Discovery of immunodominant T cell targets in COVID-19 patients and design of novel T cell-based vaccines

July 22, 2021

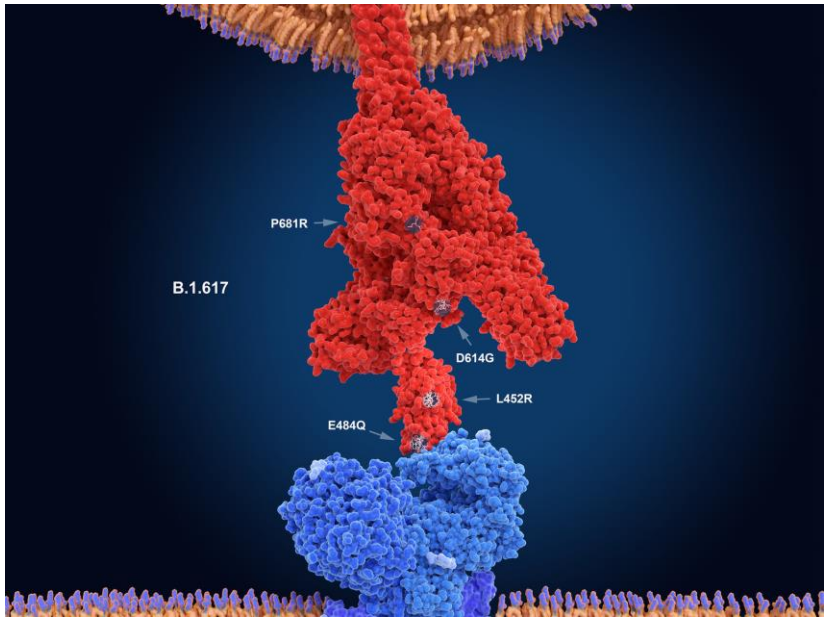
Gavin MacBeath, CSO, TScan Therapeutics

Disclaimers and Forward-Looking Statements

This presentation and the accompanying oral presentation contain forward-looking statements. All statements other than statements of historical fact contained in this presentation, including statements regarding possible or assumed future results of operations of TScan Therapeutics, Inc. (the "Company", “we”, “our” and “us”), expenses and financing needs, business strategies and plans, research and development plans or expectations, the structure, timing and success of the Company’s planned preclinical development and clinical trials, expected milestones, market sizing, competitive position, regulatory matters, industry environment and potential growth opportunities, among other things. Forward-looking statements are inherently subject to risks and uncertainties, some of which cannot be predicted or quantified. In some cases, you can identify forward-looking statements by terms such as “may,” “might,” “will,” “objective,” “intend,” “should,” “could,” “can,” “would,” “expect,” “believe,” “anticipate,” “project,” “target,” “design,” “estimate,” “predict,” “potential,” “plan” or similar expressions or the negative of those terms. The Company has based these forward-looking statements largely on its current expectations and assumptions and on information available as of the date of this presentation. The information in this presentation is provided only as of July 22, 2021 and the Company assumes no obligation to update any forward-looking statements after the date of this presentation, except as required by law.

The forward-looking statements contained in this presentation and the accompanying oral presentation are subject to known and unknown risks, uncertainties, assumptions and other factors that may cause actual results or outcomes to be materially different from any future results or outcomes expressed or implied by the forward-looking statements. These risks, uncertainties, assumptions and other factors include, but are not limited to, including the development, clinical and regulatory plans or expectations for the Company’s TCR-T therapy candidates, as well as the risks described in the "Risk Factors" and "Management's Discussion and Analysis of Financial Condition and Results of Operations" sections of the Company's Final Prospectus for its initial public offering, which is on file with the Securities and Exchange Commission (SEC) and available on the SEC's website at www.sec.gov. Additional factors may be described in those sections of the Company's Quarterly Report on Form 10-Q for the quarter ended June 30, 2021, expected to be filed with the SEC in the third quarter of 2021. You should not put undue reliance on any forward-looking statements. Forward-looking statements should not be read as a guarantee of future performance or results and will not necessarily be accurate indications of the times at, or by, which such performance or results will be achieved, if at all. It is not possible for the Company to predict all risks, nor can the Company assess the impact of all factors on its business or the markets in which it operates or the extent to which any factor, or combination of factors, may cause actual results or outcomes to differ materially from those contained in any forward-looking statements the Company may make.

The delta variant of SARS-CoV-2 is now widespread



- The delta variant is now responsible for more than 58% of new infections in the United States

- Six New York Yankee players just tested positive for COVID-19
- Five were fully vaccinated

The SARS outbreak of 2002/2003 suggests that CD8$^+$ T cells may be important for establishing long-term immunity

- Long-term follow up studies of SARS patients (2, 6, 11, and 17 years later) showed that convalescent patients rapidly lost their anti-viral antibodies and memory B cells but retained their memory T cells[1–4].
- Animal studies showed that vaccination with a single immunodominant CD8$^+$ T cell epitope conferred complete protection from lethal exposure to SARS-CoV[5,6].

References

1. Peng, H. et al. (2006) *Virology* 351, 466-475.
2. Tang, F. et al. (2011) *J. Immunol.* 186, 7264-7268.
3. Ng, O.W. et al. (2016) *Vaccine* 34, 2008-2014.
4. Le Bert, N. et al. (2020) *Nature* 584, 457-462.
5. Zhao, J. et al. (2010) *J. Virol*. 84, 9318-9325.
6. Channappanavar, R. et al. (2014) *J. Virol.* 88, 11034-11044.

