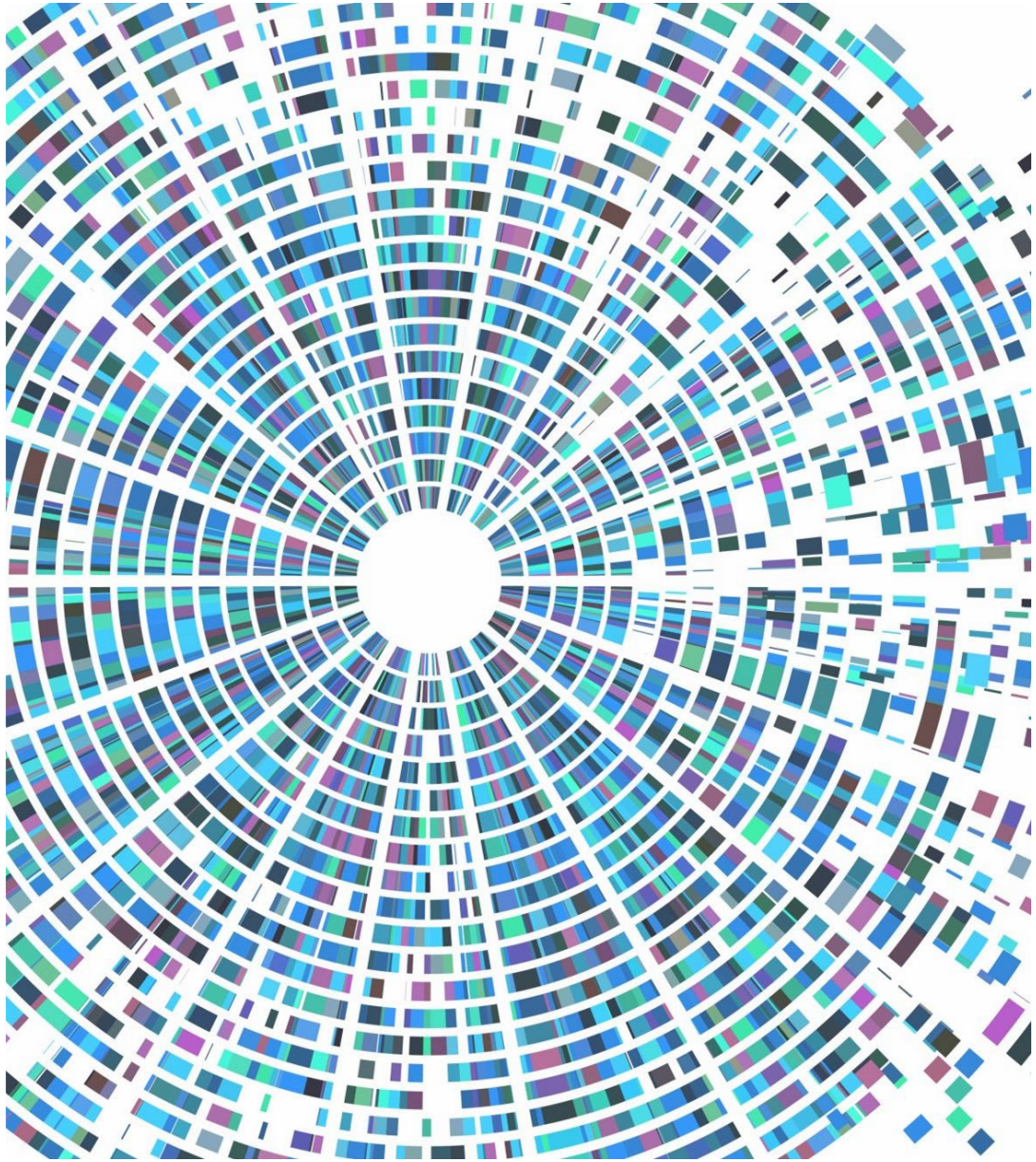
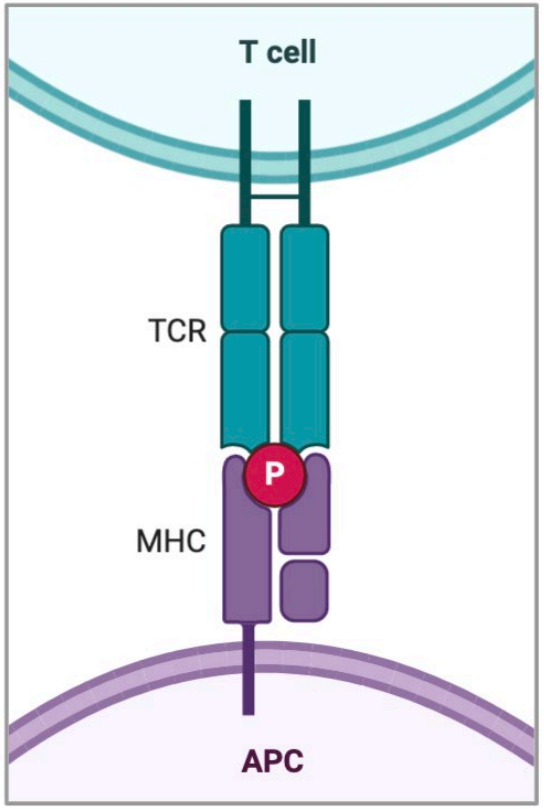
scanning T cell targets



TJC November 2020

Chryssa Zografou

antigen recognition by T cells



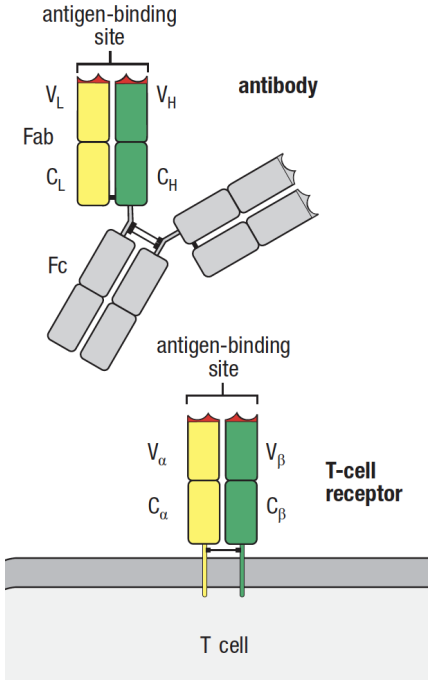
TCR

- T cell receptors (TCR) are the antigen-recognition molecules of T cells
- TCRs are related to immunoglobulins but differ in **protein structure, mechanism of variability production** but most importantly in **antigen recognition: short peptides** instead of antigens presented as **MHC molecules**

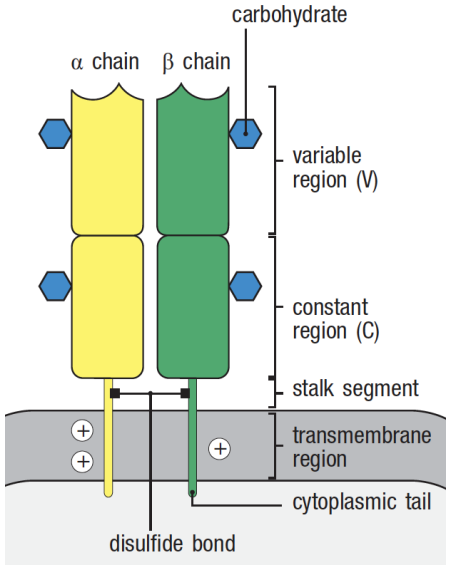
MHC

- MHC (major histocompatibility complex) molecules: **highly polymorphic** transmembrane glycoproteins that are encoded by different alleles
- TCRs recognize **both** the peptide antigens and the MHC molecules to which they are bound – only when they are displayed on the surface of a cell
- infected cells display peptide fragments of the pathogen's proteins on their surface

structure of the TCR



- a TCR has **only 1** antigen-binding site, whereas a B-cell receptor has 2, and TCRs are never secreted, whereas immunoglobulins can be secreted as antibodies
- each T cell bears about 30,000 antigen receptor molecules on its surface
- each TCR is composed of two transmembrane glycoprotein chains, α and β, similar to a Fab
- a minority of T cells have γ:δ TCRs (different antigen recognition properties)



MHC molecules bind many different peptides

MHC class I

- HLA-A, HLA-B, and HLA-C
- peptides from pathogens (viruses) to CD8 cytotoxic T cells
- almost all cells express MHC I

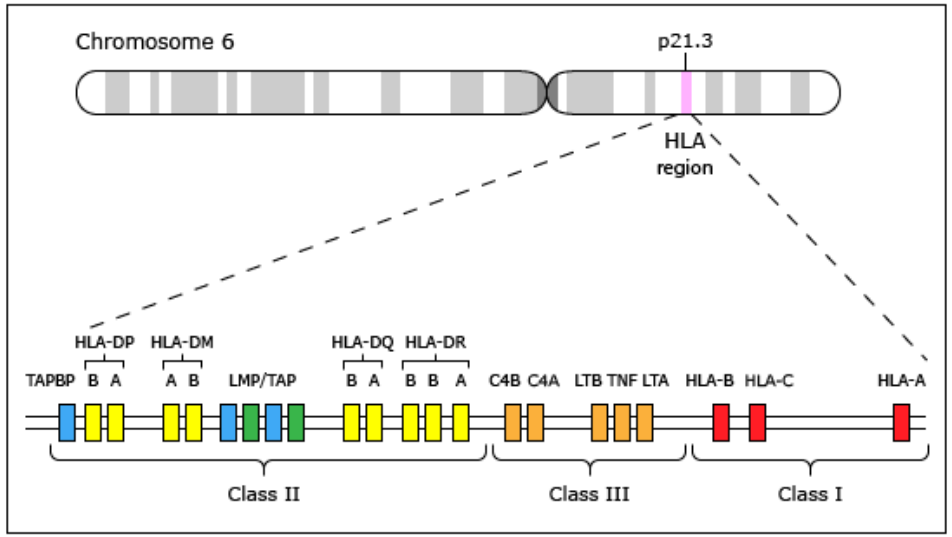
MHC class II

- HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, and HLA-DR
- CD4 T cells – activation of B cells, macrophages and dendritic cells
- in the brain, most cell types are MHC class II-negative, but microglia are MHC class II-positive

MHC class III (does not contain any HLA genes)

- complement components C2, C4, and factor B
- tumor necrosis factor (TNFa)
- heat shock proteins

