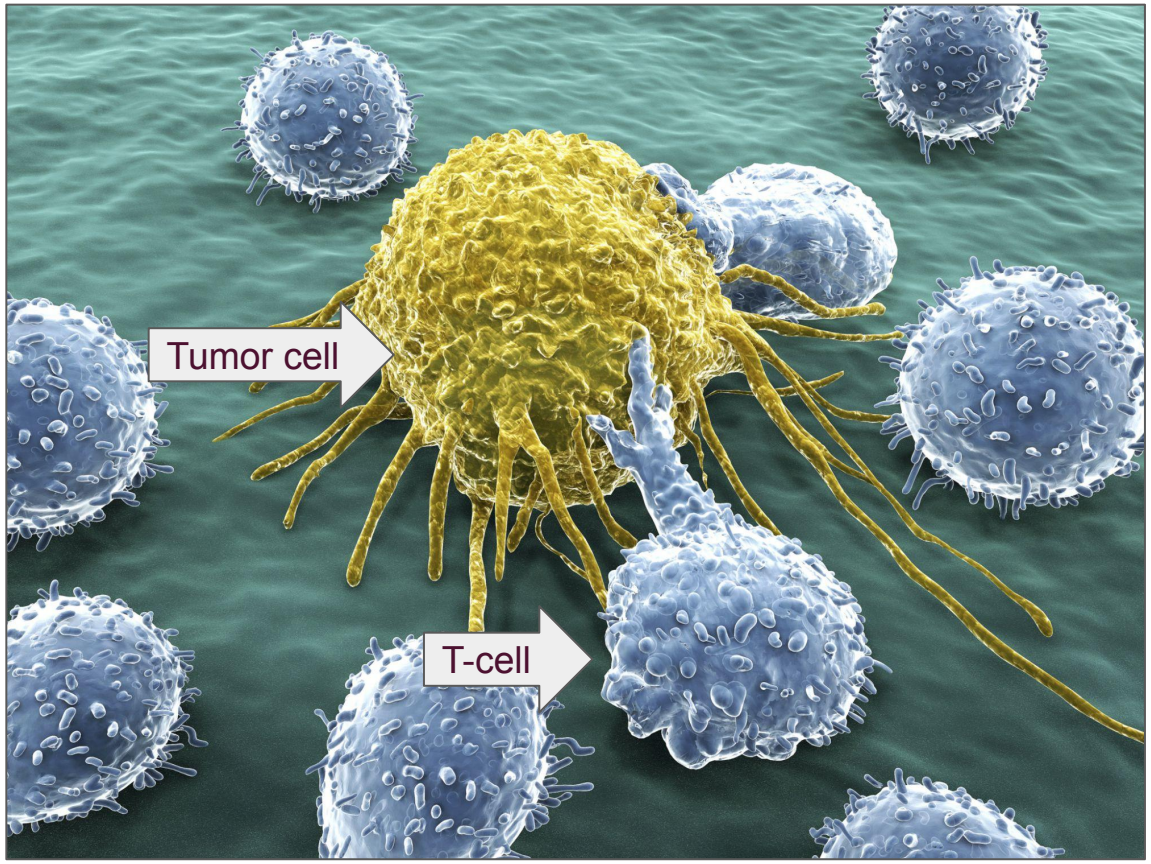
Do we get enough mutations?



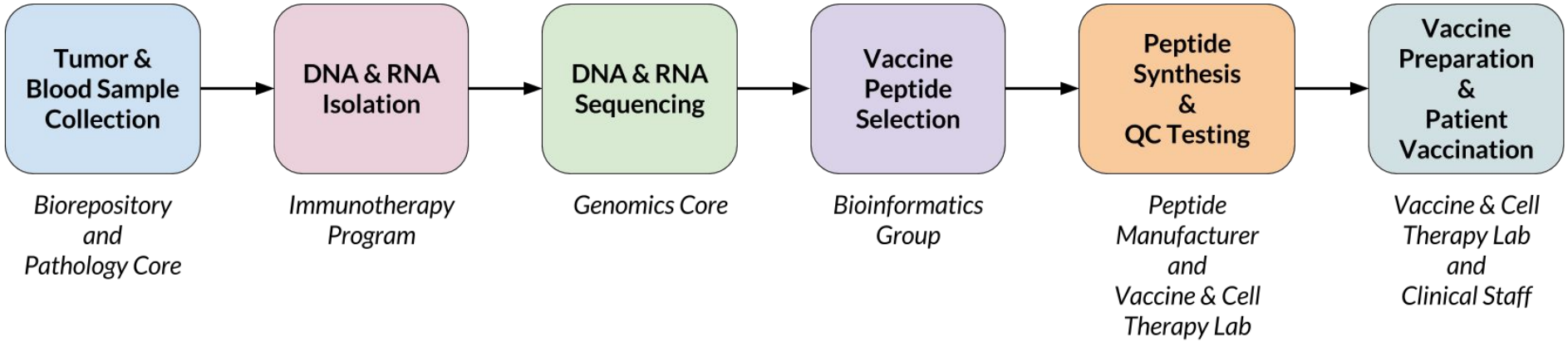
Source: Julia Kodysh

What is a T-cell?

- Patrols the body, looks for cells making “unexpected” proteins
  - Viruses and cancer
- Each T-cell has a randomly generated receptor
- When a (cytotoxic) T-cell finds its target: kill! kill!