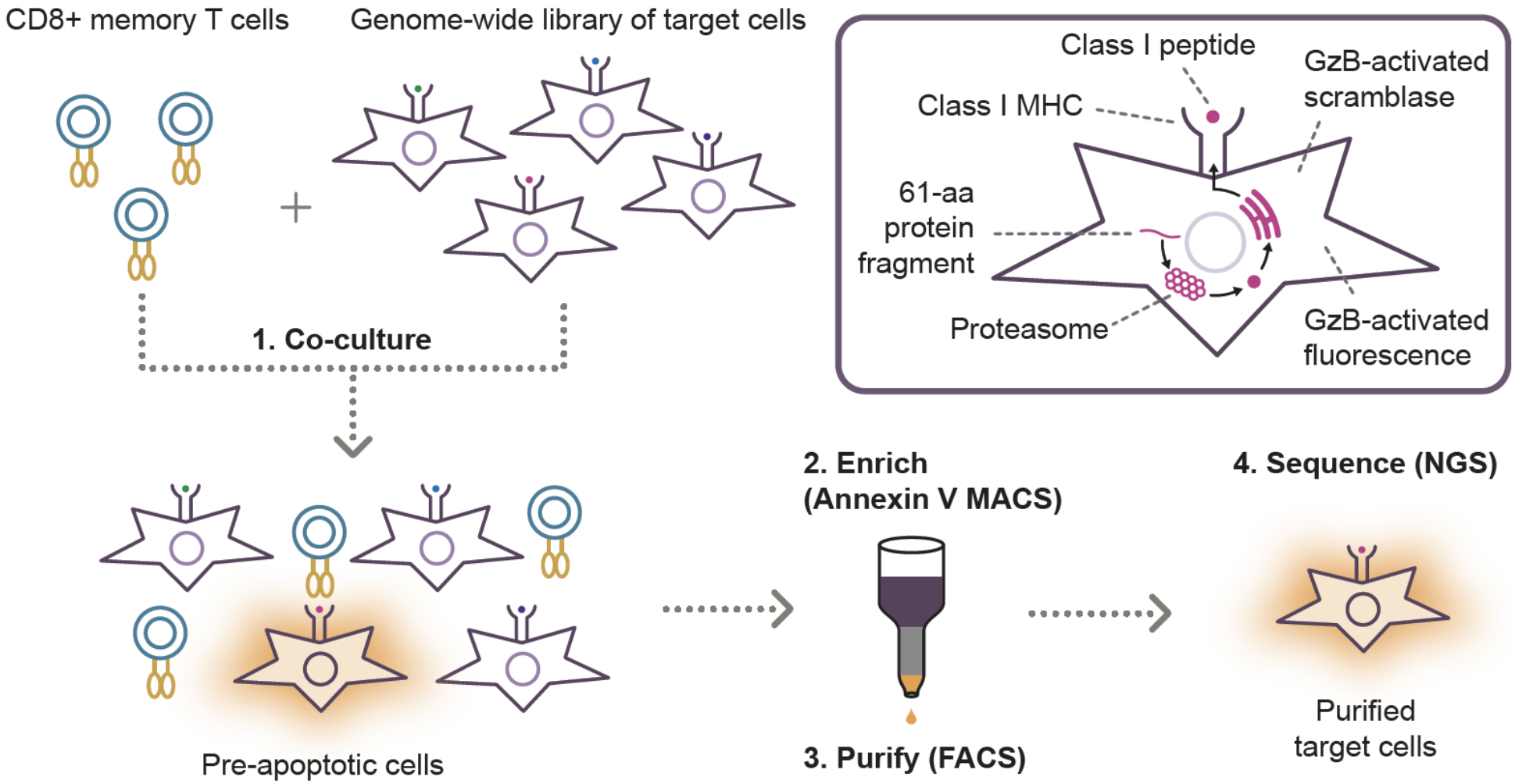
Studies of COVID-19 patients also suggest that a T cell-eliciting vaccine may be necessary for long-term immunity

- Neutralizing antibodies against the spike protein rapidly wane following infection with SARS-CoV-2[1].
- Germinal centers are largely absent in patients with acute COVID-19, impairing the formation of memory B cells and long-lived plasma cells[2].
- SARS-CoV-2-specific memory T cells are found in most convalescent individuals, including asymptomatic cases and those with undetectable antibody responses[3].

References

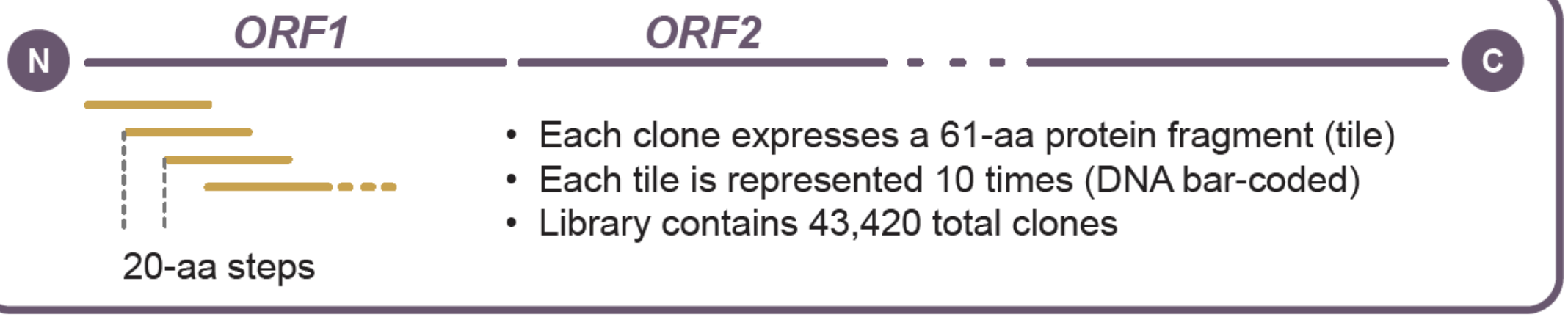
1. Seow, J. et al. (2020) *Nature Microbiology* doi: 10.1038/s41564-020-00813-8.
2. Kaneko, N. et al. (2020) *Cell* 183, 143-157.
3. Sekine, T. et al. (2020) *Cell* 183, 158-168.

Unbiased genome-wide screen enables identification of the targets of CD8+ memory T cells in COVID-19 patients



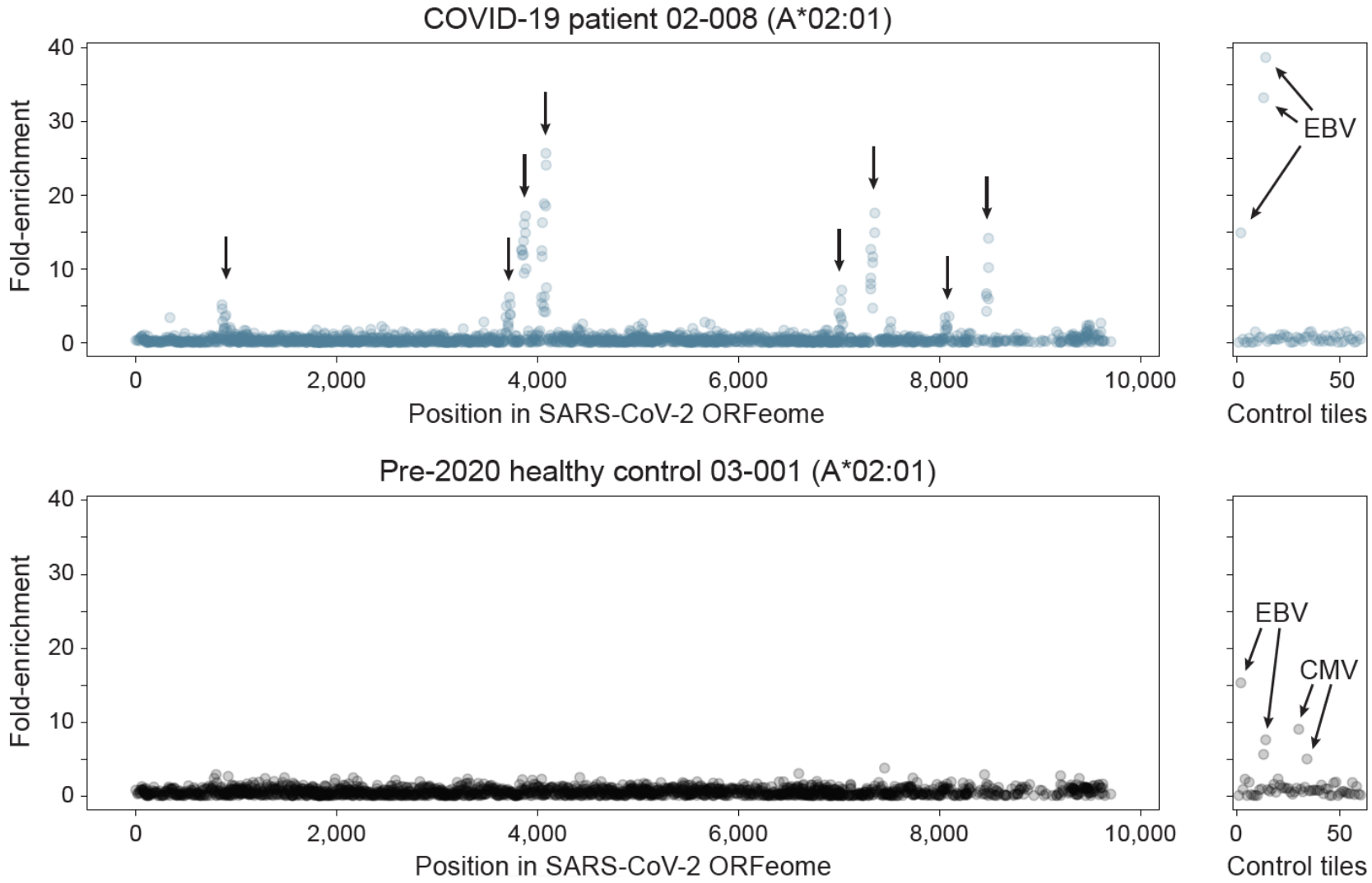
Unbiased genome-wide screen enables identification of the targets of CD8+ memory T cells in COVID-19 patients