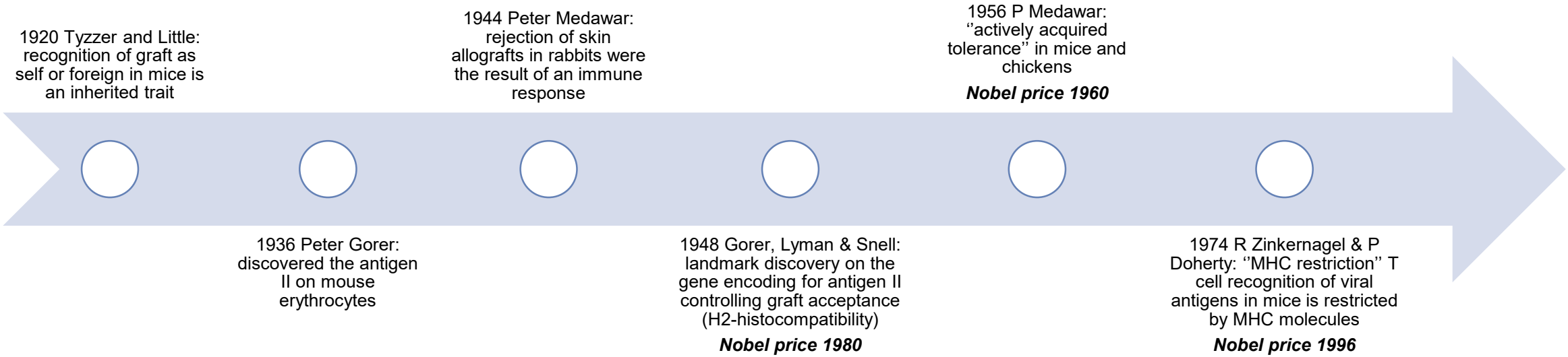
genetic structure of the MHC



- the human MHC region, also referred to as the HLA region (pink), is located on the short arm at position p21.3
- the MHC region spans 3.6 megabases and includes more than 200 genes

- MHC class I & II molecules are the most immunogenic antigens that are recognized during rejection of an allogeneic transplant
- autoimmunity is linked to specific HLA alleles:
  i. type 1 diabetes mellitus to HLA-DQ2 and HLA-DQ8
  ii. multiple sclerosis to HLA-DR2
  iii. myasthenia gravis & grave's disease to HLA-DR3
  iv. rheumatoid arthritis to HLA-DR4

timeline of MHC discovery



HLA and susceptibility to disease

HLA polymorphisms:

- shape the T-cell repertoire diversity
- influence antigen processing and presentation (foreign or self-peptides to autoreactive T cells)
- determine which peptides to bound and present to the immune system
- can generate molecular mimicry between self-antigens and either the HLA molecule itself or peptides that it recognizes
- affect immune suppression and cancer development through the loss of HLA gene expression (viral infection, somatic mutations)

cytotoxic T-cell response

- cytotoxic T cells use TCRs to survey antigens presented on MHC class I on the surface of cells
- TCR recognition of MHC-antigen complexes – elimination of pathogens by cytolytic molecule or cytokine secretion. Examples: HIV, CMV, malaria, SARS-CoV2

cross-reactive T cells

- number of distinct TCRs are estimated at 10^5–10^8, but they recognize a far greater number (>10^{15}) of possible foreign antigens
- robust immunity but may also contribute to autoimmune diseases and cancer immunotherapy toxicities

studying T cell specificities

challenges of studying TCR-pMHC interactions

- interaction of TCR to MHC is of low affinity
- T cell antigens are short peptides non-covalently bound to MHC molecules

classic approaches

predetermined antigens (up to 100)

- pMHC tetramers; fluorescently labeled streptavidin molecule bound to 4 biotinylated pMHC monomers
- pMHC dodecamers
- T cell cytotoxicity/proliferation assays in the presence of antigens
- immunoprecipitation/mass spectrometry

Article | Published: 08 January 2020

Clonally expanded CD8 T cells patrol the cerebrospinal fluid in Alzheimer's disease

David Gate, Naresha Saligrama, Olivia Leventhal, Andrew C. Yang, Michael S. Unger, Jinte Middeldorp, Kelly Chen, Benoit Lehallier, Divya Channappa, Mark B. De Los Santos, Alisha McBride, John Pluvinage, Fanny Elahi, Grace Kyin-Ye Tam, Yongha Kim, Michael Greicius, Anthony D. Wagner, Ludwig Aigner, Douglas R. Galasko, Mark M. Davis & Tony Wyss-Coray

Nature **577**, 399–404(2020) | Cite this article

Fig. 3: Clonal expansion of CD8$^+$ T_{EMRA} cells in the CSF of patients with AD.

From: Clonally expanded CD8 T cells patrol the cerebrospinal fluid in Alzheimer's disease

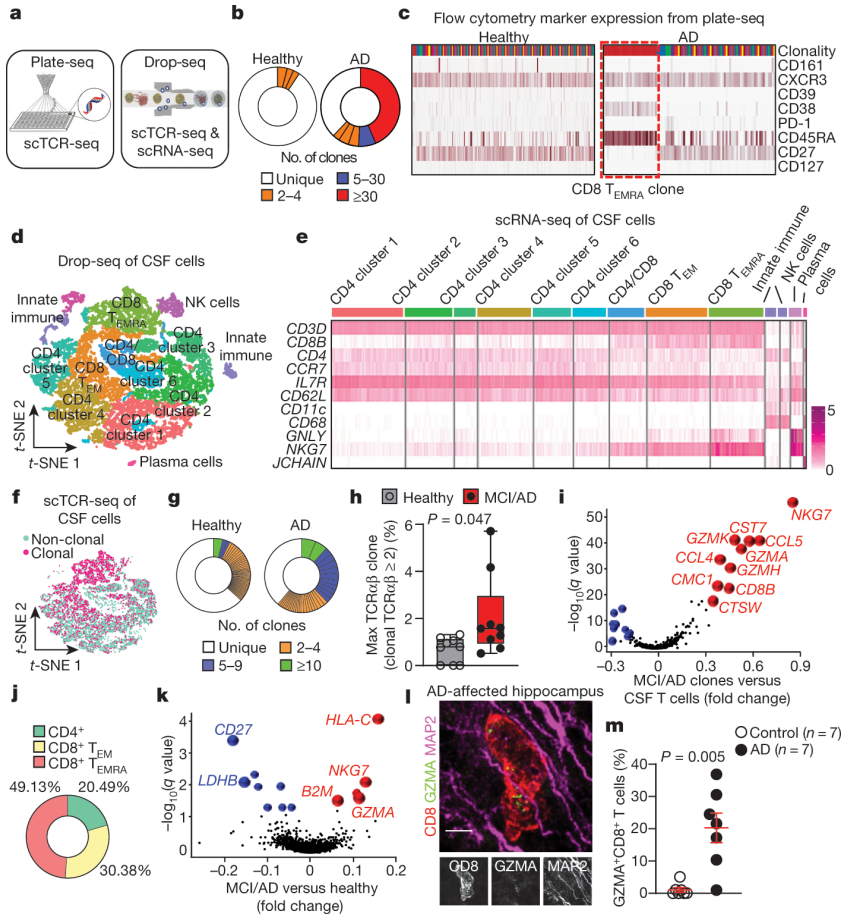


Fig. 4: Antigen identification of clonally expanded TCRs in the CSF of patients with AD.

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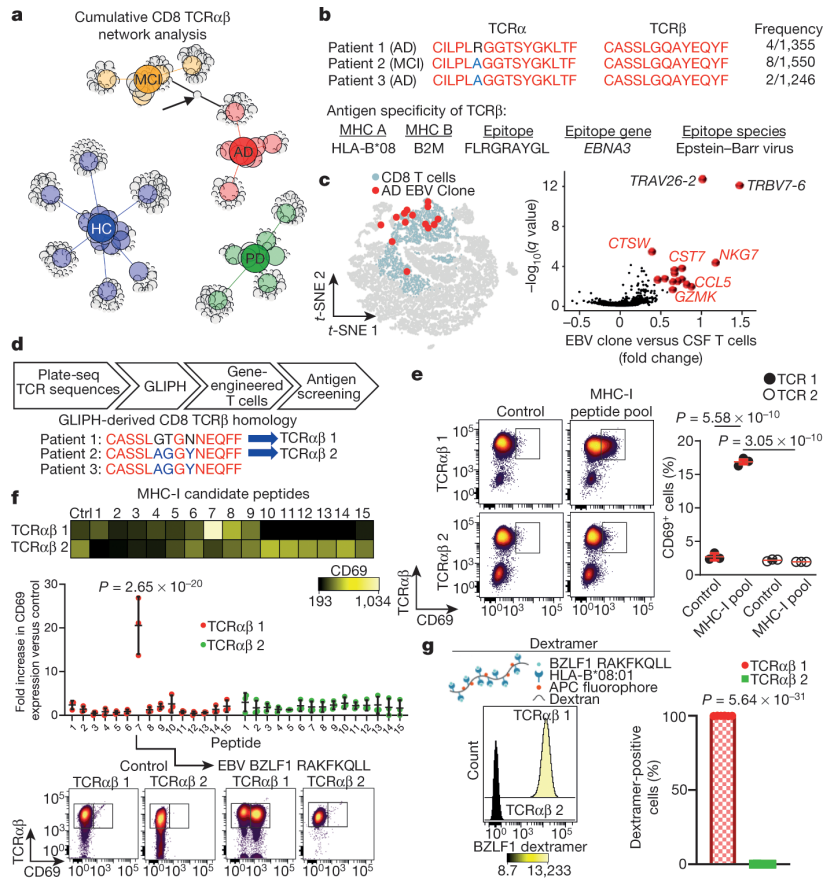

Published: 12 November 2018

High-throughput determination of the antigen specificities of T cell receptors in single cells

Shu-Qi Zhang, Ke-Yue Ma, Alexandra A Schonnesen, Mingliang Zhang, Chenfeng He, Eric Sun, Chad M Williams, Weiping Jia ✉ & Ning Jiang ✉

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T cell receptor fingerprinting enables in-depth characterization of the interactions governing recognition of peptide–MHC complexes

Amalie K Bentzen, Lina Such, Kamilla K Jensen, Andrea M Marquard, Leon E Jessen, Natalie J Miller, Candice D Church, Rikke Lyngaa, David M Koelle, Jürgen C Becker, Carsten Linnemann, Ton N M Schumacher, Paolo Marcatili, Paul Nghiem, Morten Nielsen & Sine R Hadrup ✉

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- evaluate TCR cross-reactivity
- provide new insights into TCR–pMHC interactions
- strategies for characterizing the safety of T-cell immunotherapies

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- pMHC tetramers coupled to DNA barcodes to analyze TCR binding to more than 1,000 different antigens in a sample

- both groups used ultraviolet-cleavable conditional ligands to make large numbers of pMHCs and overcome the time-consuming process of making individual pMHCs

- Bentzen et al. applied their DNA-barcoded pMHC multimer technology to study TCR cross-reactivity and recognition patterns, by using peptide synthesis

- Zhang et al. synthesized the peptides from DNA oligonucleotides through in vitro transcription and translation (reducing cost & time) compared to synthesizing peptides chemically