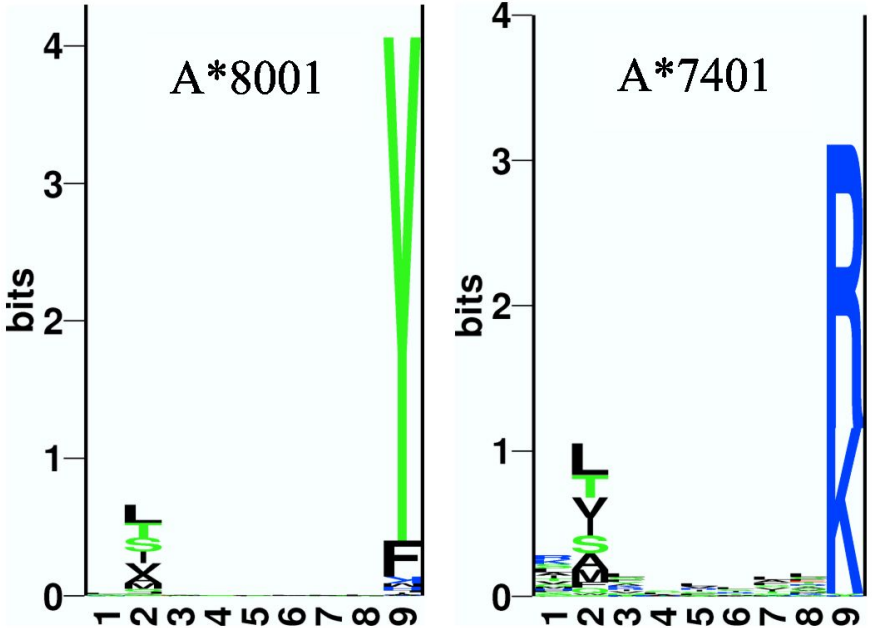
Trial logistics



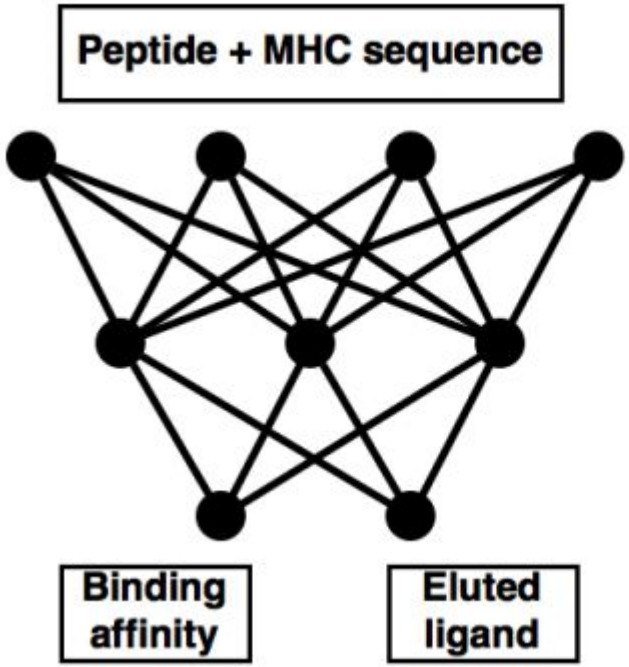
- 1-2 weeks from surgery to sequencing data
- 1 week to run computational pipeline and manually review results
- 6-8 weeks peptide synthesis
- 10 immunizations over 6 months

MHC: Most Diverse Gene in the Human Genome

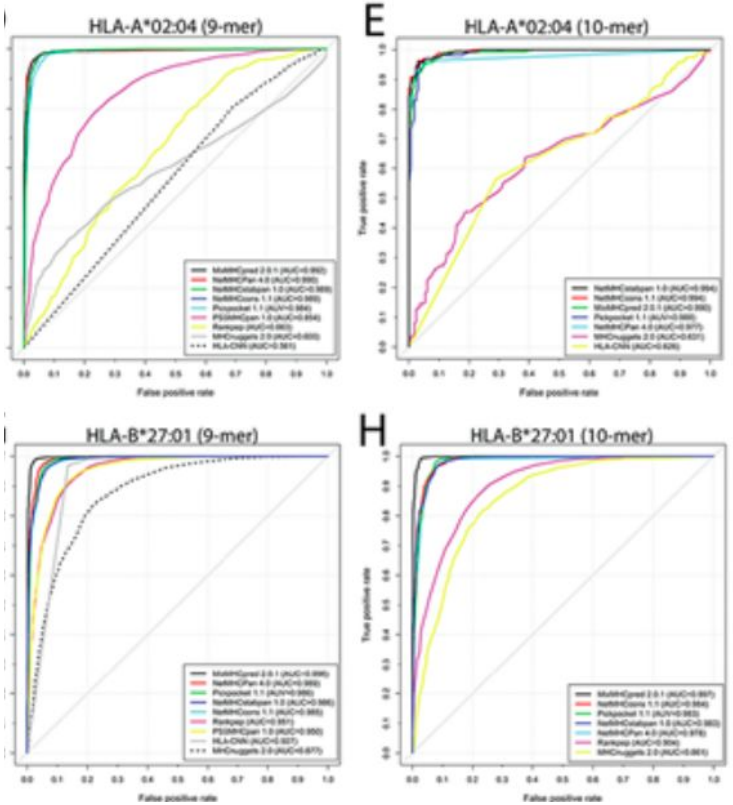
- 3 genes (HLA-A/B/C)
- Everyone has two copies of each gene
- Thousands of distinct versions (“alleles”)
- Each allele has a distinct pattern recognition specificity



MHC binding prediction



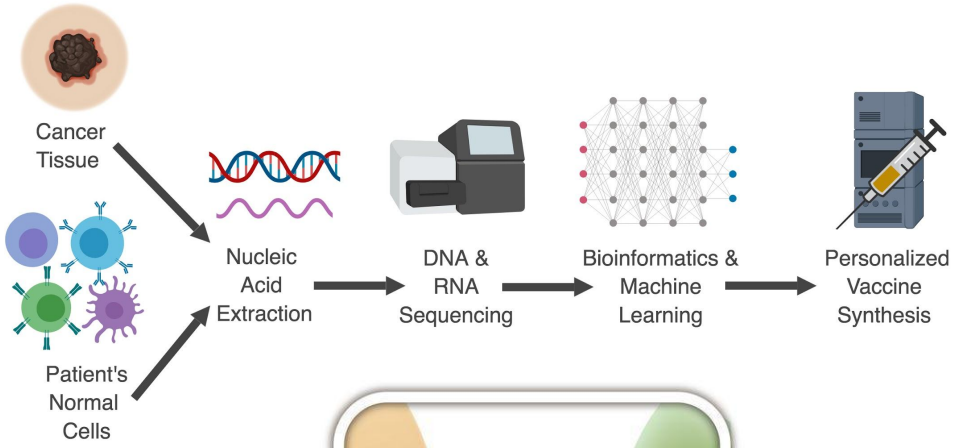
NetMHCpan 4.0

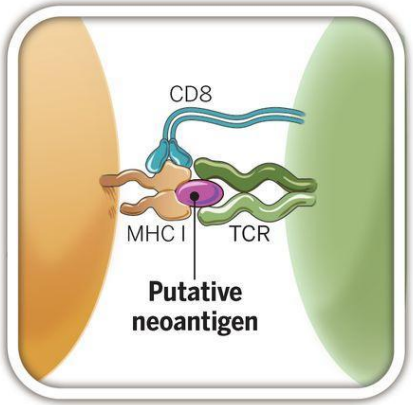


A comprehensive review and performance evaluation of bioinformatics tools for HLA class I peptide-binding prediction

Neoantigen vaccination: simple!