SARS-CoV-2 (104 sequenced isolates), SARS-CoV, HKU1, OC43, 229E, NL63

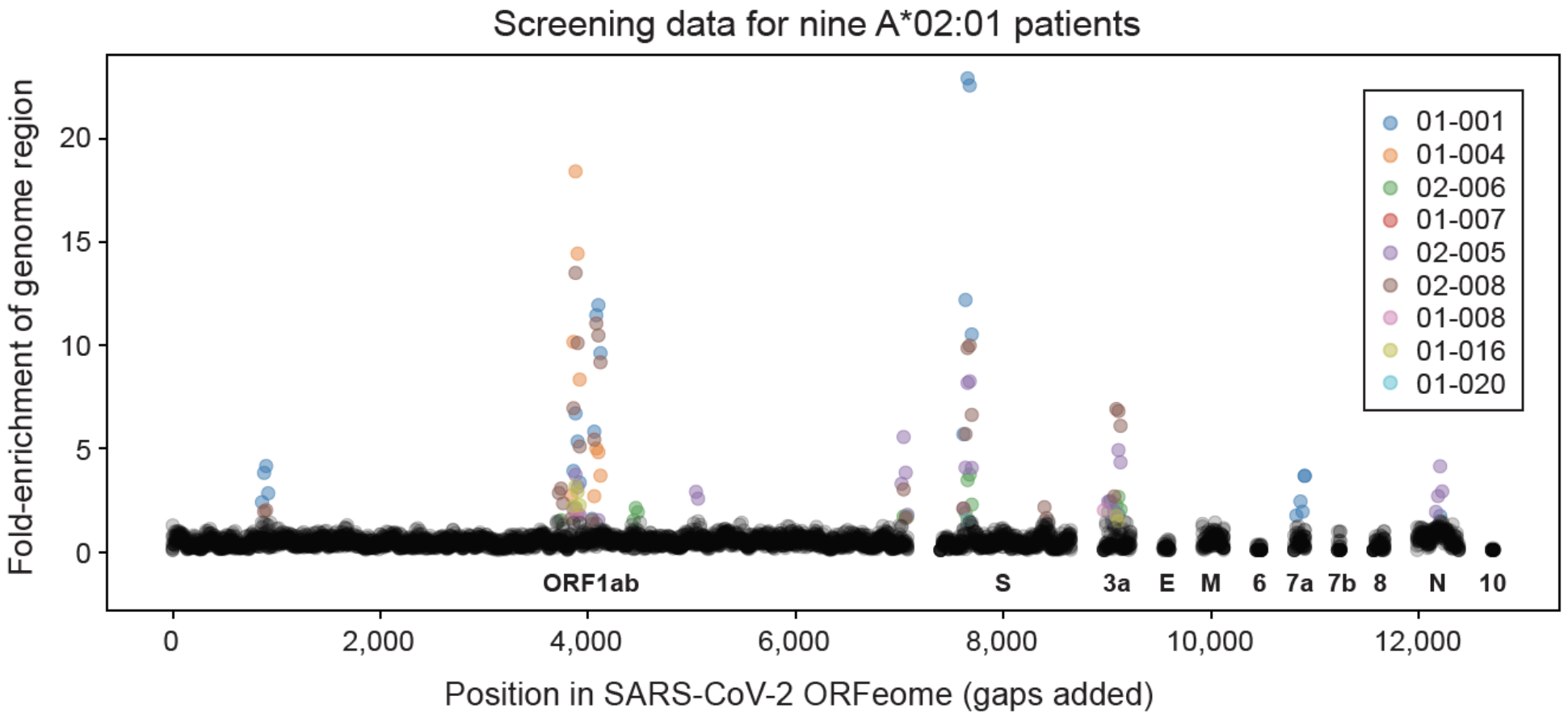


- Each clone expresses a 61-aa protein fragment (tile)
- Each tile is represented 10 times (DNA bar-coded)
- Library contains 43,420 total clones

TScan screen identified eight dominant targets in an HLA A*02:01 patient

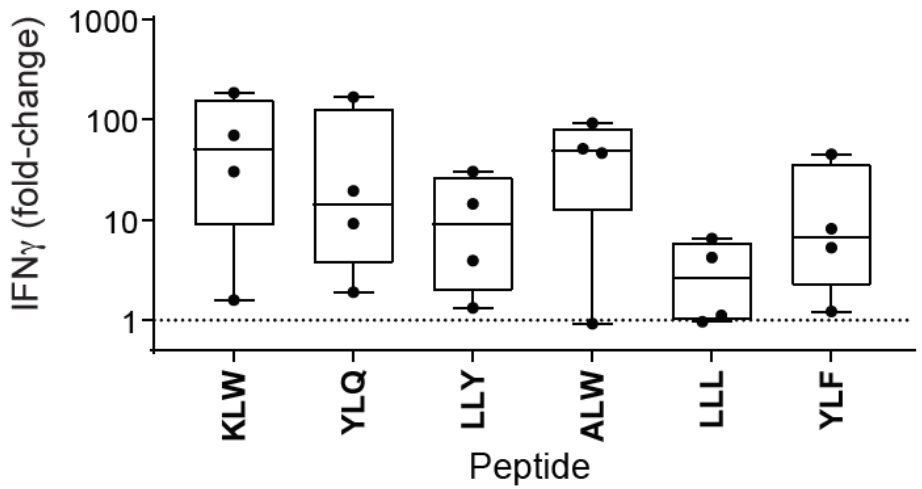


TScan screens of nine A*02:01 patients show that their T cells are largely recognizing the same epitopes