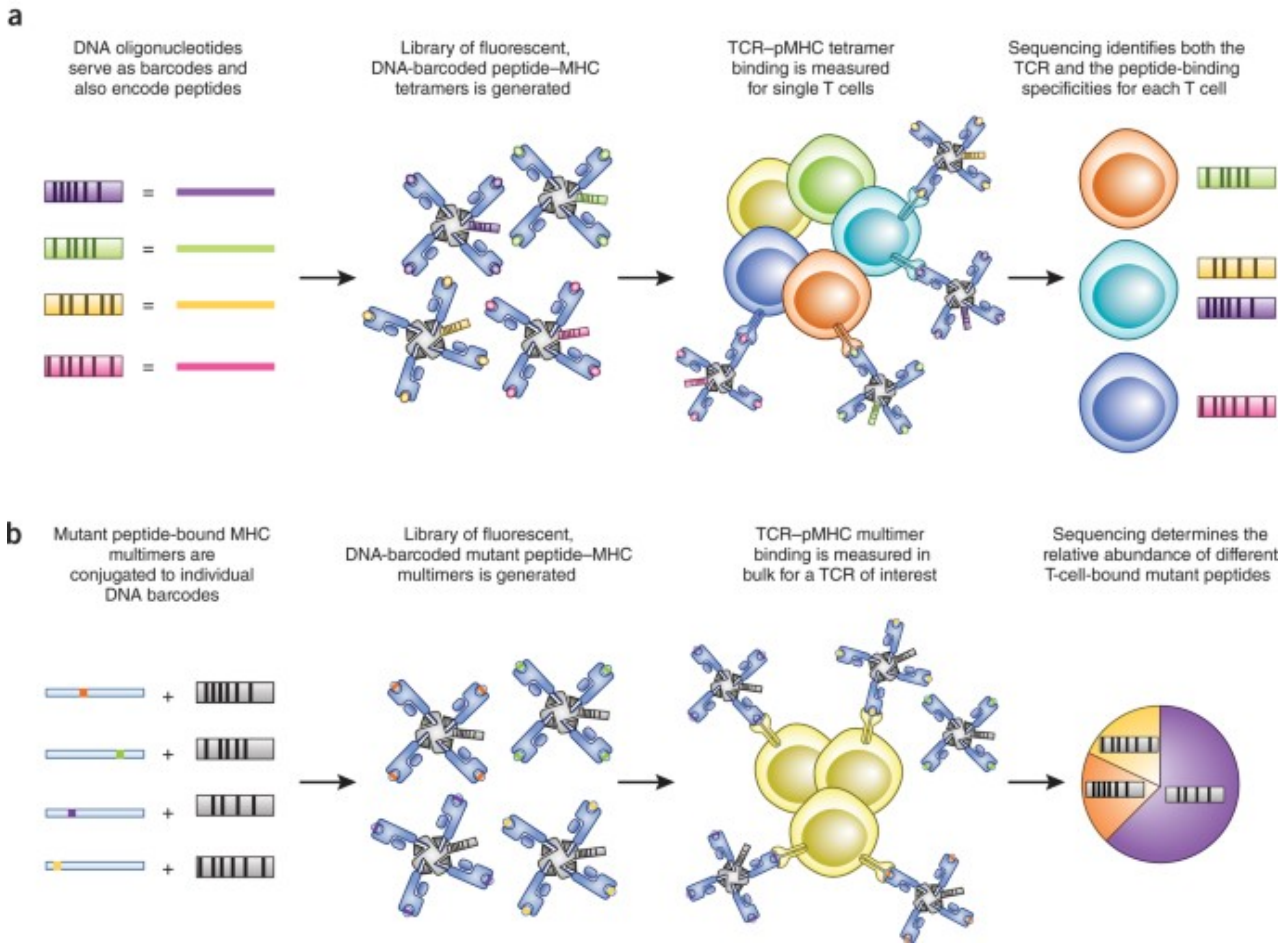
two similar methods for assessing T cell–pMHC binding patterns

Zhang *et al*. (a):

tetramer-associated TCR sequencing (TetTCR-seq)

the oligonucleotides that encode antigenic peptides are also used as DNA barcodes, which are conjugated to the fluorescent streptavidin coupled to the pMHC tetramers

they tested the method using pMHC tetramers made with a WT hepatitis C virus peptide and 4 related altered peptide ligands in conjunction with peripheral human CD8 T cells and a spiked-in WT peptide-reactive T-cell clone



results

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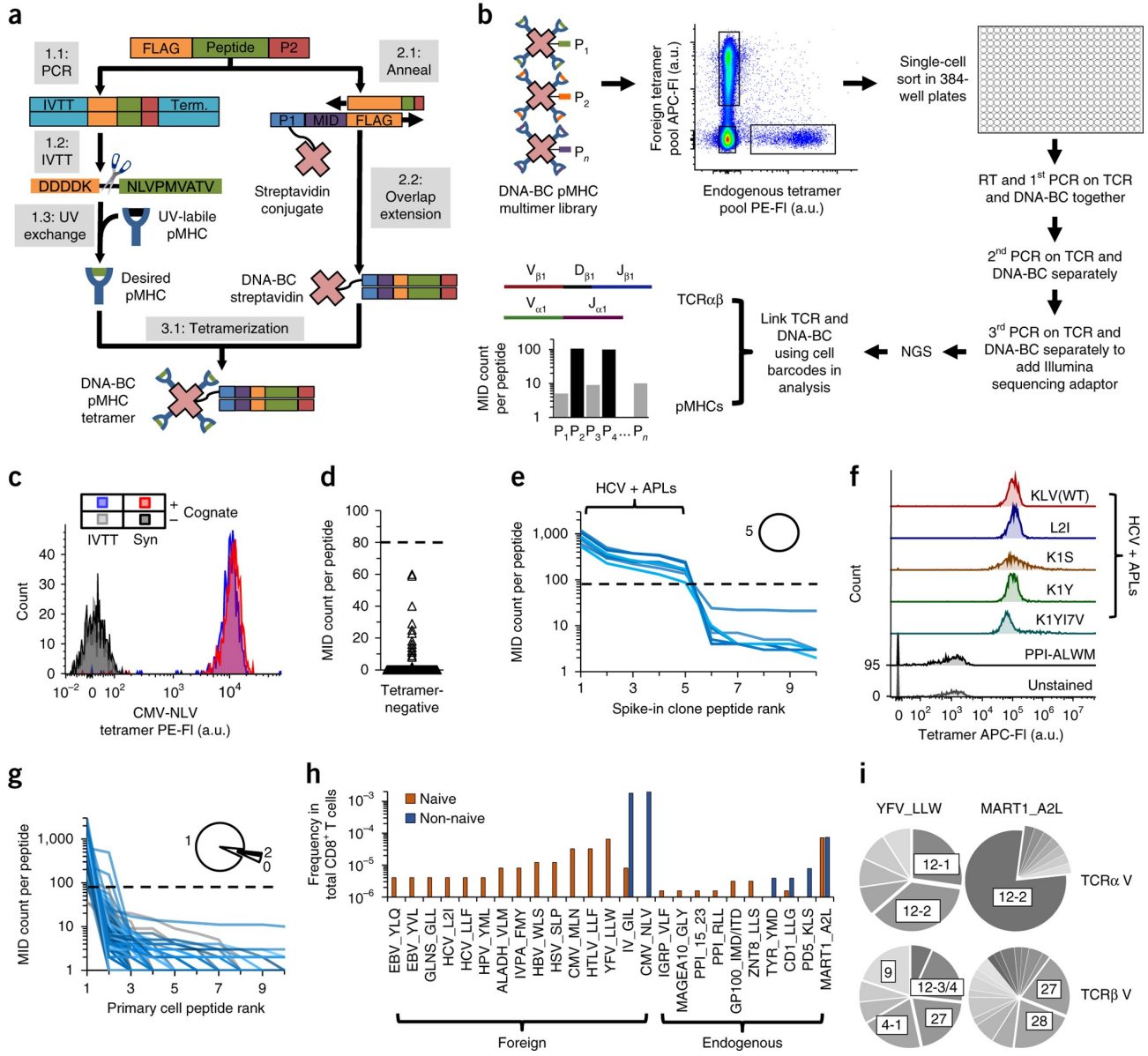

- a set of peptide-encoding oligonucleotides that serve as both the DNA-BCs are used to identify antigen specificities and as DNA templates for peptide generation via IVTT
- tetramer-stained cells are single-cell sorted and the DNA-BC and TCR genes amplified by RT-PCR
- high peptide diversity in the foreign-antigen-binding naive T cells: two dominant peptides for CMV and influenza in the non-naive repertoire
- TCRα genes dominated in MART1-A2L- and YFV-LLW-specific TCRs

results

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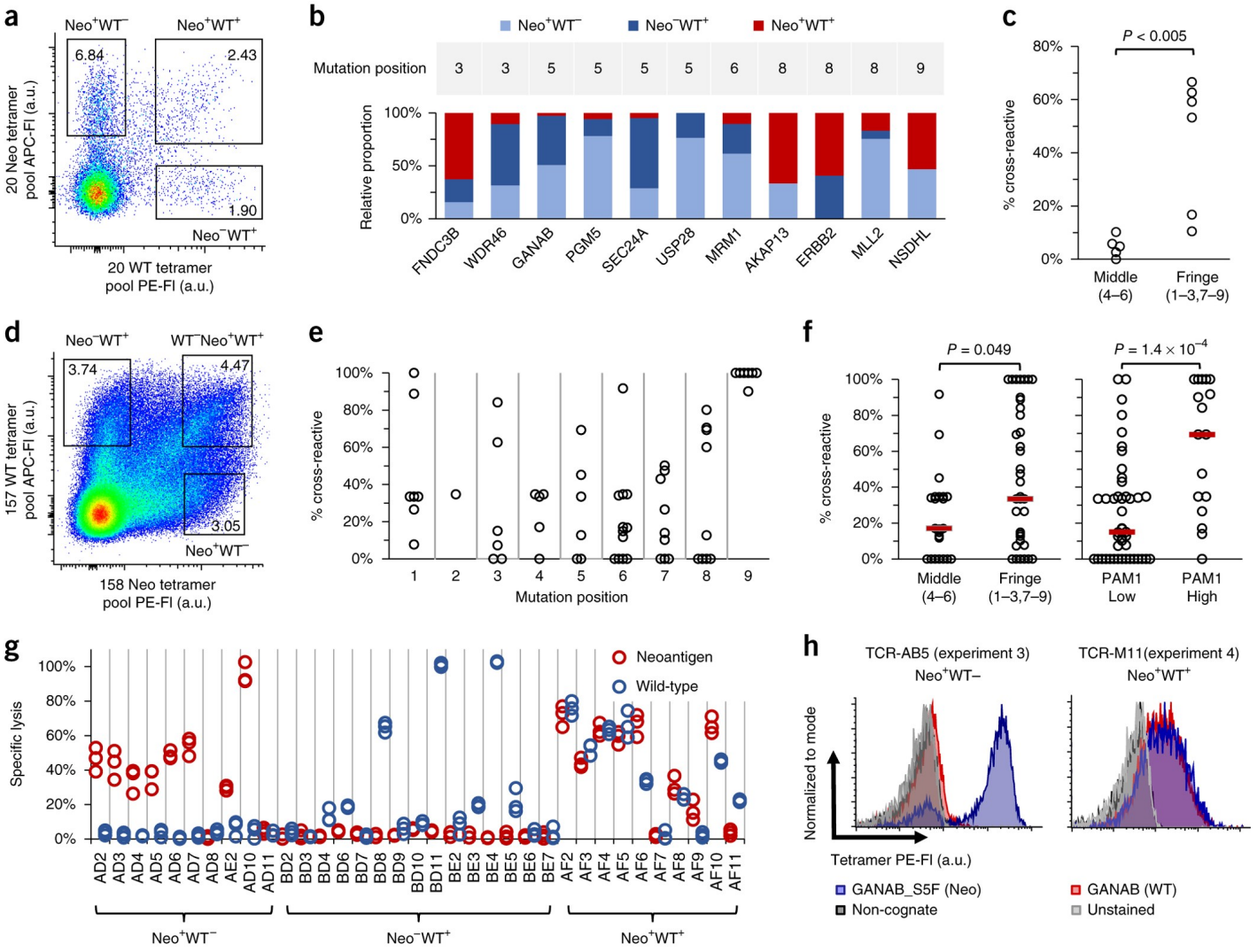