- **Inputs**
  - Tumor + Normal DNA
  - Tumor RNA
- **Selection**
  - Predict which mutant peptides bind patient MHC
- **Vaccine**
  - Peptides + adjuvant, mRNA, DNA, viral vector, bacterial vector, &c

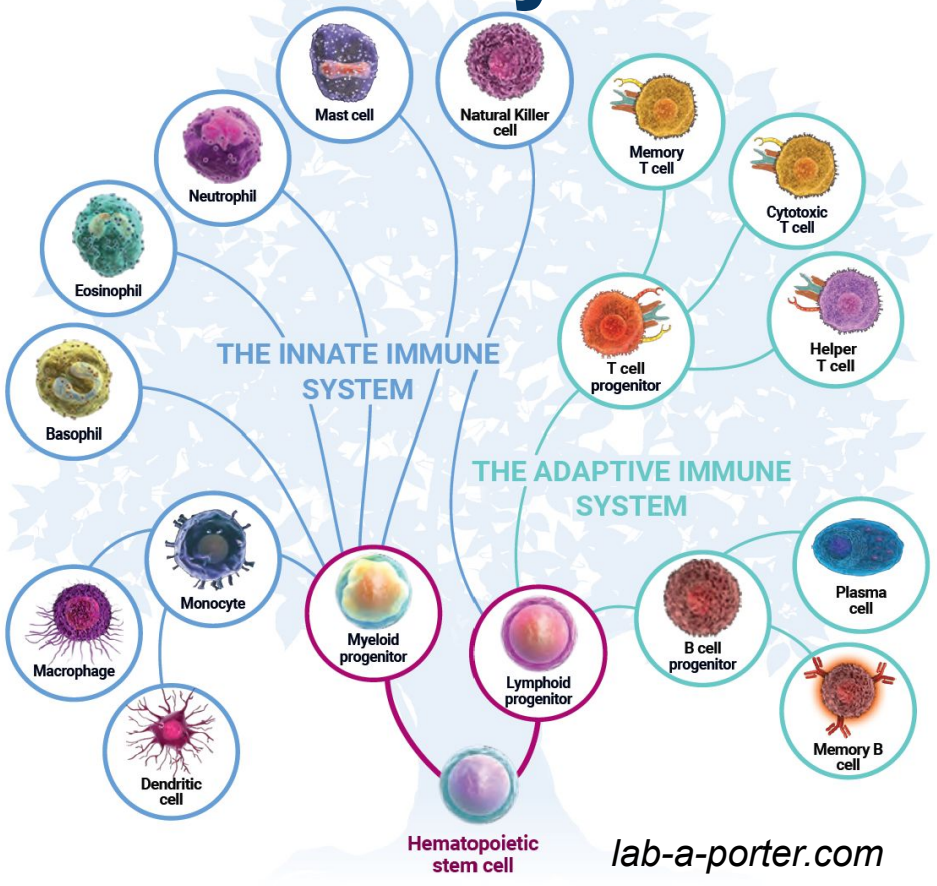




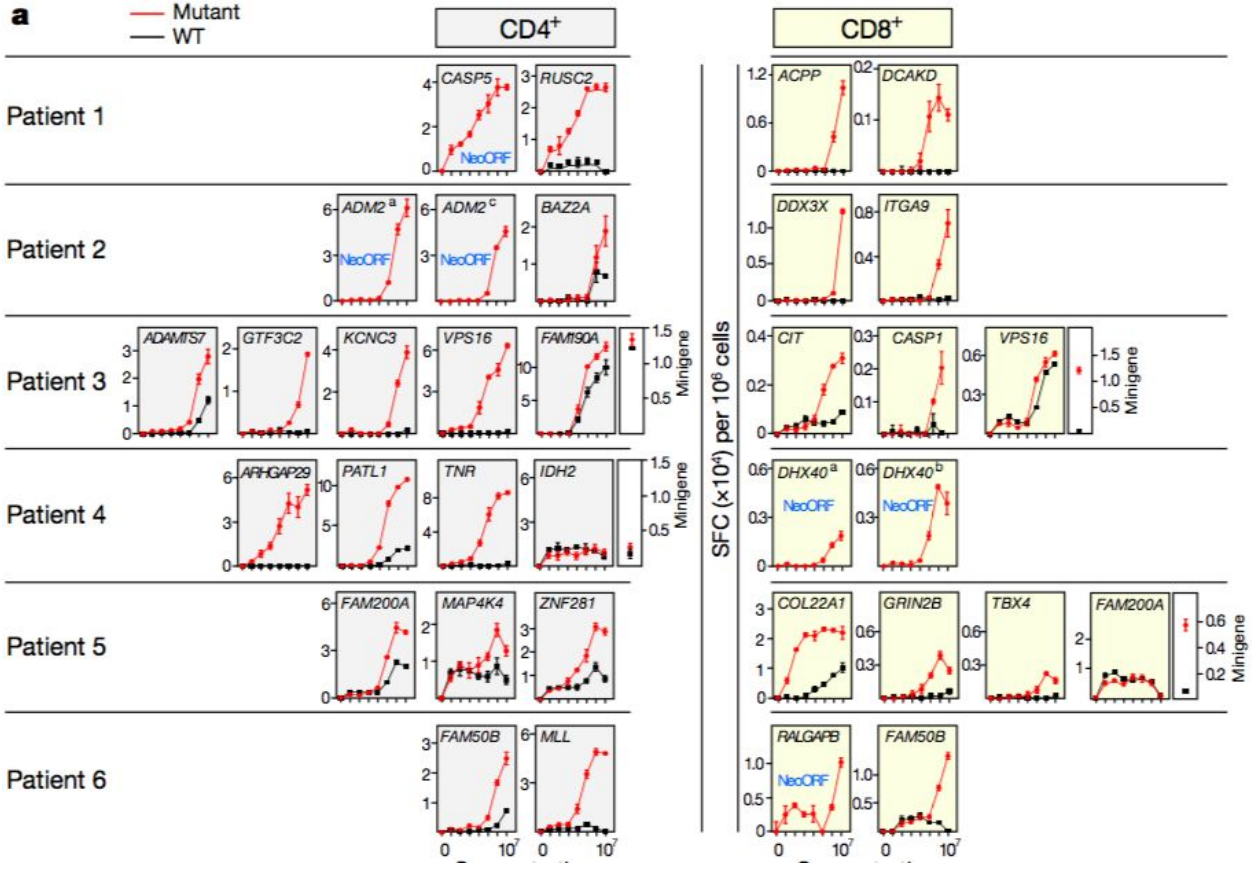
Schumacher & Schreiber 2015

Quick Intro to the Immune System

- **Innate Immune System**
  - Recognize pathogens through evolved pattern recognition receptors
- **Adaptive Immune System**
  - Learn outlier self vs. non-self detection
  - Kill non-self when it's causing damage