The precise T cell epitopes were identified and found to be immunodominant (shared across patients)

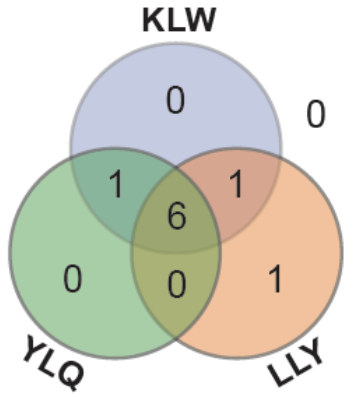
Validation by IFNγ secretion



- Also validated by CD137 expression and tetramer staining

Top three epitopes are broadly shared among patients

Nine patients from screen



27 patients including independent test-set

Immunodominant epitopes were observed in five additional common HLA types

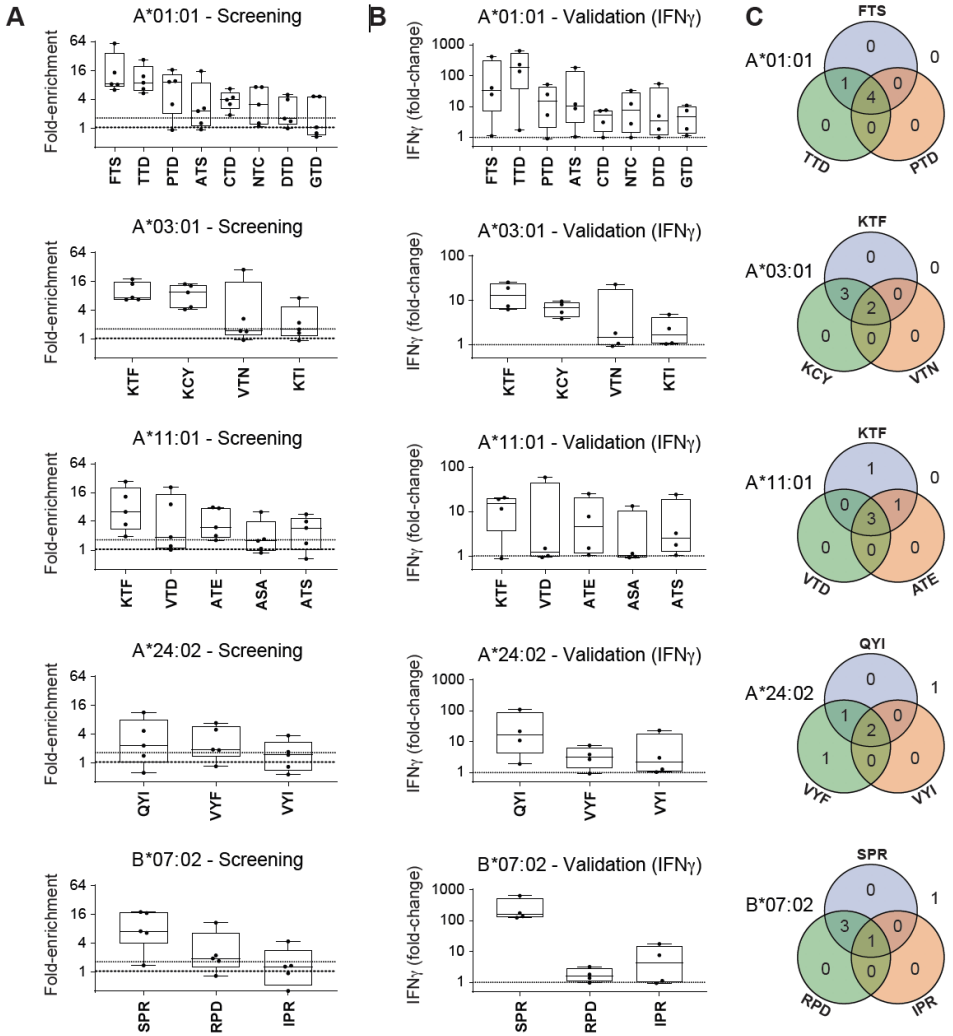
HLA-A*01:01

HLA-A*03:01

HLA-A*11:01

HLA-A*24:02

HLA-B*07:02



TScan discovered a total of 29 immunodominant epitopes

Table 1. Shared CD8+ T Cell Epitopes Identified in COVID-19 Convalescent Patients