- cross-reactivity of high-affinity tumor neoantigens with CD8 T cells isolated from healthy donors:
  - neoantigens with mutations near the termini of peptides (positions 3, 8 and 9) yield more cross-reactive T cells than do mutations in center positions (4, 5 and 6)
- the authors also identified neoantigen-specific TCRs that do not cross-react with healthy tissues
- also showed that cross-reactive clones are functionally reactive by measuring TCR-mediated cytotoxicity

summary

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High-throughput determination of the antigen specificities of T cell receptors in single cells

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the TetTCR-seq method:

- links TCR sequences with multiple antigenic pMHC binders in a high-throughput manner
- identifies functionally relevant neoantigen-specific TCRs with no cross-reactivity to wild-type antigens, and identifies cross-reactivity patterns in neoantigen-specific TCRs
- can be integrated with single-cell transcriptomics and proteomics to gain further insights into the connections between a single T cell phenotype and TCR sequence/pMHC-binding

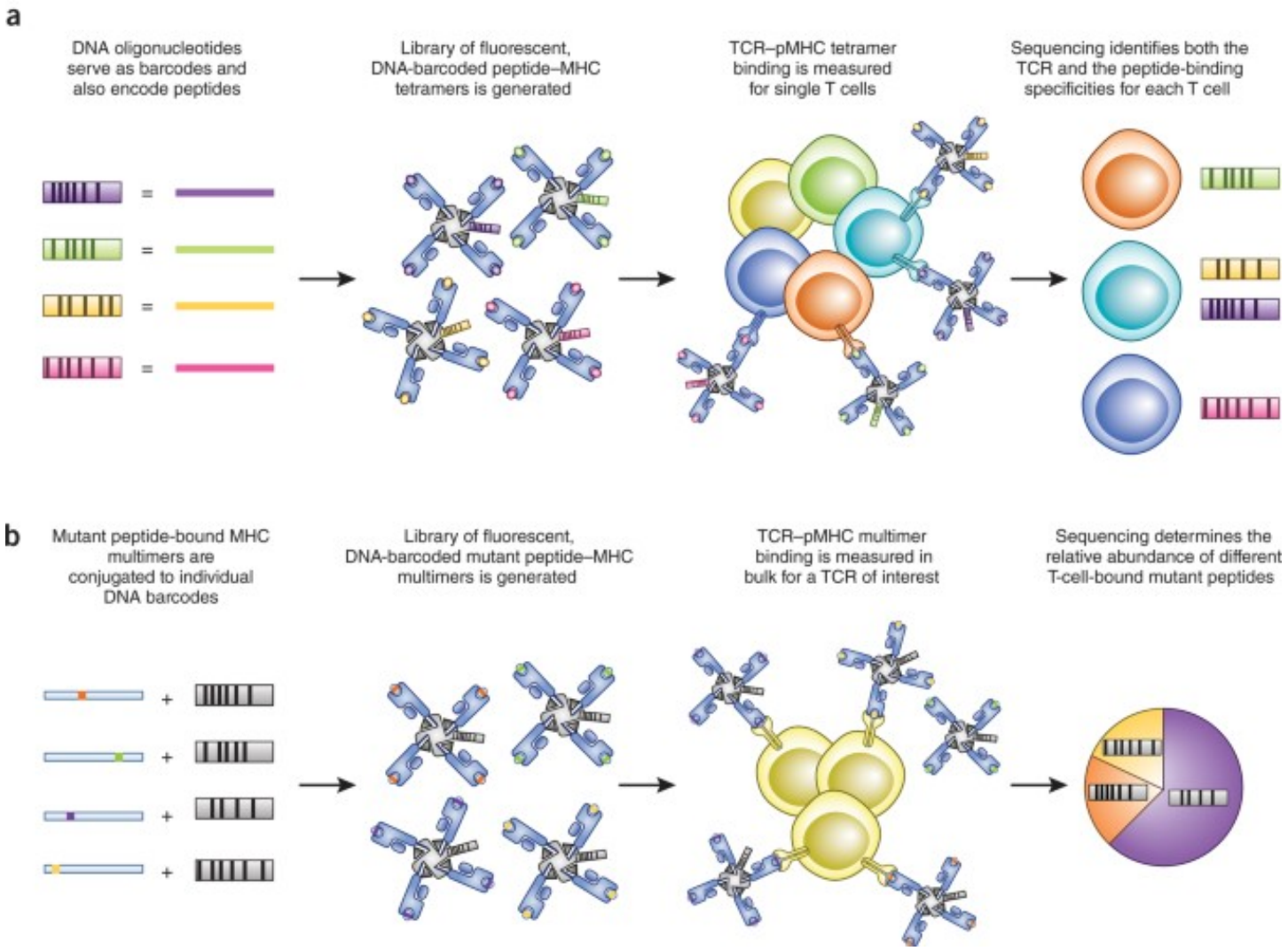
two methods for assessing T cell–pMHC binding patterns

Bentzen *et al*. (b):

TCR recognition pattern characterization on pMHC interactions: ‘’fingerprinting’’

based on the group's earlier work on developing DNA-barcoded pMHC multimers, they identified low-frequency CD8 T cells as well as neoantigen-specific T cells

they mutated every amino acid position of two HLA-bound polyomavirus peptides to all naturally occurring amino acids (191 for each peptide) and then tested the peptides in bulk for binding to two TCRs isolated from patients with Merkel cell carcinoma



approach for detecting antigen-specific T cells

Published: 19 November 2018

T cell receptor fingerprinting enables in-depth characterization of the interactions governing recognition of peptide–MHC complexes

Amalie K Bentzen, Lina Such, Kamilla K Jensen, Andrea M Marquard, Leon E Jessen, Natalie J Miller, Candice D Church, Rikke Lyngaa, David M Koelle, Jürgen C Becker, Carsten Linnemann, Ton N M Schumacher, Paolo Marcatili, Paul Nghiem, Morten Nielsen & Sine R Hadrup

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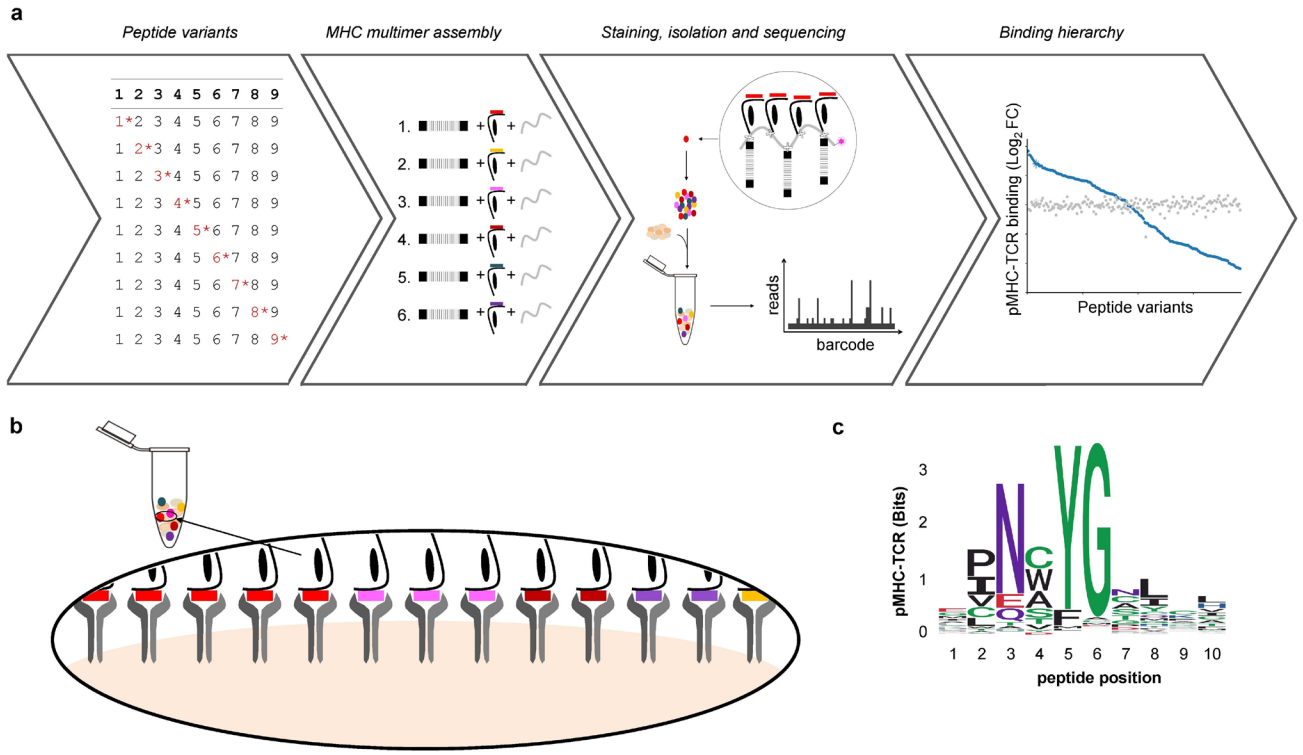
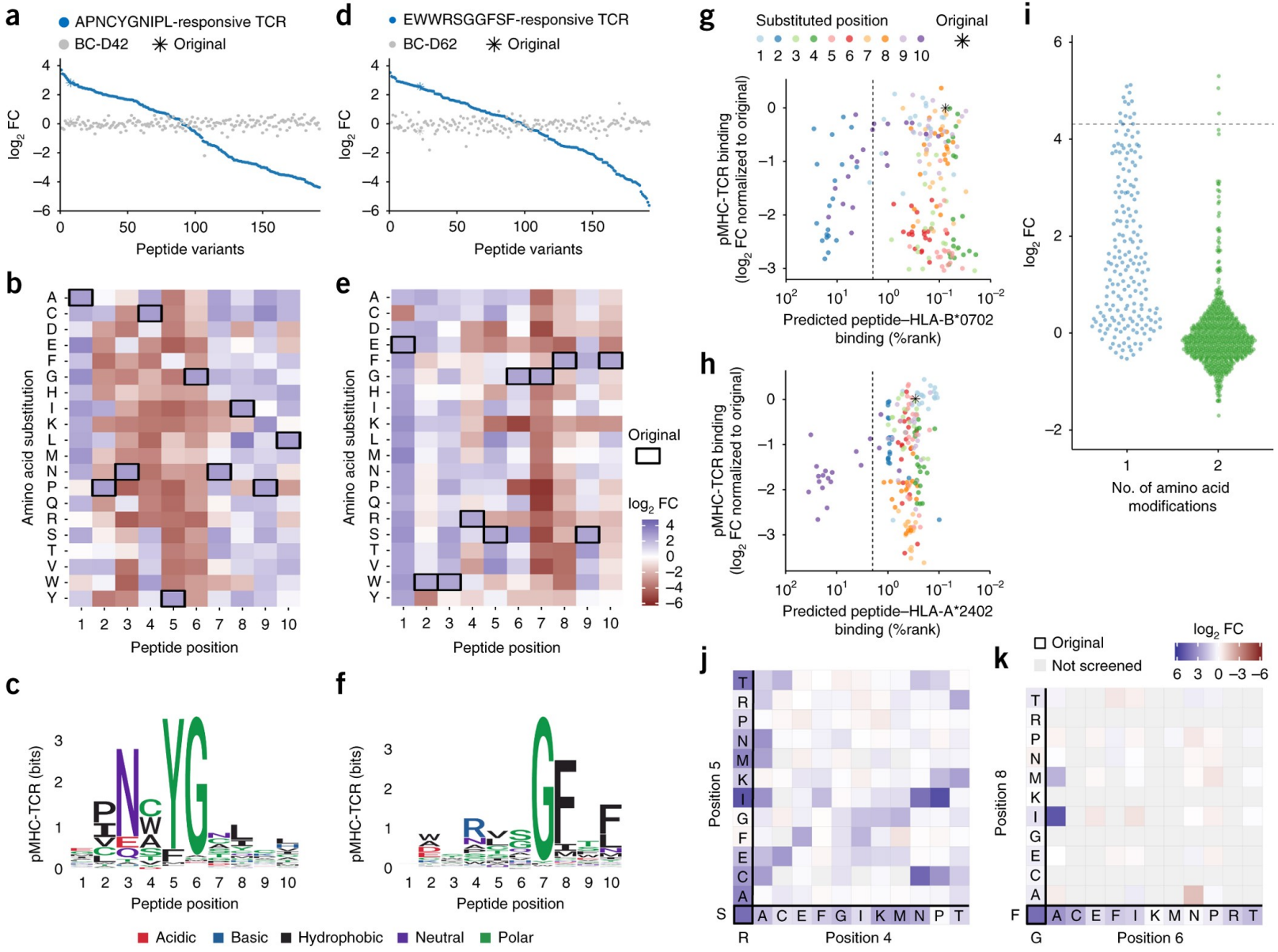