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]*

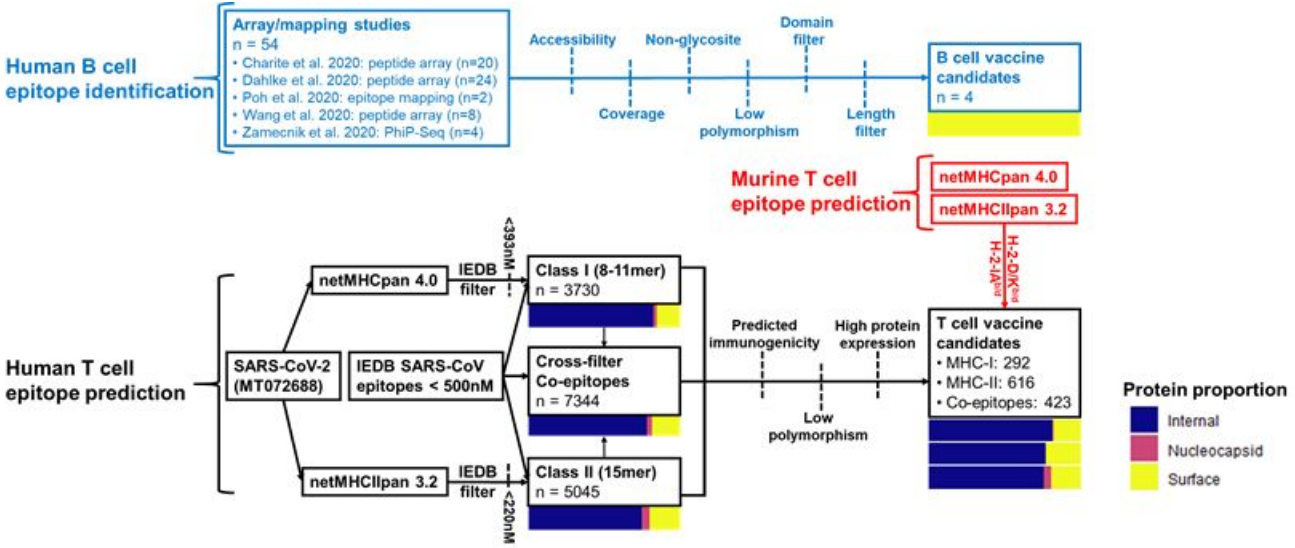
- 10 enrolled glioblastoma patients, 8 w/ enough mutations
- All eight vaccinated patients eventually died
- 6/8 were given steroids during priming: no T-cell responses!

Peptide vaccines for pathogens

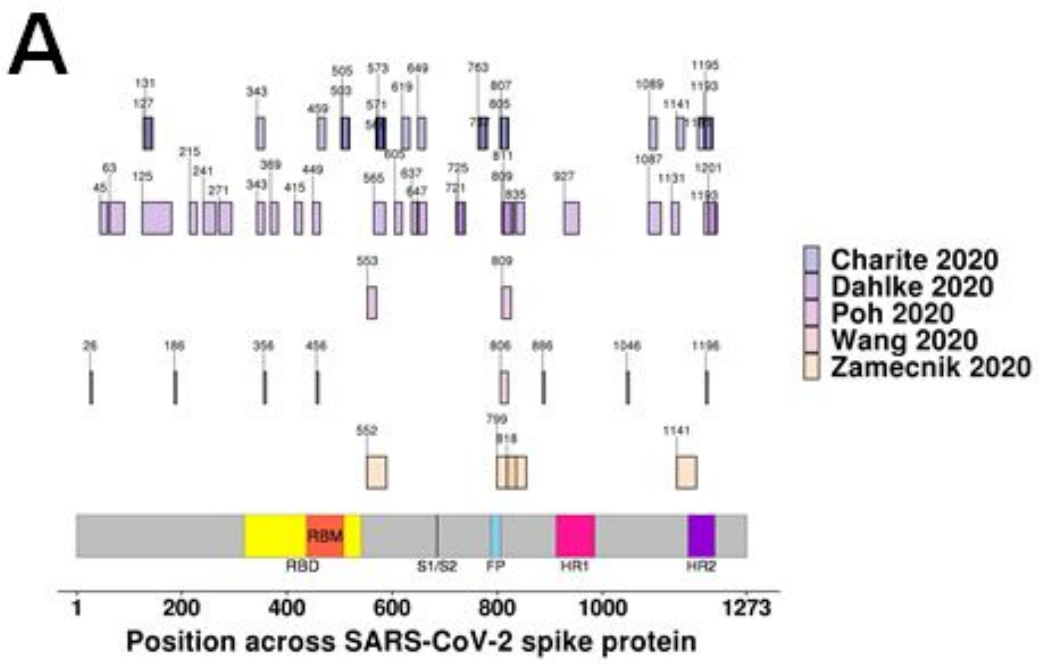
- Potential problems with whole virus or whole protein vaccination:
  - Diffuse T-cell responses; will immunodominant epitopes match presented epitopes of infected cells?
  - Responses to polymorphic regions of virus
  - Unlikely (but worrying) possibility of antibody dependent enhancement (ADE), mediated 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)

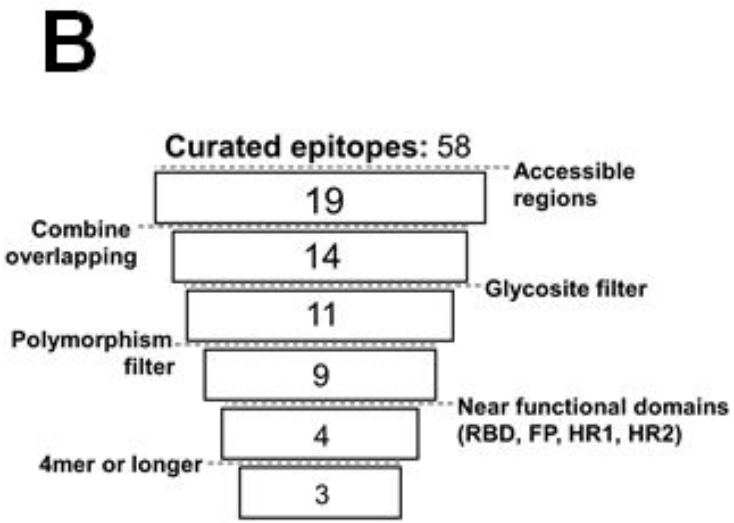
Integrating Predicted T-Cell Epitopes With Measured Linear B-cell Epitopes

- 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

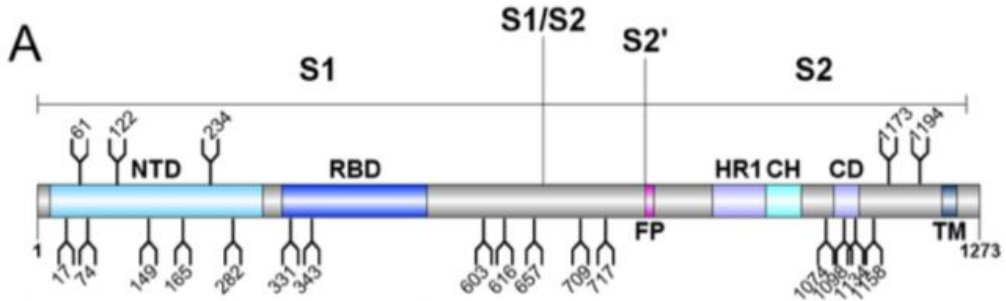


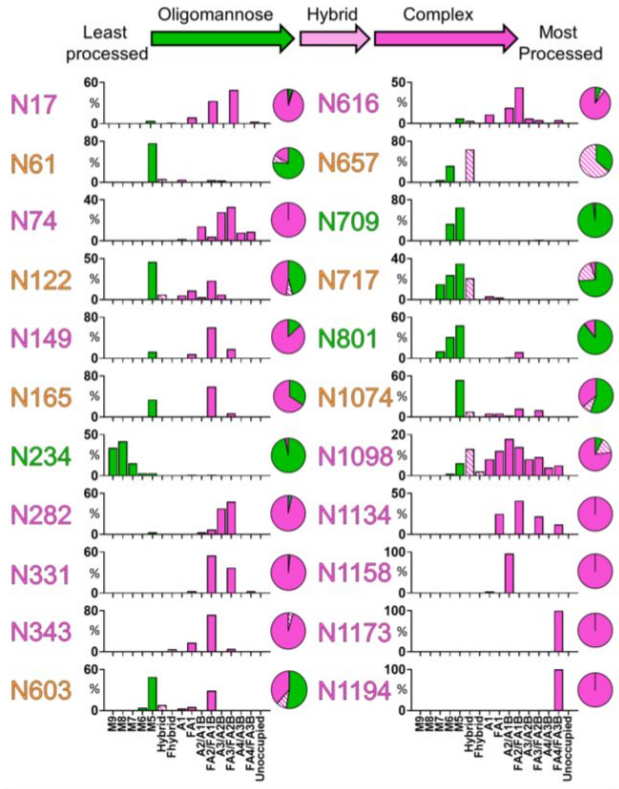


Source for Glycosites (Watanabe et al.)