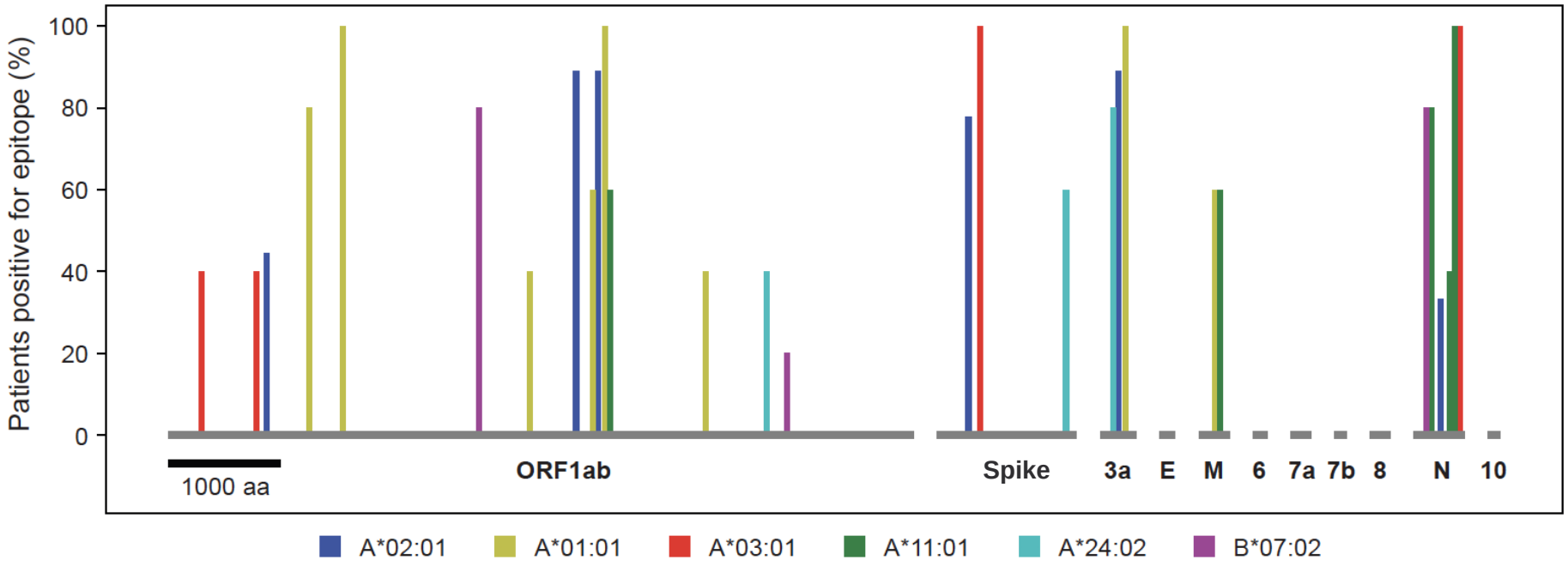
	Allele	Peptide Name	Full Peptide	Parent Protein	Start	End	Affinity[a] (nM)	% of Patients (Screen)
1	A*02:01	KLW	KLWAQCVQL	ORF1ab	3,886	3,894	17.7	88.9
2	A*02:01	YLQ	YLQPRTFLL	S	269	277	5.4	77.8
3	A*02:01	LLY	LLYDANYFL	ORF3a	139	147	3.1	88.9
4	A*02:01	ALW	ALWEIQQVV	ORF1ab	4,094	4,102	7.8	88.9
5	A*02:01	LLL	LLLDRLNQL	N	222	230	14.8	33.3
6	A*02:01	YLF	YLFDESGEFKL	ORF1ab	906	916	22.2	44.4
7	A*01:01	FTS	FTSDYYQLY	ORF3a	207	215	3.2	100
8	A*01:01	TTD	TTDPSFLGRY	ORF1ab	1,637	1,646	7.2	100
9	A*01:01	PTD	PTDNYITTY	ORF1ab	1,321	1,329	6.1	80
10	A*01:01	ATS	ATSRTLSYY	M	171	179	16.7	60
11	A*01:01	CTD	CTDDNALAYY	ORF1ab	4,163	4,172	5.3	100
12	A*01:01	NTC	NTCDGTTFTY	ORF1ab	4,082	4,091	121.8	60
13	A*01:01	DTD	DTDFVNEFY	ORF1ab	5,130	5,138	2.8	40
14	A*01:01	GTD	GTDLEGNFY	ORF1ab	3,437	3,445	6	40
15	A*03:01	KTF	KTFPPTEPK	N	361	369	20.8	100
16	A*03:01	KCY	KCYGVSPTK	S	378	386	152.6	100
17	A*03:01	VTN	VTNNTFTLK	ORF1ab	808	816	19.8	40
18	A*03:01	KTI	KTIQPRVEK	ORF1ab	282	290	113.2	40
19	A*11:01	KTF	KTFPPTEPK	N	361	369	6.3	100
20	A*11:01	VTD	VTDTPKGPK	ORF1ab	4,216	4,224	160.6	60
21	A*11:01	ATE	ATEGALNTPK	N	134	143	55.5	80
22	A*11:01	ASA	ASAFFGMSR	N	311	319	14.4	40
23	A*11:01	ATS	ATSRTLSYYK	M	171	180	7.9	60
24	A*24:02	QYI	QYIKWPWYI	S	1,208	1,216	13.2	60
25	A*24:02	VYF	VYFLQSINF	ORF3a	112	120	47.4	80
26	A*24:02	VYI	VYIGDPAQL	ORF1ab	5,721	5,729	206	40
27	B*07:02	SPR	SPRWYFYYL	N	105	113	6.3	80
28	B*07:02	RPD	RPDTRYVL	ORF1ab	2,949	2,956	56.9	80
29	B*07:02	IPR	IPRRNVATL	ORF1ab	5,916	5,924	5.1	20

[a]Affinity (equilibrium dissociation constant) predicted by using NetMHC4.0.

TSCAN THERAPEUTICS

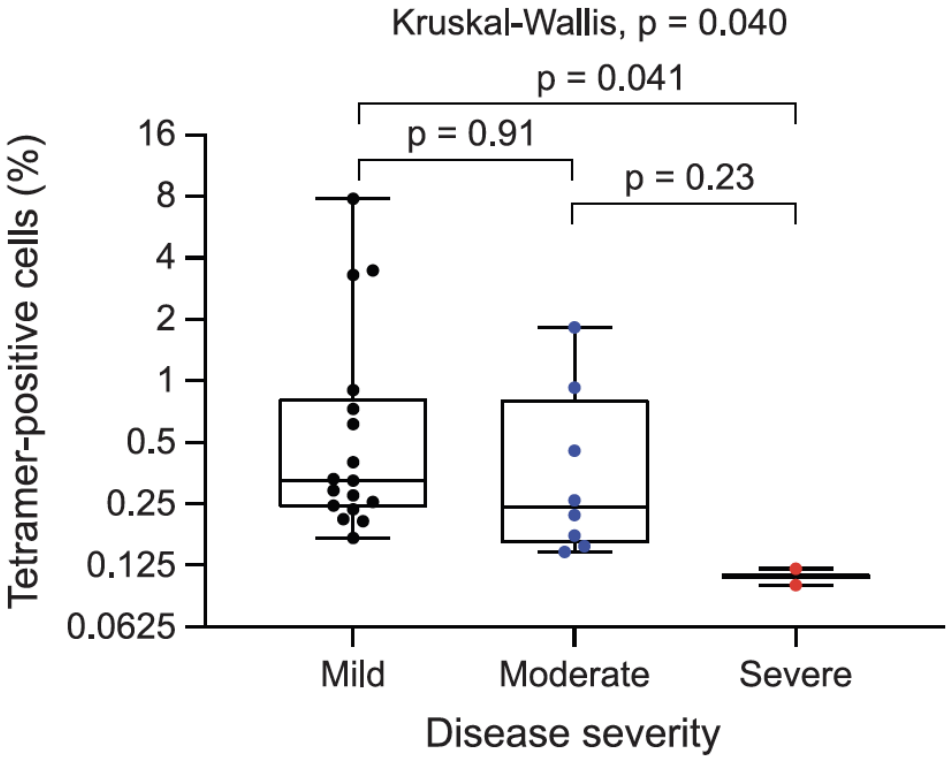
Of the 29 immunodominant epitopes in SARS-CoV-2, only 3 are found in the Spike protein

- ~90% of immunodominant epitopes are located outside the Spike protein
- No mutations with frequency >1% are observed in 27 of the 29 epitopes (>10,000 sequenced isolates)
- None of the mutations in the UK, South African, Brazilian, or Delta variants occur in these epitopes