- biotinylated DNA barcodes and pMHC molecules are attached to a PE-labeled backbone carrying streptavidin
- MHC multimers have a given DNA barcode and MHC multimer-binding T cells are FACS sorted as PE positive. DNA barcodes are amplified and sequenced, and the relative number of DNA barcode reads is used to determine the composition of antigen-responsive T cells

results



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- characterized recognition patterns of two different TCRs isolated from patients with Merkel cell carcinoma each recognizing a different peptide:
  1. APNCYGNIPL, restricted to HLA-B*0702
  2. EWWRSGGFSF (EWW), restricted to HLA-A*2402
- DNA barcode sequencing showed an affinity-based hierarchy of pMHC interactions with the TCRs
- amino acid residues critical for binding were identified for both peptides

results

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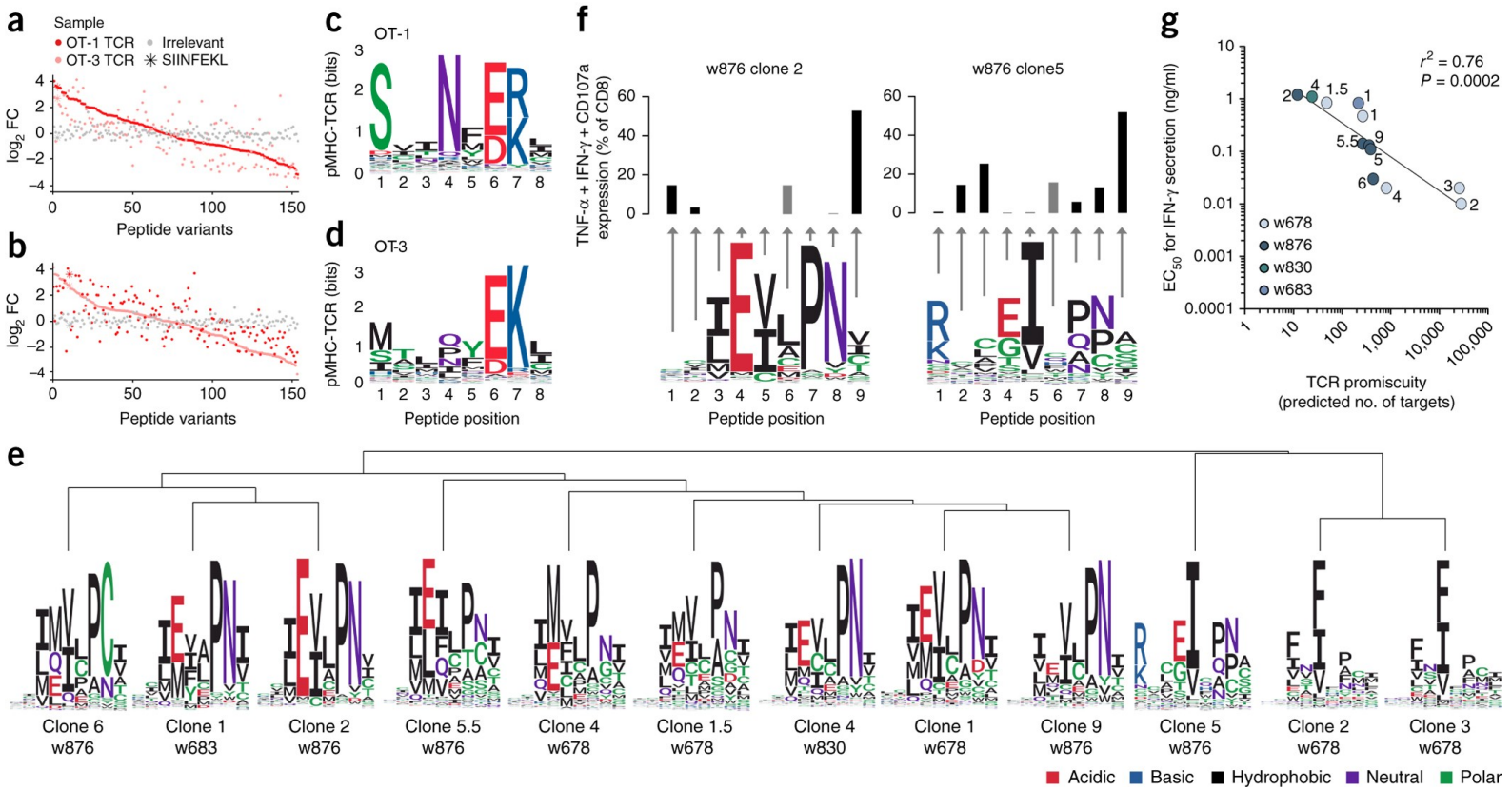

- recognition patterns of different TCRs to the same target: two murine transgenic TCR cell lines, OT-1 and OT-3, which are known to have high and low functional avidity, respectively, to the SIINFEKL peptide
- screened with a library of 153 DNA barcode-labeled MHC multimers with a single aa substitution
- 12 different TCRs derived from four patients with MCC
- variance in the TCR fingerprints of these TCRs, even among those derived from the same patient; however there was preference pattern for position 5

summary

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- the DNA barcode sequencing revealed an affinity-based hierarchy of pMHC interactions with the TCRs, and amino acid residues critical for binding were identified for both peptides
- this highlights the utility of the technique to analyze multiple TCR–pMHC interactions, as in the method described by Zhang et al.
- mutations in peptide 'anchor' residues did not hinder peptide–MHC interactions as predicted, suggesting that these residues do not have a major role in TCR recognition

Cell

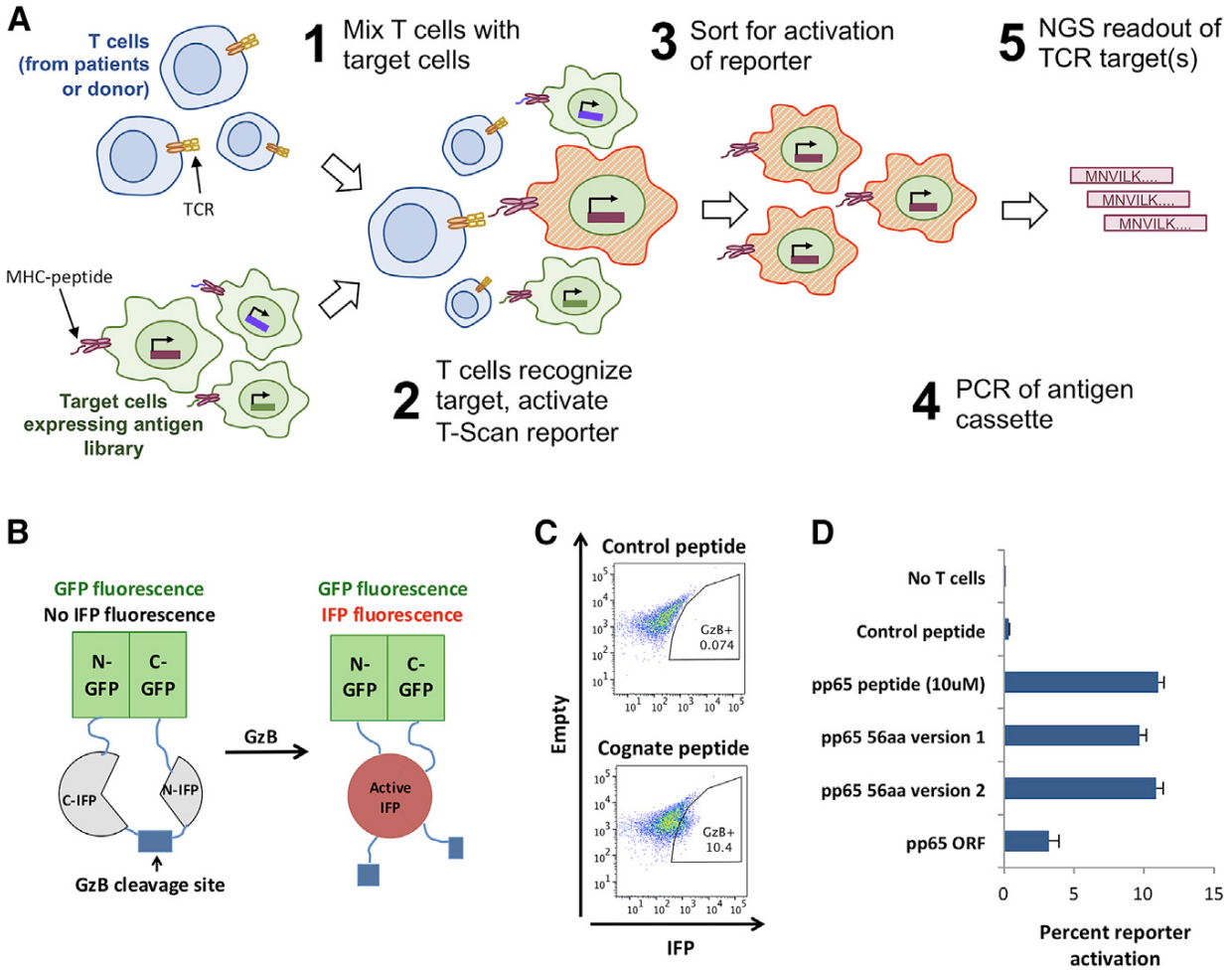
Volume 178, Issue 4, 8 August 2019, Pages 1016-1028.e13