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%)

Personalized cancer vaccines

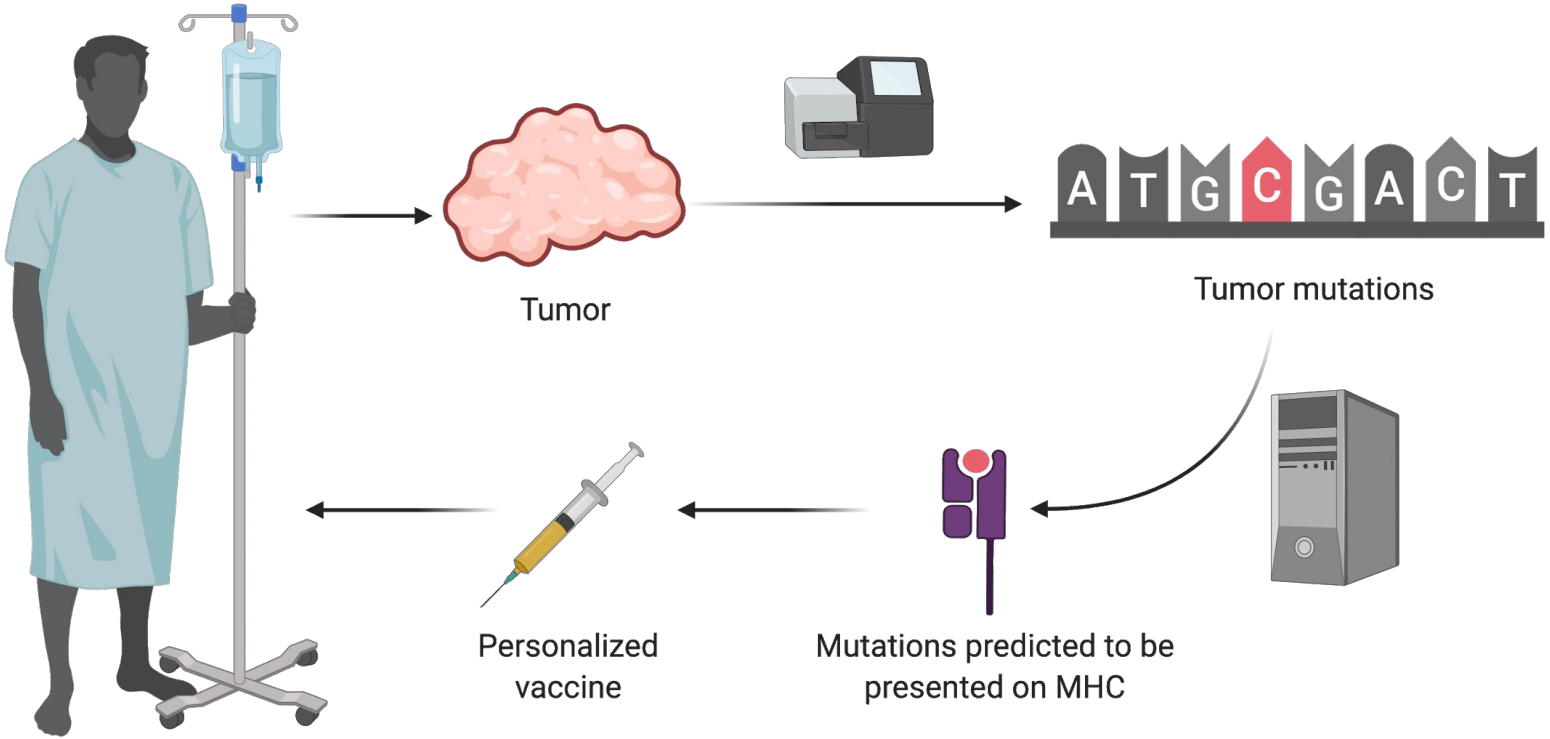


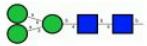
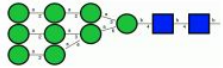
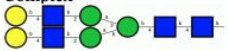
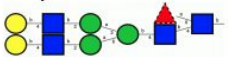
Figure adapted from Tim O'Donnell's PhD defense

Source for Accessibility (Grant et al.)

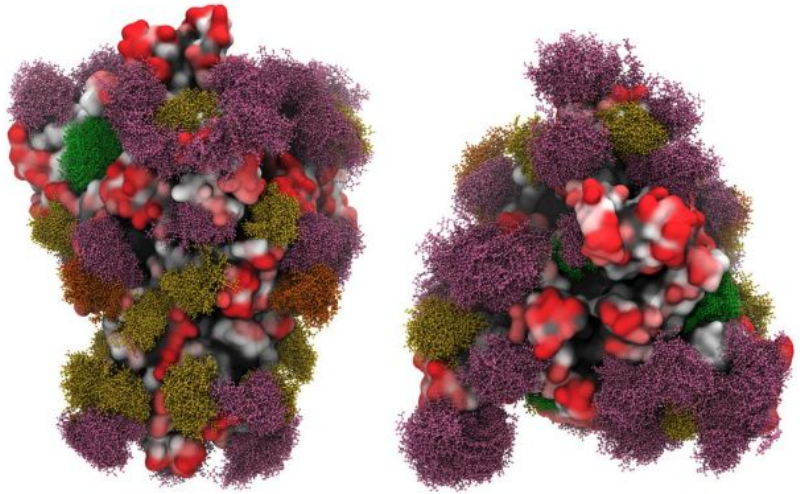
3D Models of glycosylated SARS-CoV-2 spike protein suggest challenges and opportunities for vaccine development