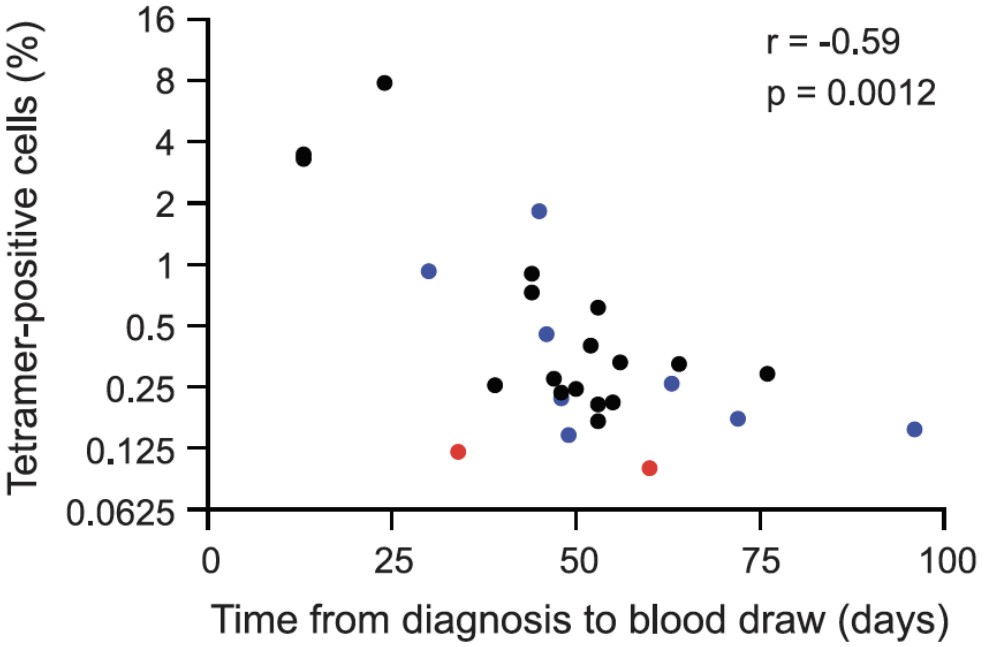


Trend observed between anti-viral T cells and disease severity

Virus-specific T cells negatively correlate with disease severity



T cell contraction is not driving the correlation

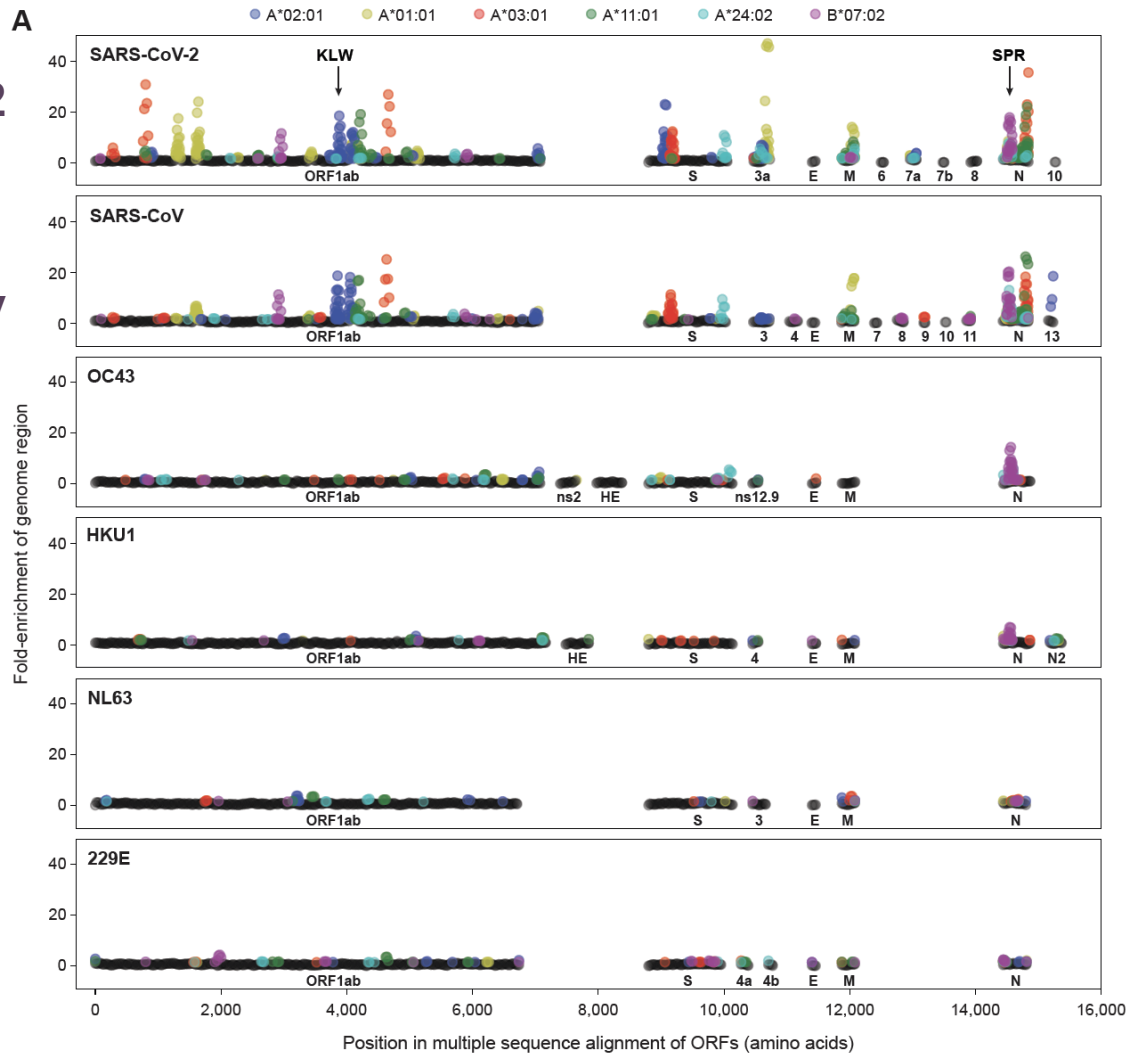


T cells don’t cross-react with other coronaviruses

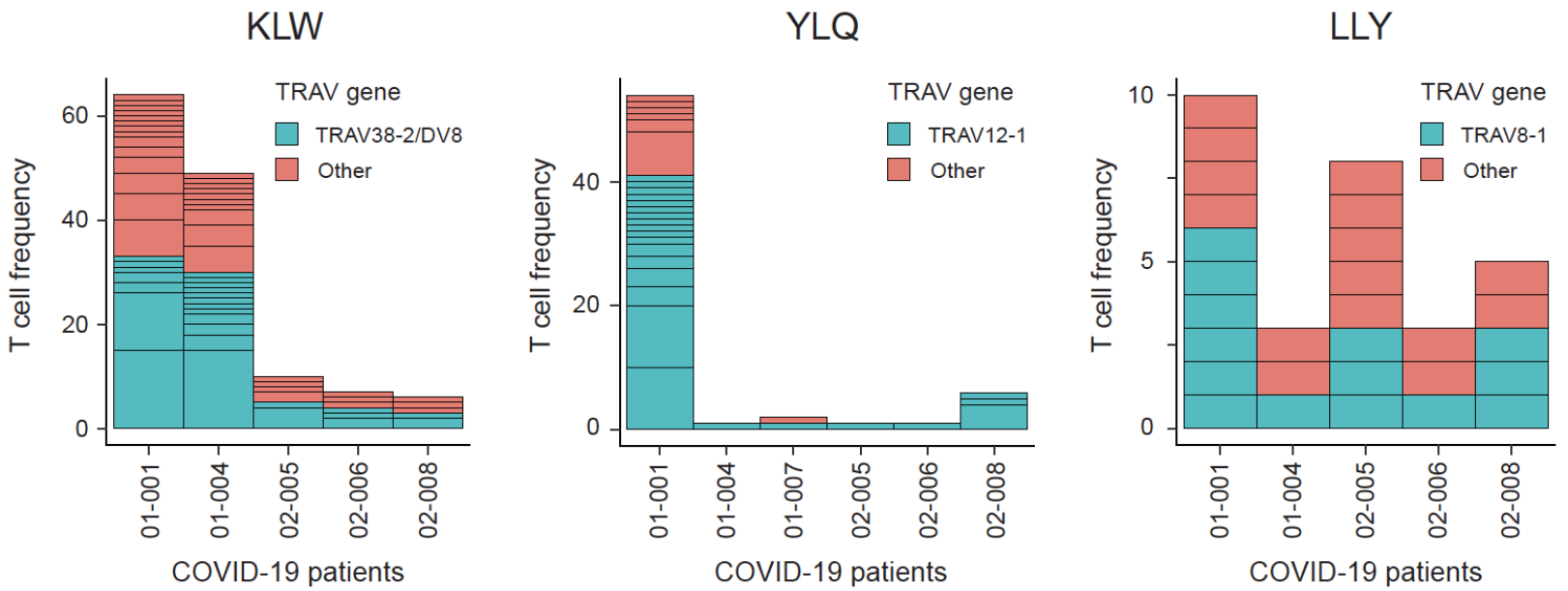
SARS-CoV-2

SARS-CoV

‘Common Cold’ Coronaviruses

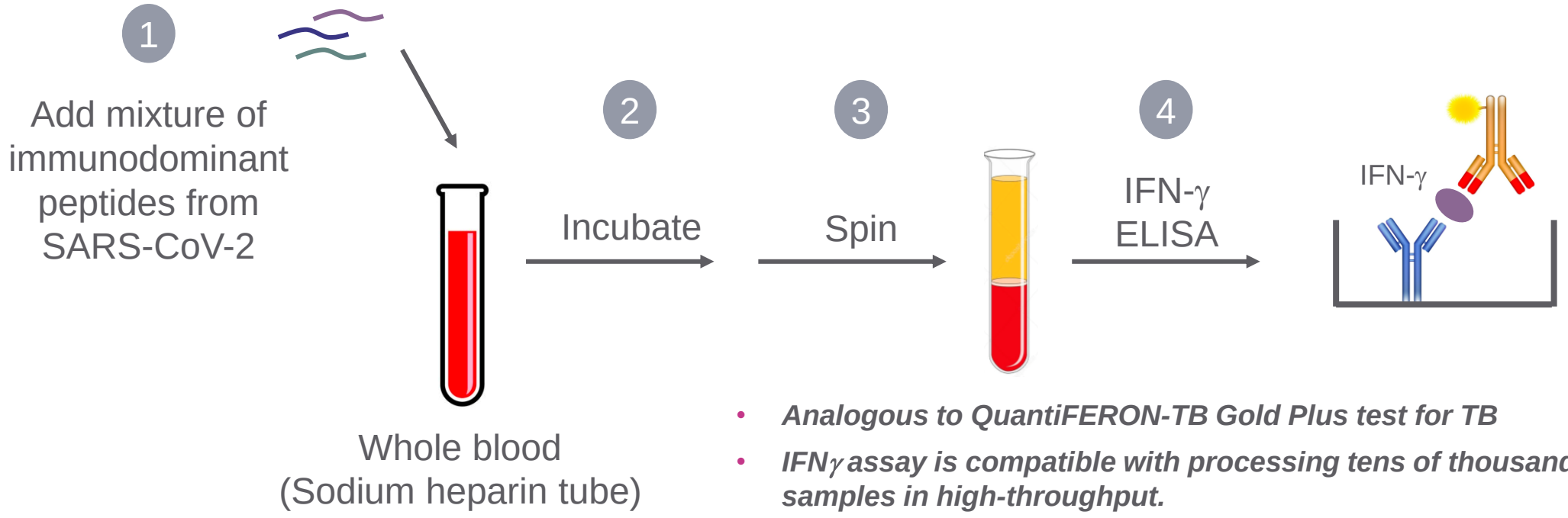


>400 TCRs for SARS-CoV-2 were discovered, explaining immunodominance and enabling T cell-based therapeutics