Resource

T-Scan: A Genome-wide Method for the Systematic Discovery of T Cell Epitopes

Tomasz Kula [1, 2], Mohammad H. Dezfulian [1, 2], Charlotte I. Wang [1, 2, 3], Nouran S. Abdelfattah [1, 2], Zachary C. Hartman [4], Kai W. Wucherpfennig [5], Herbert Kim Lyerly [6], Stephen J. Elledge [1, 2, 7]

T scan platform workflow



- the platform employs a cell-based pooled screen to identify the cognate antigens of T cells
- target cells express a library of lentivirally delivered candidate antigens that are processed and presented endogenously on MHC molecules
- PCR and NGS to identify the antigens that these cells are programmed to express
- a GzB reporter is used to detect the target cells that receive these cytotoxic granules and to enable their isolation by FACS
- GzB activity is used to isolate target cells functionally recognized by a T cell
- IFP^{GZB}, an infrared fluorescent protein (IFP)-based GzB is activated in cells co-cultured with cytotoxic T cells in the presence of a cognate antigen

apoptotic cell identification (EDCs)

GzB delivery into cells results in the activation of caspases and apoptosis:

- caspases cleave the inhibitor of caspase-activated DNase (ICAD) protein, releasing active CAD nuclease which fragments genomic DNA
- expressed a caspase resistant version of ICAD protein, in the target cells to prevent genomic DNA fragmentation during apoptosis, enabling more efficient recovery of antigen information from the genomic DNA of GzB-positive cells

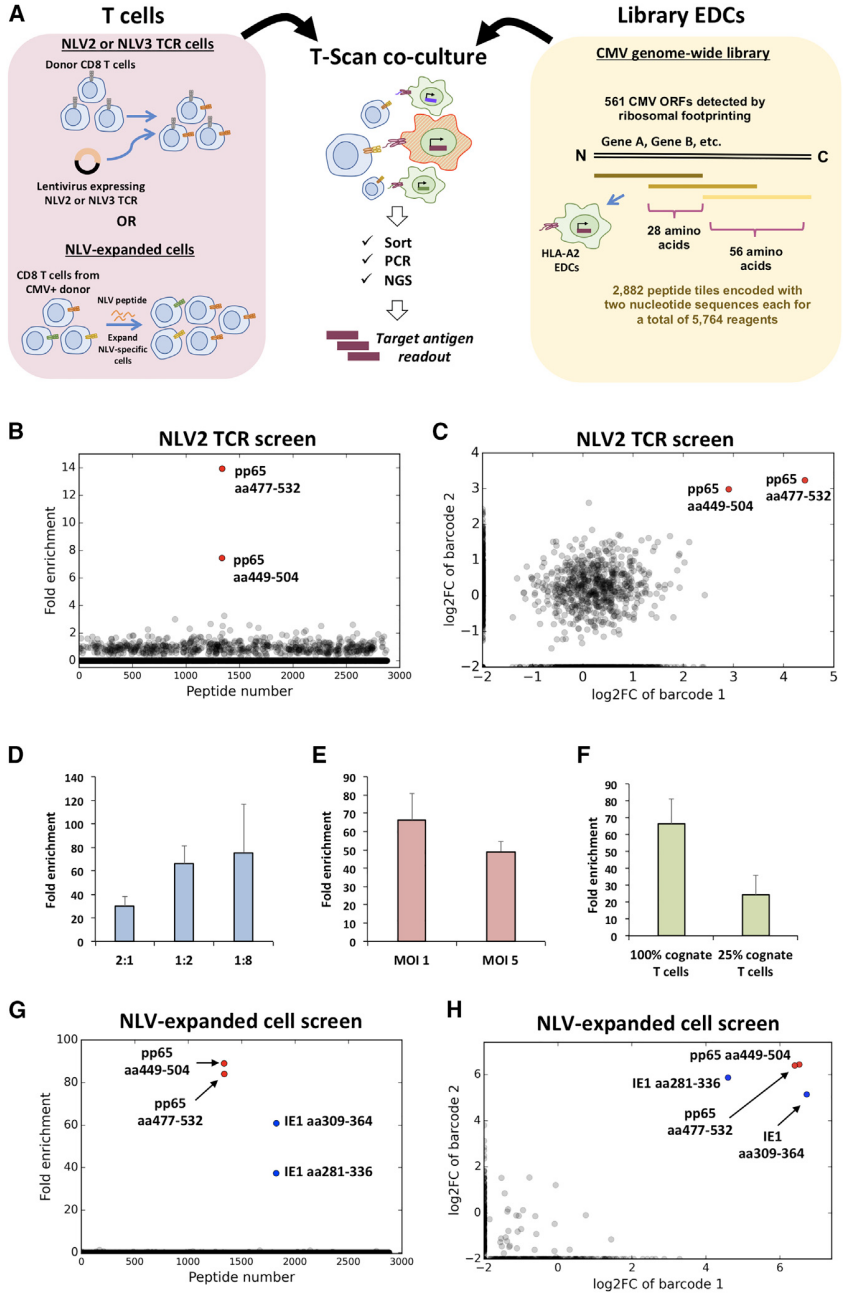
epitope discovery cells

CRISPR:

- mutated all 6 endogenous HLA-A, HLA-B, and HLA-C MHC-encoding genes and re-expressed the individual HLA allele of interest
- these cells express IFPGZB and ICADCR

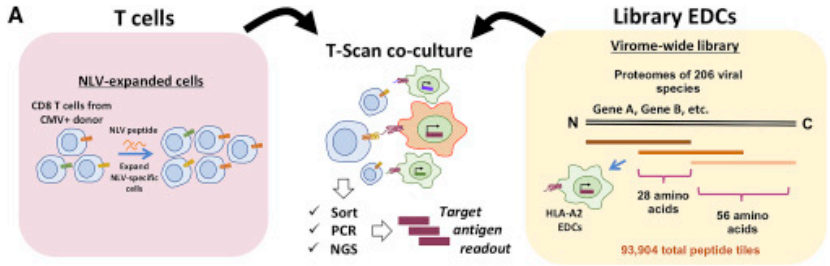
CMV epitope discovery

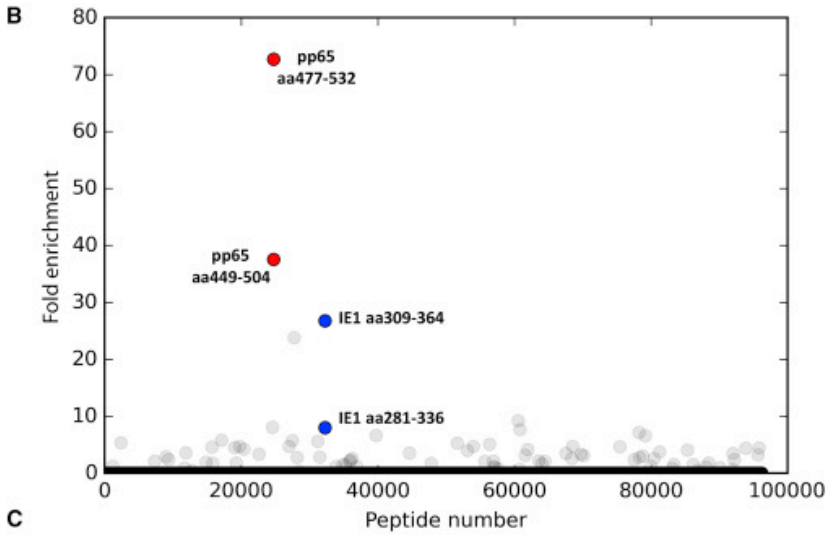
- CMV as a model for testing the T-scan platform (known immunodominant epitopes, antigen-specific TCRs)
- library of 5,764 oligonucleotides encoding 2,882 56-amino acid fragments that tiled across the entire CMV proteome with 28-aa overlap between adjacent fragments (including ORFs identified by ribosome footprinting)
- library: lentiviral vector and transduced into cells expressing HLA-A2
- CD8 T cells were transduced with a vector expressing the CMV pp65 protein-specific NLV2 TCR
- the most enriched peptides identified were the only two antigens with the NLV epitope



virome-wide T cell antigen discovery

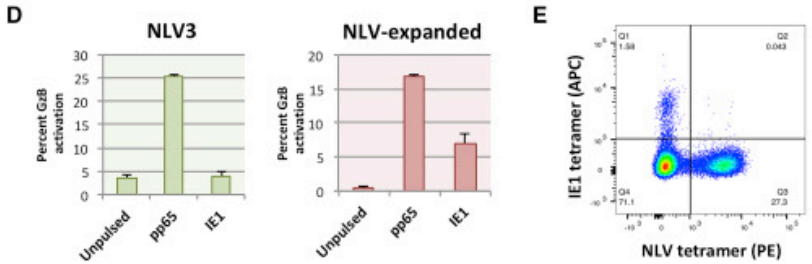
- virome-wide screen for far larger numbers of candidate antigens (proteome-wide peptide library from all human viruses)
- a library a library of 93,904 56-aa fragments that tiled across the entire human virome
- same previously identified peptides popped up together with two novel 56-mers from the IE1 gene of CMV
- the NLV-expanded T cells, but not the NLV3 TCR-transduced T cells, were reactive to the IE1 peptides