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

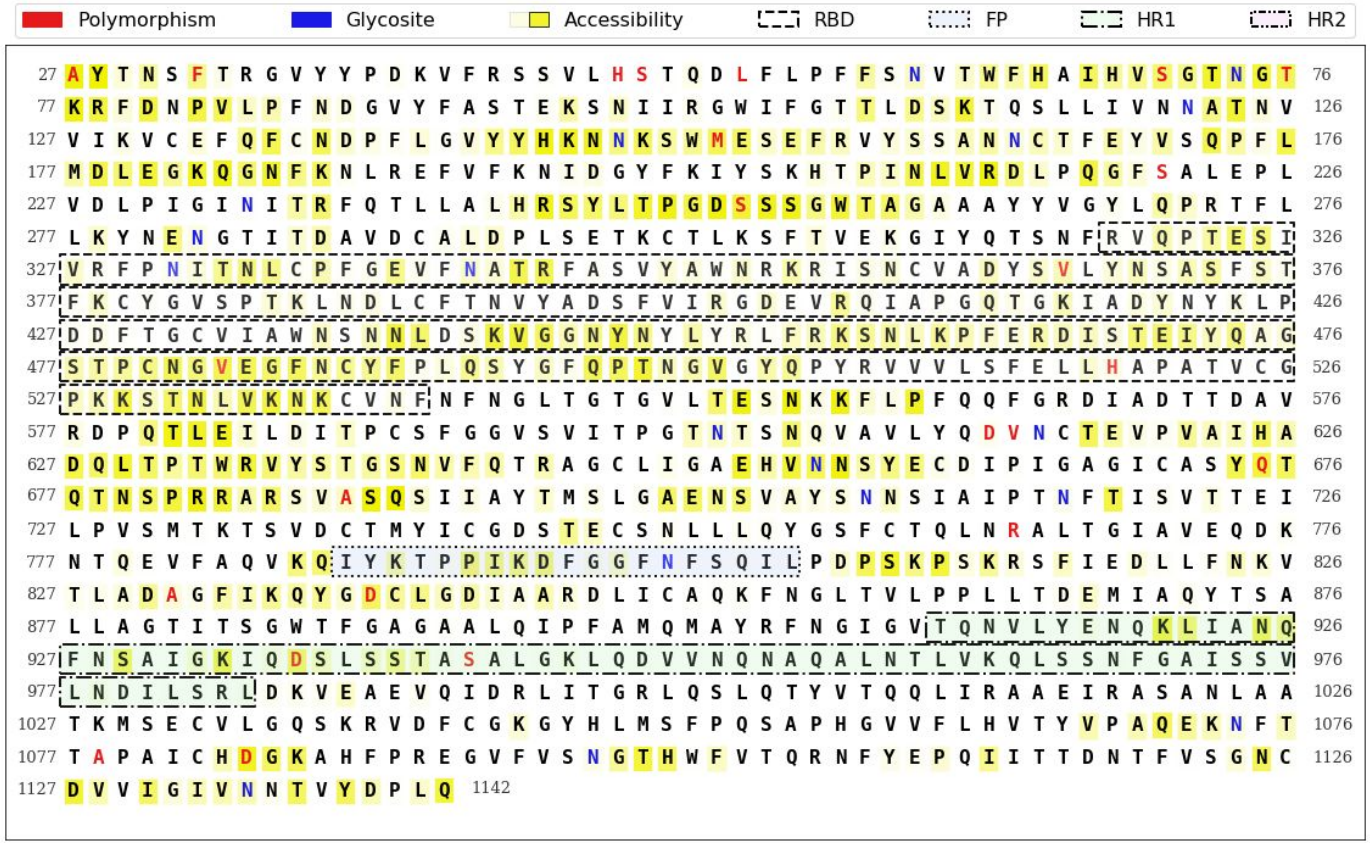
Table 1. SARS-CoV-2 S glycoprotein antigenic surface areas (Å^2) as a function of glycoform.

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.



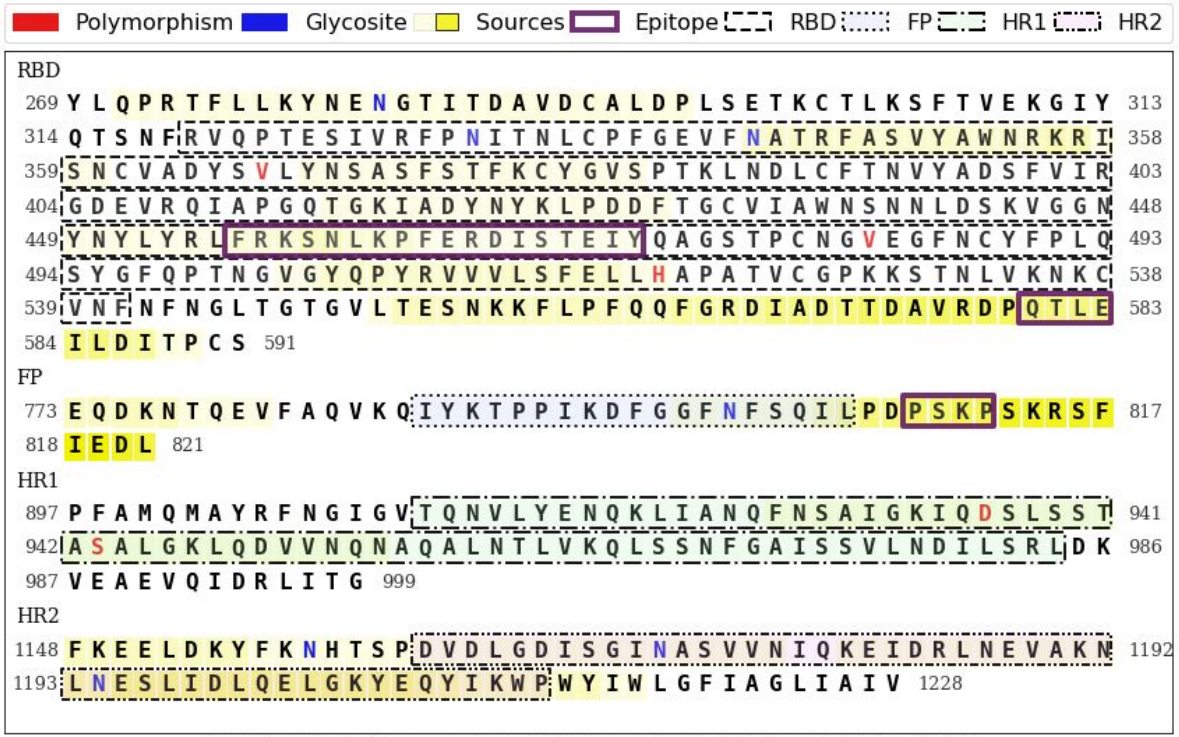
Accessible residues near functional features



Only 3 B-cell linear epitopes regions

Filters:

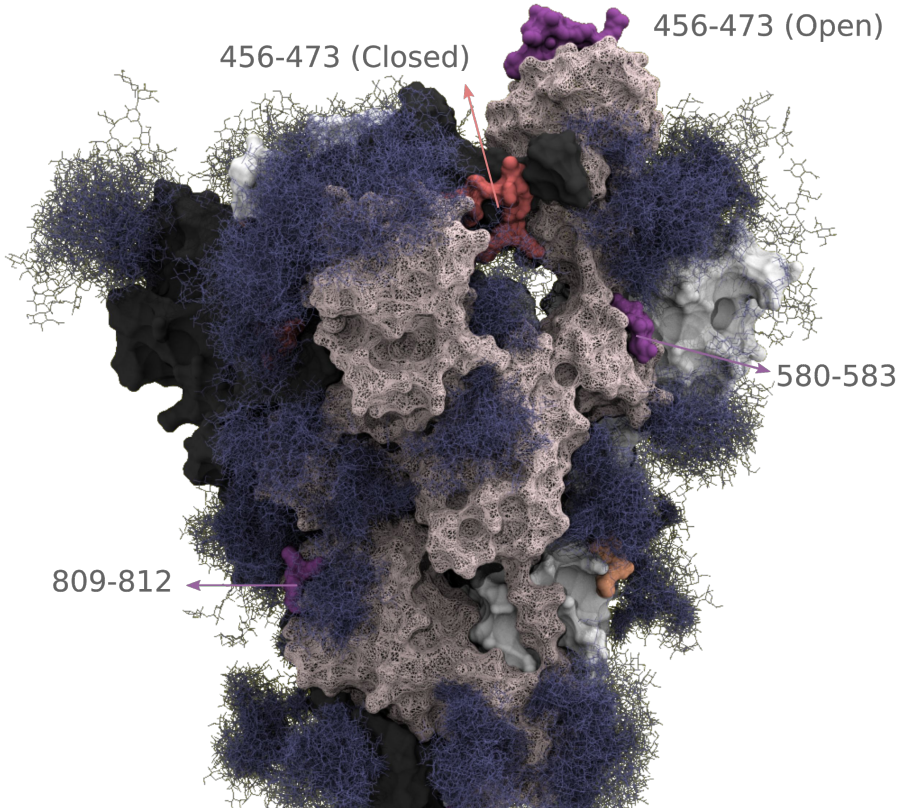
- >=4mer region
- Accessibility > 25%
- Does not contain glycosites
- Does not contain polymorphic sites
- Within 50aa of RBD or 15aa of fusion peptide (FP) or HR1/HR2 regions



Number of data sources supporting each residue as antibody epitope

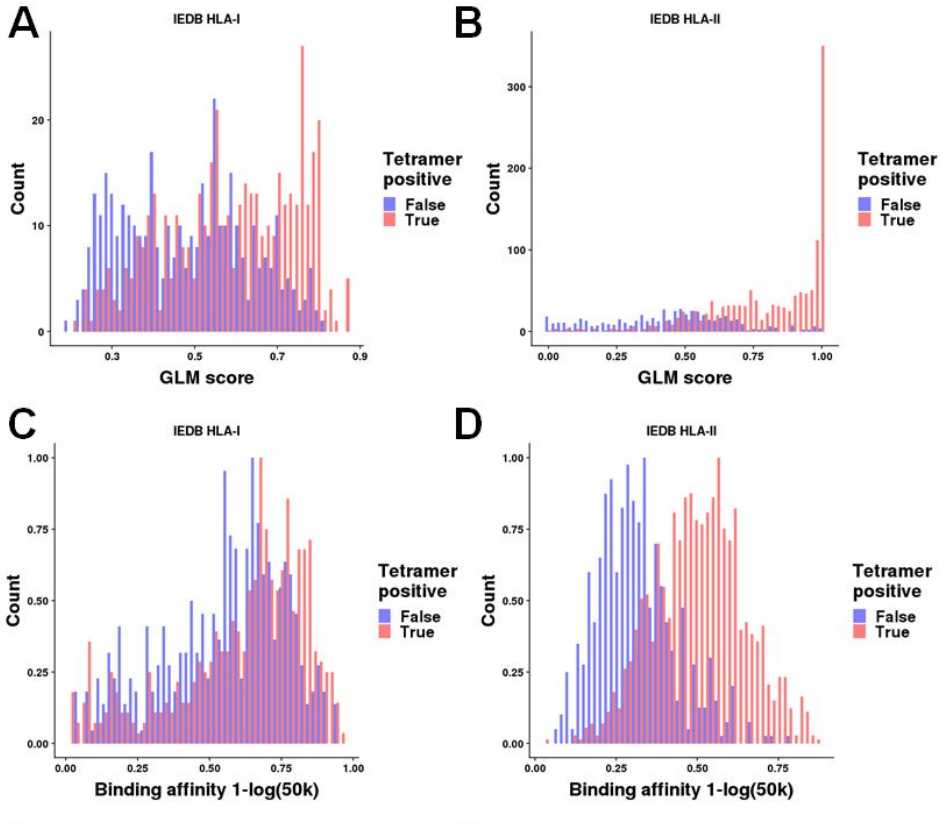
Location of predicted linear B-cell epitopes

- **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

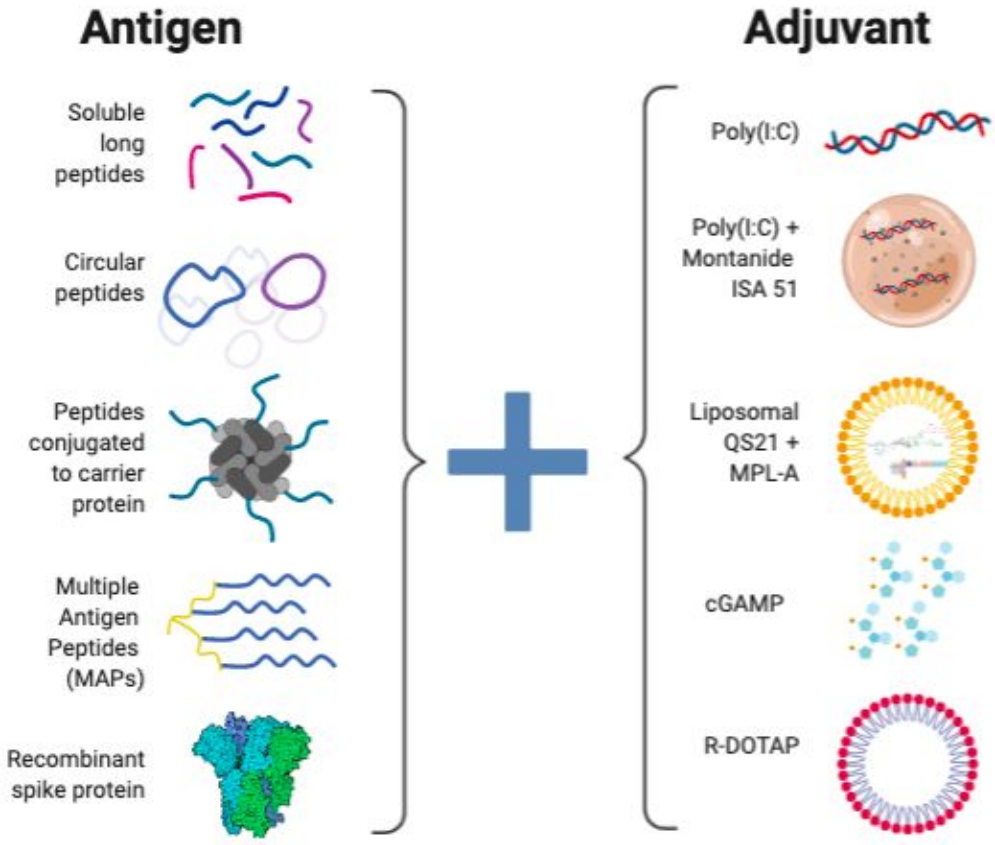
	Sequence	Protein	Start	End	B-cell Epitope Region	HLA-I Coverage	HLA-II Coverage	$H2^b$ I	$H2^b$ II	$H2^d$ I	$H2^d$ II	Selection Sets
1	LLQFAYANRNRFLYIIKLIFLWLLWPV	M	34	60		89.0%	36.0%	+	+	+	+	
2	PVTLACFVLAAVYRINWITGGIAIAMA	M	59	85		42.0%	76.0%	+	+	-	+	
3	YFIASFRLFARTRSMWSFNPETNILLN	M	95	121		78.0%	53.0%	+	+	+	+	
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%	+	-	-	+	