See: “An Allogeneic TCR-T Cell Therapy for COVID-19” – Poseida Therapeutics

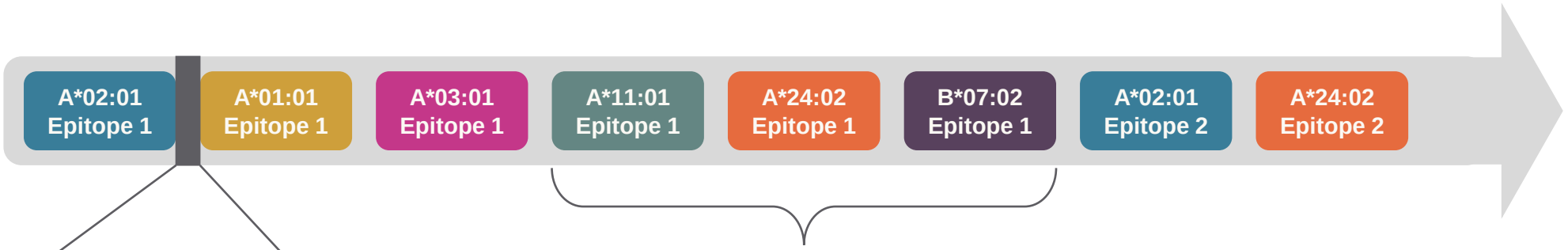
Assay developed by QIAGEN to detect prior exposure to SARS-CoV-2 based on anti-viral T cells



- ***Analogous to QuantiFERON-TB Gold Plus test for TB***
- ***IFNγ assay is compatible with processing tens of thousands of samples in high-throughput.***

- Immunodominant peptides provide specificity, as they are unique to SARS-CoV-2 and not endemic coronaviruses.