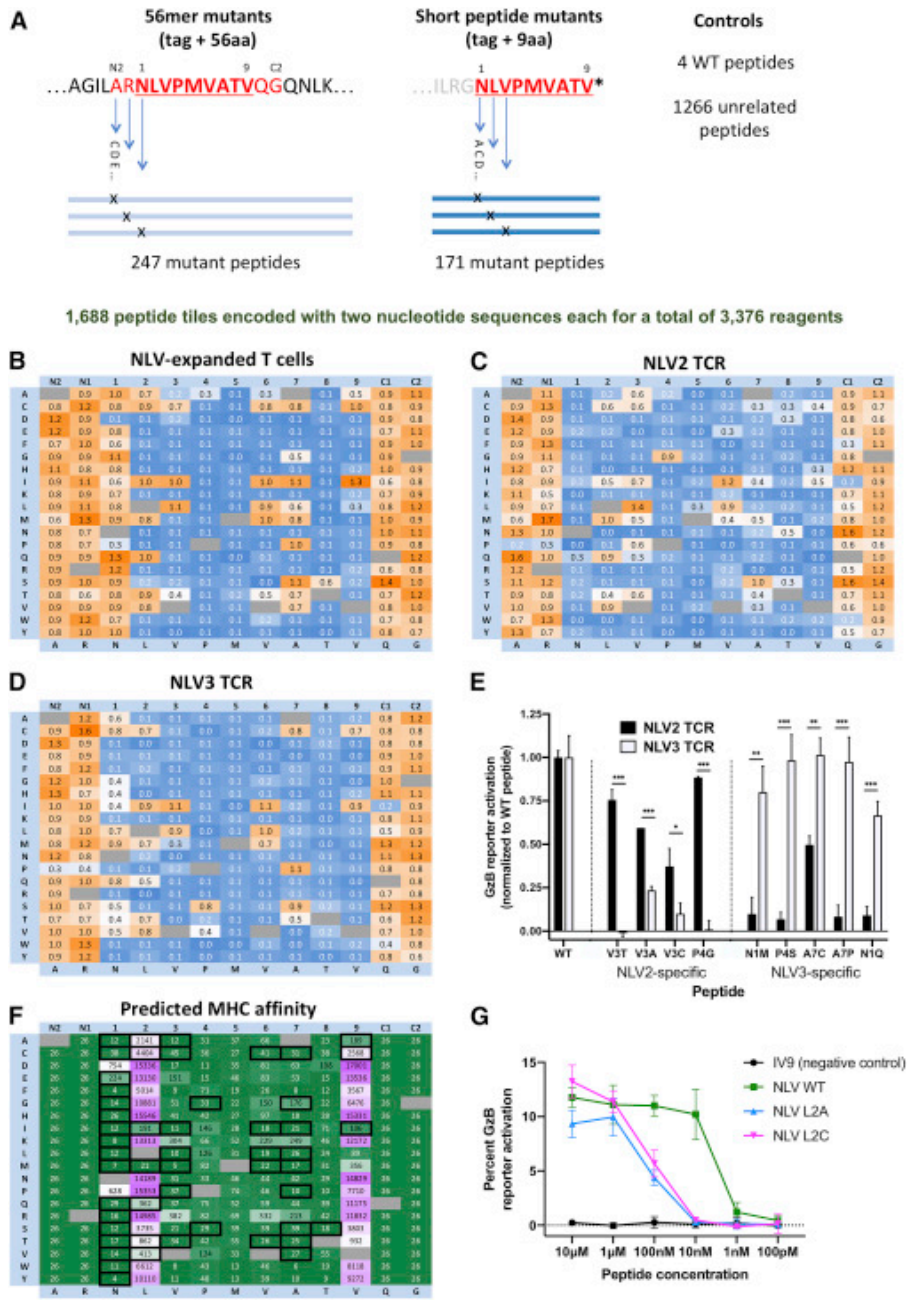
C

Gene	Fragment	Peptide
pp65	449-504	GVMTRGRLKAESTVAPEEDTDEDSDNEIHNPAVFTWPPWQAGILARNLVPMVATVQ
pp65	477-532	HNPAVFTWPPWQAGILARNLVPMVATVQGQNLKYQEFFWDANDIYRIFAELEGVWQ
IE1	281-336	ETMCNEYKVTSDACMMTMYGGISLLSEFCRVLCCYVLEETSVMLAKRPLITKPEVI
IE1	309-364	CRVLCCYVLEETSVMLAKRPLITKPEVISVMKRRIEEICMKVFAQYILGADPLRVC



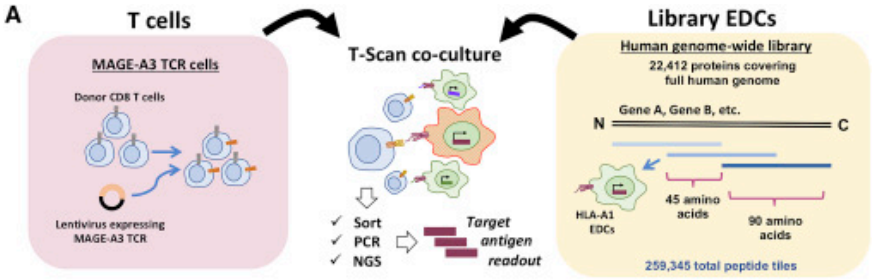
epitope mutagenesis

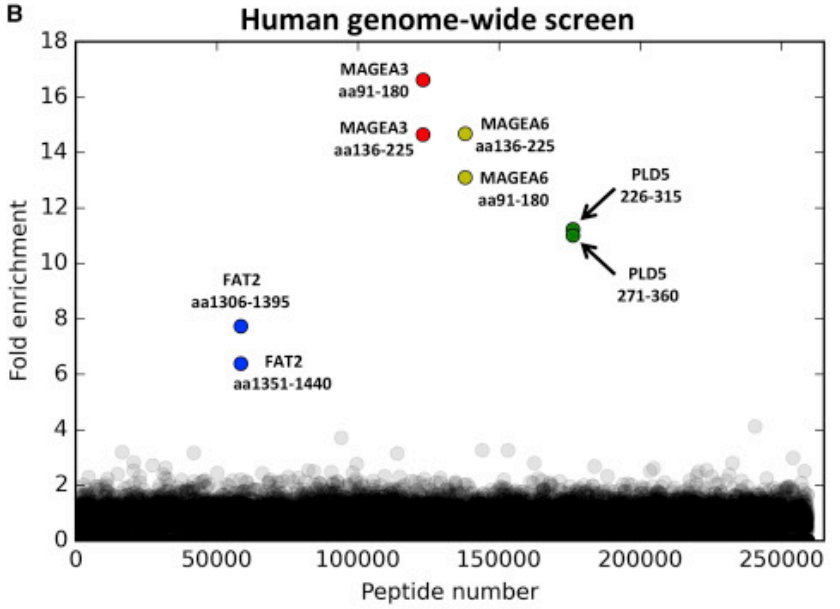
- use of a single mutant library of the NLV epitope, with each mutant epitope present as a 56-aa fragment and a 9-aa fragment
- set of 418 mutants
- mutant library was transduced into cells expressing HLA-A2
- NLV-expanded T cells
- most mutations abrogated T cell killing:
  - almost all mutations at position one were tolerated, while any substitution at position four and five prevented recognition



human genome-wide T cell antigen discovery

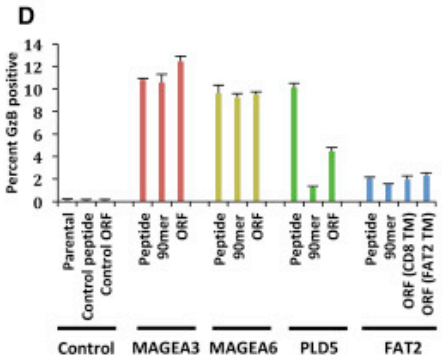
- applications of T-scan in anti-tumor immunity: genome-wide screen using a tumor-derived TCR specific for an HLA-A1-restricted epitope of MAGE-A3
- a library of 259,345 antigens that tile across the entire human proteome in 90-aa fragments with 45-aa overlap into EDCs expressing HLA-A1
- CD8 T cells were transduced with the MAGE-A3 TCR
- enrichment of only 8 antigens in the library, which encoded 4 sets of overlapping peptides
- identified epitopes with a single leucine to valine substitution
- same ExDP motif



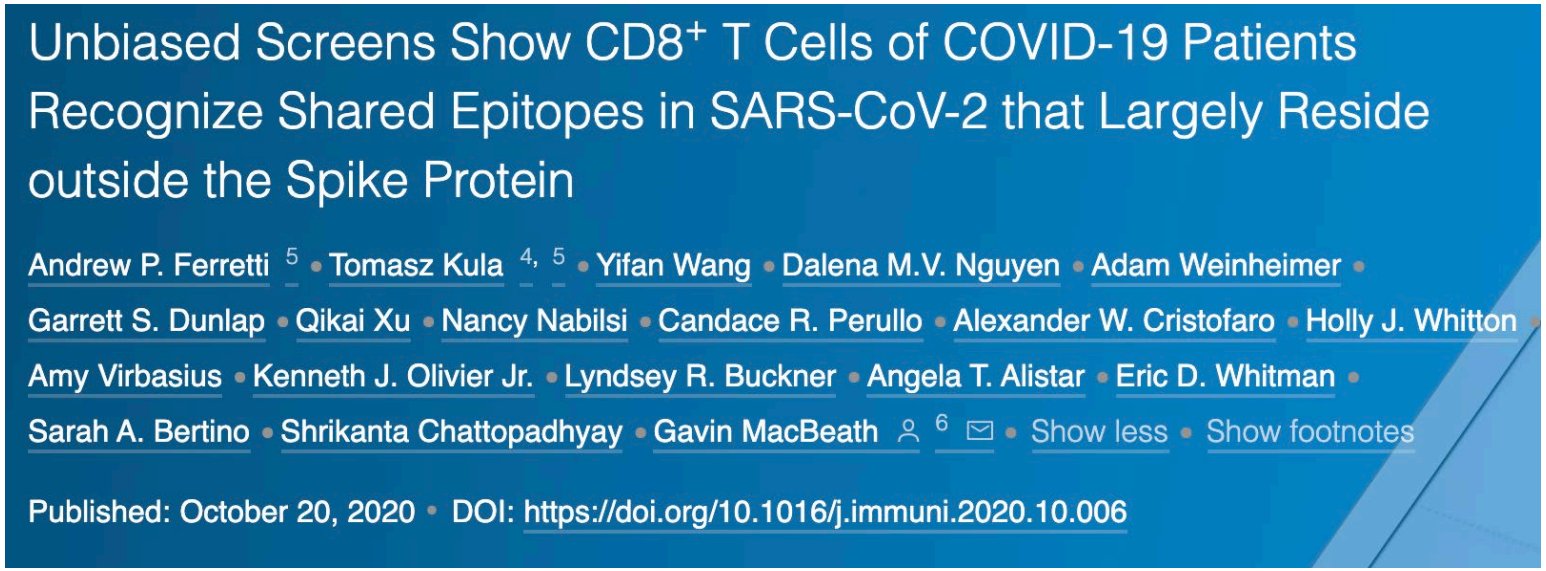


C

Epitope	Sequence
$MAGEA3_{168-176}$	EVDPIGHLY
$MAGEA6_{168-176}$	EVDPIGHVY
$PLD5_{179-187}$	ETDPLTFNF
$FAT2_{1360-1368}$	ETDPVNHMV



T cell immunity in COVID-19 with T-scan



Unbiased Screens Show CD8+ T Cells of COVID-19 Patients Recognize Shared Epitopes in SARS-CoV-2 that Largely Reside outside the Spike Protein

Andrew P. Ferretti [5] • Tomasz Kula [4, 5] • Yifan Wang • Dalena M.V. Nguyen • Adam Weinheimer • Garrett S. Dunlap • Qikai Xu • Nancy Nabilsi • Candace R. Perullo • Alexander W. Cristofaro • Holly J. Whitton • Amy Virbasius • Kenneth J. Olivier Jr. • Lyndsey R. Buckner • Angela T. Alistar • Eric D. Whitman • Sarah A. Bertino • Shrikanta Chattopadhyay • Gavin MacBeath [6] • Show less • Show footnotes

