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PRECISION IMMUNE MONITORING

Monitoring Long Term T-Cell Immunity In COVID-19

Monitoring of naturally acquired and vaccine-induced SARS-CoV-2-specific cellular immune responses using SARS-CoV-2 Dextramer® Panels

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Highlights

- We selected epitopes for Spike and Non-Spike proteins of the coronavirus and developed SARS-CoV-2 Dextramer® Panels to monitor the dynamics of CD8+ T cell responses over time in pre-pandemic, post-infection and post-vaccination samples from an individual with hybrid immunity
- The SARS-CoV-2 Dextramer® Panels can be used to evaluate new vaccine candidates and assess long term cellular immunity
- The selected epitopes are largely conserved in Delta and Omicron variants, enabling immune monitoring across SARS-CoV-2 variants
- Monitoring long term T-cell immunity has the potential to identify sub-populations of individuals with low or waning immunity, and support booster vaccination decision making

Introduction

The global COVID-19 pandemic has highlighted our need to understand the dynamics of the immunological response and provide guidance to clinicians and health authorities about the type, magnitude, and duration of immunity.

Historically, measurement of immunity has relied on antibody levels. However, antibody levels alone do not provide sufficient information about long-term immunity. In order to support decision making about future vaccination programs in the context of the COVID-19 pandemic, and evaluate new vaccine candidates, immune markers need to be identified that correlate with vaccine effectiveness in the long term[1].

Focus On T-Cell Immunity

Attention is turning towards cellular immunity in an effort to understand potential correlates of protective immunity.

Spike-specific T cells appear around 10 days after the first dose of the Pfizer mRNA vaccine, which coincides with the start of protective immunity[2,3]. SARS-CoV-2-specific CD4+ and CD8+ T cells expand over the first month after infection[4] or vaccination[5,6], and gradually decline over subsequent months[4]. Neutralizing antibodies only start to be present later in high titers, at 21 days after the first dose of vaccine[2], and begin to decline around 3 months after vaccination[7].

Studies are now increasingly investigating T-cell responses against SARS-CoV-2, to better understand the dynamics and duration of cellular immunity.

Long-term T-Cell Immunity

One of the key questions is to what extent infection with or vaccination against SARS-CoV-2 results in the generation of long-term immunity capable of protecting the individual against severe disease in the case of re-infection. In respiratory viruses, a subset of antigen-specific memory CD8+ T cells persist after infection and provide long-term protective immunity[8]. It is promising to note that SARS-CoV-specific T cells have been detected 17 years after infection with SARS[9], although it is not known whether this would be sufficient to confer protective immunity.

The duration of immunity against COVID-19 is an area of active research (**Fig. 1**). SARS-CoV-2-specific CD4+ and CD8+ T cells are present for at least a year after infection[10], and at least 6 months after vaccination[11]. The half-life of SARS-CoV-2-specific T cells following natural infection is estimated to be 200 days, similar to the half-life of neutralizing antibodies reported in convalescent donors[4]. The extended half-life of the antibody response suggests the generation of longer-lived plasma cells producing antibody against SARS-CoV-2[4].

Immudex's technology has been used to demonstrate the persistence of SARS-CoV-2-specific memory CD8+ T cells up to 1 year after infection, and identify a transcriptional signature correlating with long-lived memory T cells[12]. Studying the functional phenotypes of different sub-populations of antigen-specific T cells in detail, in order to understand exactly how they contribute to long-term protective immunity, will be key to identifying the correlates of long-lasting COVID-19 protection.