Overview

- Cancer immunotherapy & personalized cancer vaccines
- OpenVax personalized cancer vaccine clinical trials
- Do personalized cancer vaccines work?
- Peptide vaccines for SARS-CoV-2
- Current work: Peptide vaccine optimization
- Current work: Immunogenicity prediction
- Future: Back to cancer

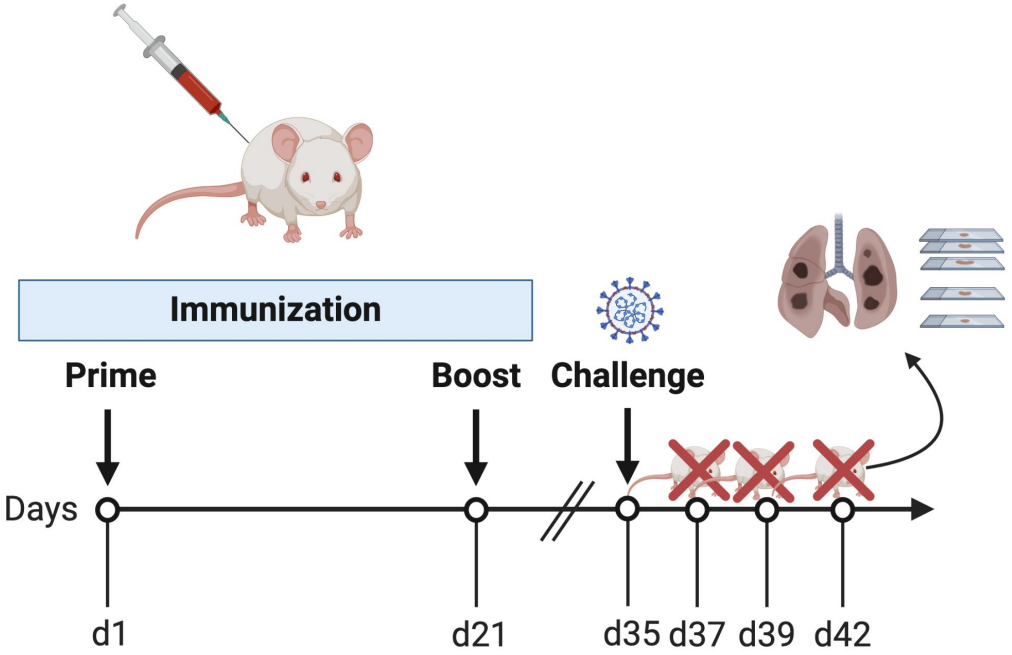
Can we make precise vaccination work for SARS-CoV-2?

- 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



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

First experiments (w/ Vincent Lab)

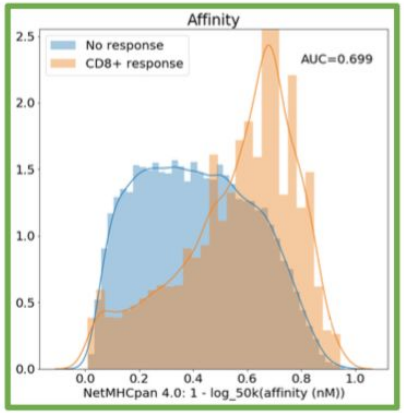
- 27mer peptides + Poly(I:C)
- BALB/c mice
- T-cell responses (ICS) & Ab binding to spike (ELISA)
- Do vaccine peptides compete other?
  - A (n=5): T-cell
  - B (n=5): T-cell
  - C (n=10): A+B
  - D (n=6): B-cell

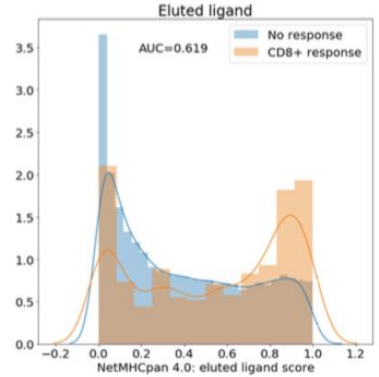
	SARSCOV2_20-121.0_002.5			N	Day				
					1	7	14		21
	Group	Peptide	Adjuvant		↓	↓	↓		↓
T Cell	1	Set A	Poly IC + M	6	Vaccinate	Cheek Bleed	Cheek Bleed	Boost	Sacc
T Cell	2	Set B	Poly IC + M	6					
T Cell	3	Set C (Set A + B)	Poly IC + M	6					
						↓ Serum	↓ Serum		↓ Serum ↓ Spleen
						Freeze	Freeze		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

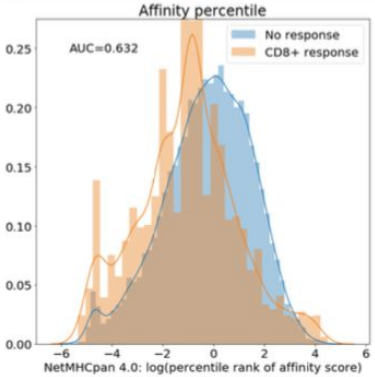
Using mass spec data: not enough

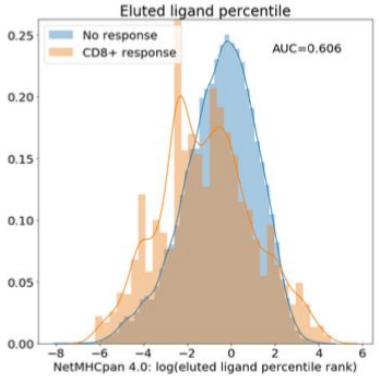
- NetMHCpan 4.0 has 2 outputs:
  - EL: Trained on mass spec data
  - BA: Binding affinity
- Can also use percentile of EL or BA
- Affinity predictive for viral epitopes

Virus	Count	% Positive
Dengue Virus	1140	98.6%
Hepacivirus C	943	75.9%
Human betaherpesvirus 5	772	81.7%
Human gammaherpesvirus 4	779	90.0%
Influenza A virus	669	90.9%
Vaccinia virus	21,899	1.2%









Fin