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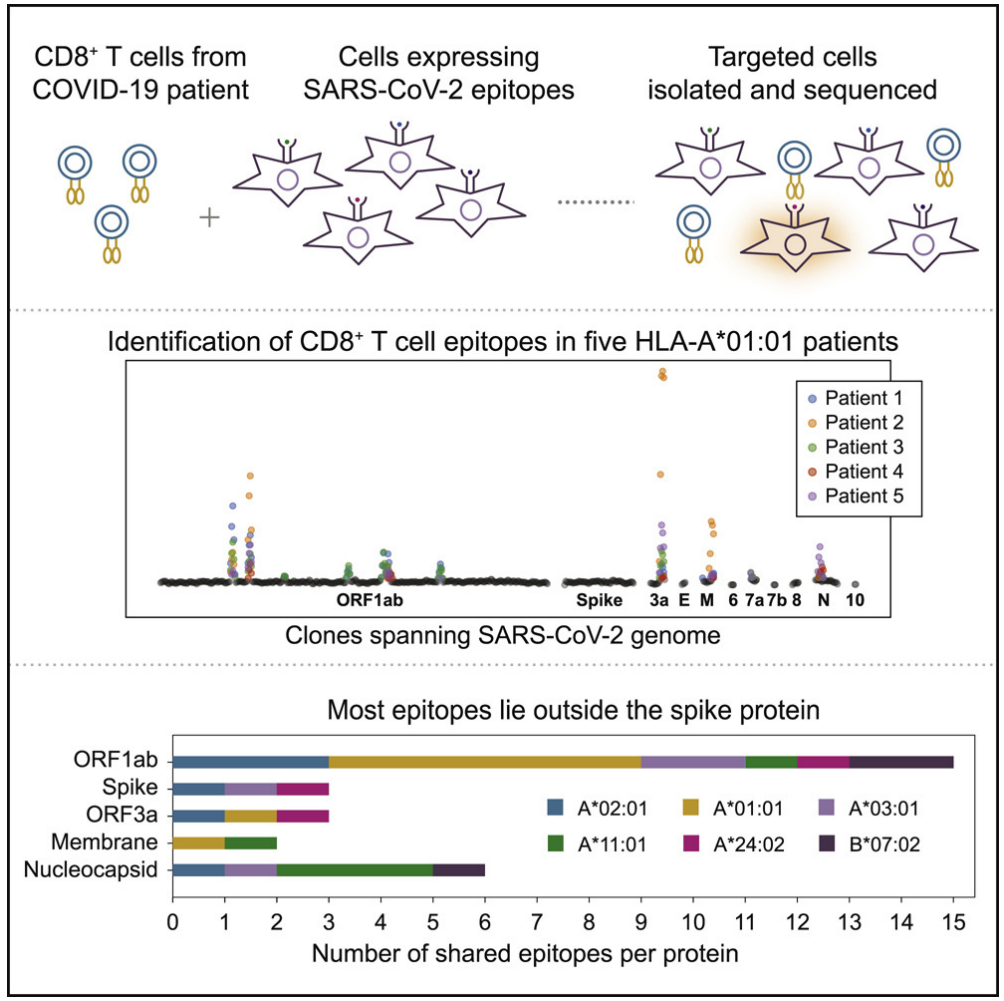
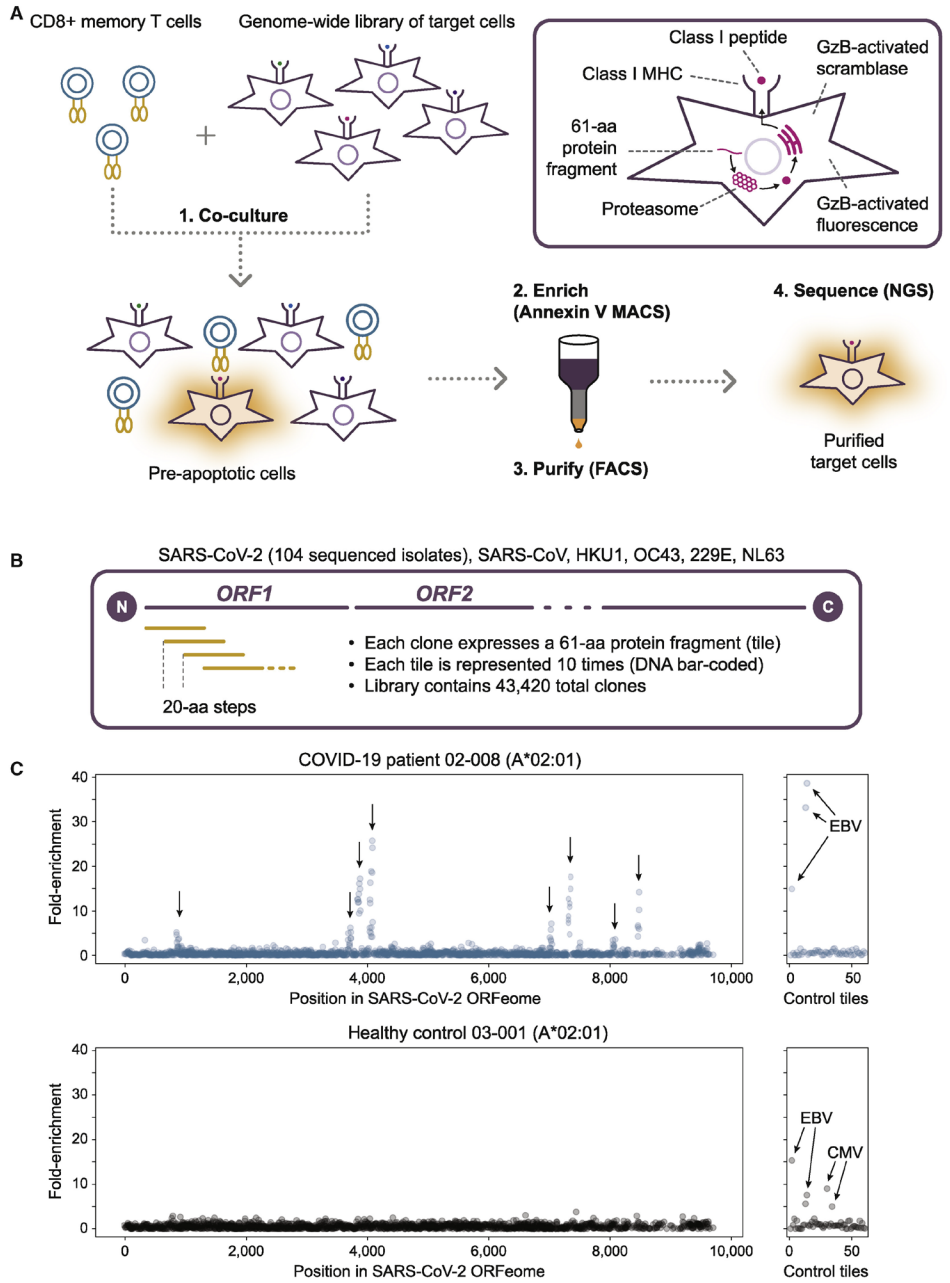
COVID-19 T-scan main findings



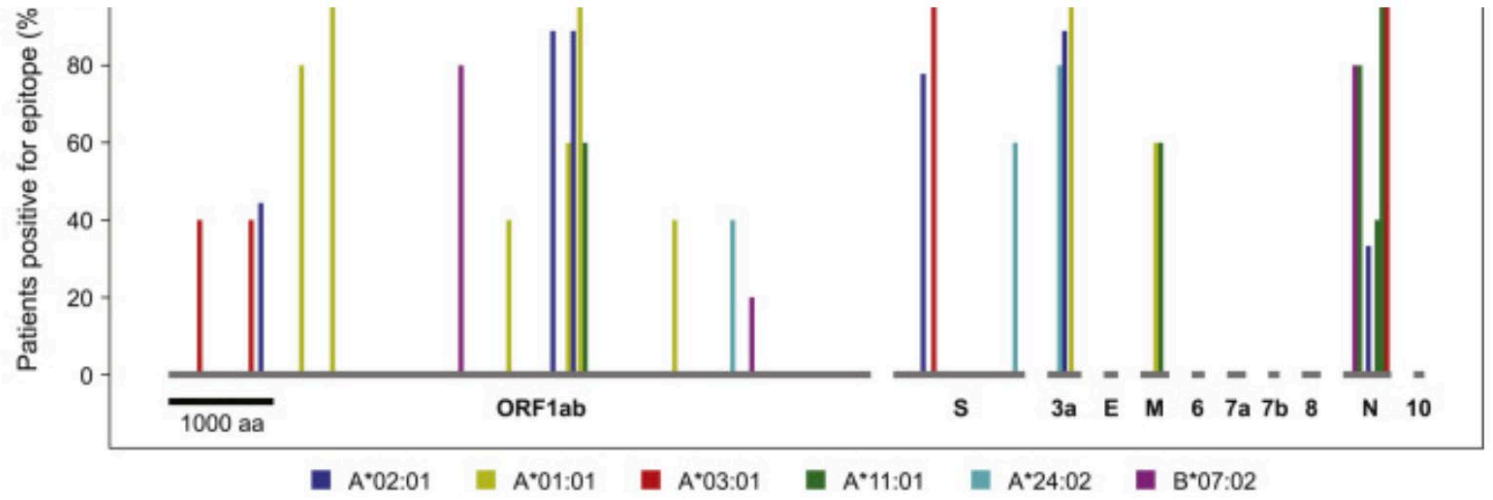
- determined the precise peptide sequences in SARS-CoV-2 that are recognized by the memory CD8 T cells of COVID-19 patients
- identified 3–8 epitopes for each of the 6 most prevalent HLA types
- CD8 T cells generally did not cross-react with epitopes in seasonal coronaviruses that cause the common cold

genome-wide screen for CD8 T cell epitopes in SARS-CoV-2

- CD8 T cells were co-cultured with a genome-wide library of target cells engineered to express a single HLA allele
- Each target cell in the library also expressed a unique coronavirus-derived 61-amino acid protein fragment
- Early apoptotic cells were enriched by MACS with Annexin V, followed by FACS with the fluorescent reporter. This modification increased the throughput of the T-Scan assay 20x and enabled rapid processing of a large number of samples



most shared CD8+ T Cell epitopes lie outside of the spike



- only 3 of the 29 epitopes are located in the S protein. Most epitopes (15 of 29) are located in ORF1ab, and the highest density of epitopes are located in the N protein

summary

T cell scan:

- enables the interrogation of highly complex epitope sets in a high-throughput manner
- unbiased approach to characterize antigens
- can be adapted to identify new targets in autoimmunity (autoreactive antigens)
- allows for a rapid discovery of novel antigen targets in viral infections, as well as in cancer immunology (solid tumors included)
- lastly, it can identify off-targets in cancer immunotherapy that lead to toxicity

limitations:

- genetic information and encoding of candidate antigens must be available
- some targets might be missed (e.g. lipids)
- endogenously expressed antigens by the EDCs cannot be screened

Thank you for your attention!