Several polyepitope vaccine candidates were designed based on the discovered immunodominant sequences



Linker

- Different lengths of native adjacent sequence
- Optimized proteasomal cleavage motif

Epitope Order

- Optimized to minimize generation of junctional neoepitopes

Epitope Number

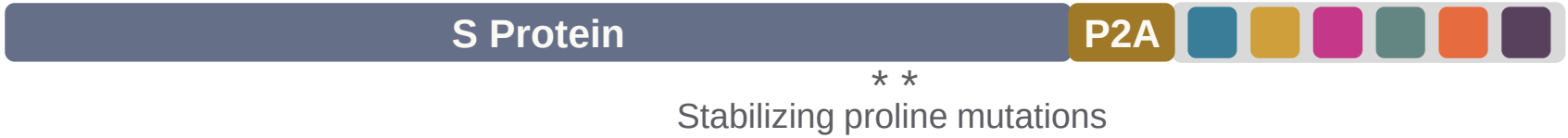
- Top 3-4 epitopes per allele (19 total)
- All immunodominant epitopes (27 total)

Next-generation vaccine constructs were designed with and without the Spike protein

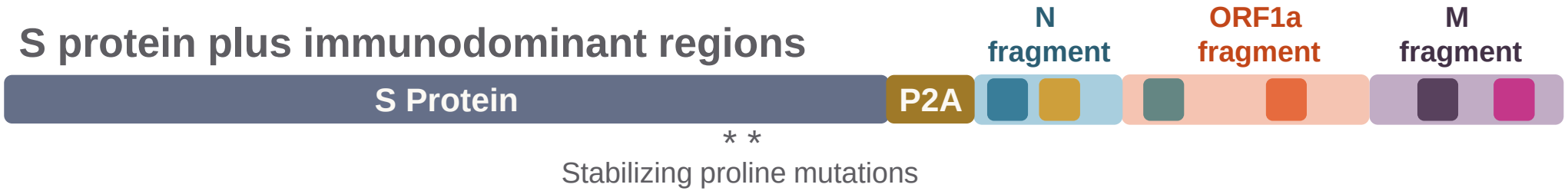
Polyepitope vaccine alone

Two versions: 19 epitopes and 27 epitopes.

S protein plus polyepitope vaccine



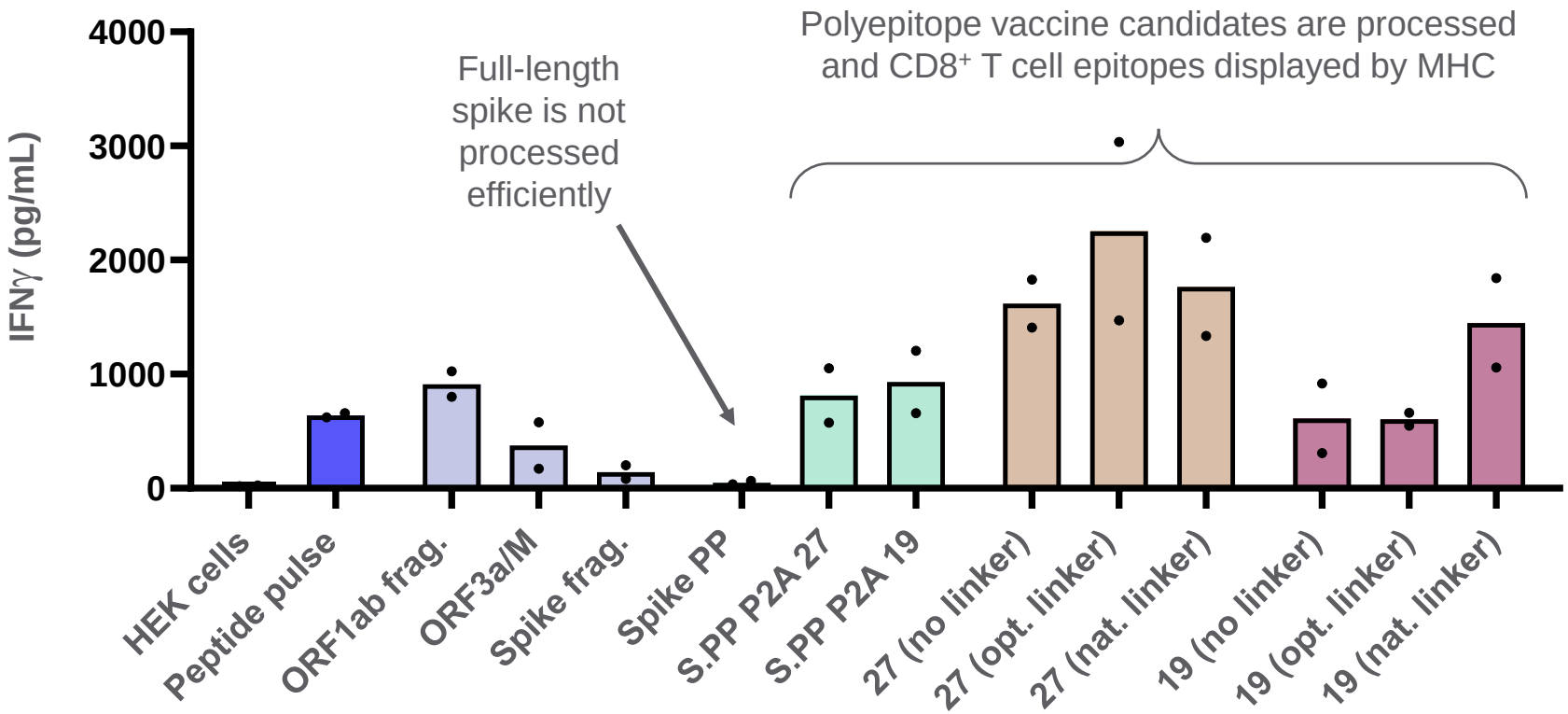
S protein plus immunodominant regions



- These constructs can be delivered using a variety of technologies, including mRNA/LNP

Human cells efficiently process and present epitopes from the polyepitope vaccine candidates, but not from full-length Spike

- HEK 293 cells engineered to express A*02:01 were transduced with lentiviral vectors delivering each vaccine candidate
- Memory CD8$^+$ T cells from two A*02:01-positive COVID-19 patients were co-cultured with the transduced HEK cells and secreted IFN-γ was measured after 18 hours



Data from 7 additional patients further support these conclusions.

Data available in *Immunity* publication

Immunity

CellPress

Article

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,[1,5] Tomasz Kula,[1,4,5] Yifan Wang,[1] Dalena M.V. Nguyen,[1] Adam Weinheimer,[1] Garrett S. Dunlap,[1] Qikai Xu,[1] Nancy Nabilsi,[1] Candace R. Perullo,[1] Alexander W. Cristofaro,[1] Holly J. Whitton,[1] Amy Virbasius,[1] Kenneth J. Olivier, Jr.,[1] Lyndsey R. Buckner,[2] Angela T. Alistar,[3] Eric D. Whitman,[3] Sarah A. Bertino,[1] Shrikanta Chattopadhyay,[1] and Gavin MacBeath[1,6,*]

[1]TScan Therapeutics, Waltham, MA 02451, USA
[2]Ochsner Medical Center, New Orleans, LA 70121, USA
[3]Atlantic Health System, Morristown, NJ 07960, USA
[4]Present address: Society of Fellows, Harvard University, Cambridge, MA 02138, USA
[5]These authors contributed equally
[6]Lead Contact
*Correspondence: gmacbeath@tscan.com
https://doi.org/10.1016/j.immuni.2020.10.006

Posted on medRxiv:
July 27, 2020

Published in *Immunity*:
October 20, 2020

Immunity 2020, **53**, 1-13.