T Cell and Antibody Responses in COVID-19

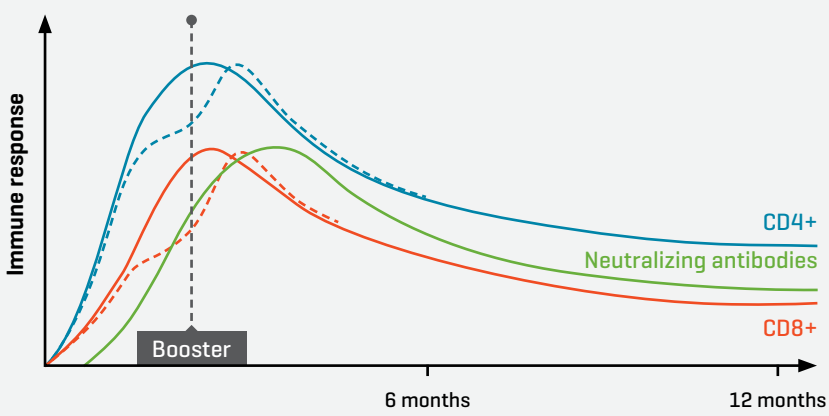


Fig. 1: The duration of T cell and antibody responses following natural infection or vaccination of naïve donors. Illustrative representation of the results presented in publications[2,4,5,6,10,11,13]. The mean magnitude of the T cell response is reported to be similar at 3 months after infection vs. 3 months after booster vaccination[14]. The magnitude of the CD4+ response is higher than that of the CD8+ response, which preferentially targets the nucleoprotein[4]. The level of neutralizing antibodies are reported to be higher in vaccinees compared to convalescent patients[15].

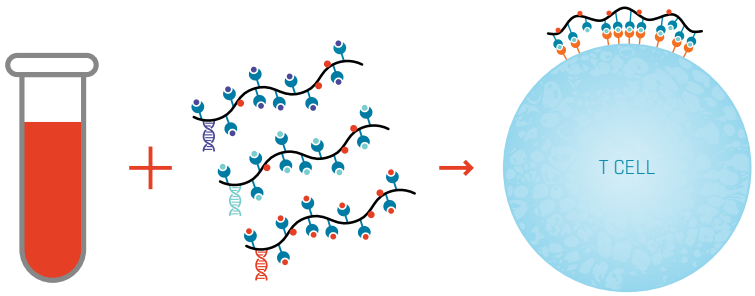
Meeting the Challenge of Large-Scale Immune Monitoring

There is a critical need for reliable immune monitoring assays to identify the correlates of protective immunity and monitor long-term immunity in large populations.

The global population is extremely variable, with different history of SARS-COV-2 infections, variant exposure, vaccination programs, and levels of prior immunity. A robust immune monitoring approach must take these variables into consideration in order to provide meaningful input for the development of future vaccine programs.

At Immudex, we have developed the Dextramer® technology to reliably monitor SARS-CoV-2-specific T cells [**Fig. 2**]. Dextramer® technology enables the direct *ex vivo* quantification of SARS-CoV-2-specific CD4+ and CD8+ T cells. Samples from COVID-19 patients can be rapidly analyzed via a standardized flow cytometry assay, without the need to culture or stimulate cells *in vitro*, which can introduce variability. The results are reproducible, even between different laboratories [**Fig. 3**], making the Dextramer technology well suited to large-scale studies.

Dextramer® SARS-CoV-2 Panels Enable Large-Scale Immune Monitoring Studies



Blood samples from COVID-19 infected patients or vaccinated people

Dextramer® Panel of validated epitopes

Dextramer® binds to TCR on virus-specific T cells

- Rapid flow cytometry assay (2 hours)
- No need to culture or stimulate cells *in vitro*
- Reproducible results, even between different laboratories [**Fig. 3**]
- Dextramer® reagents manufactured following rigorous production and quality control requirements

Fig. 2: The Dextramer® technology relies on displaying virus-specific epitopes on major histocompatibility complex (MHC) molecules for recognition by virus-specific T-cells. The Dextramer® reagents are especially suitable for large-scale studies for the reasons indicated in the figure. (GMP reagents available upon request). Dextramer® technology can be combined with functional markers or multiparametric characterization of single cells for more detailed analysis of antigen-specific T cells, if desired.

MHC Multimer Results are Most Consistent Between Different Laboratories

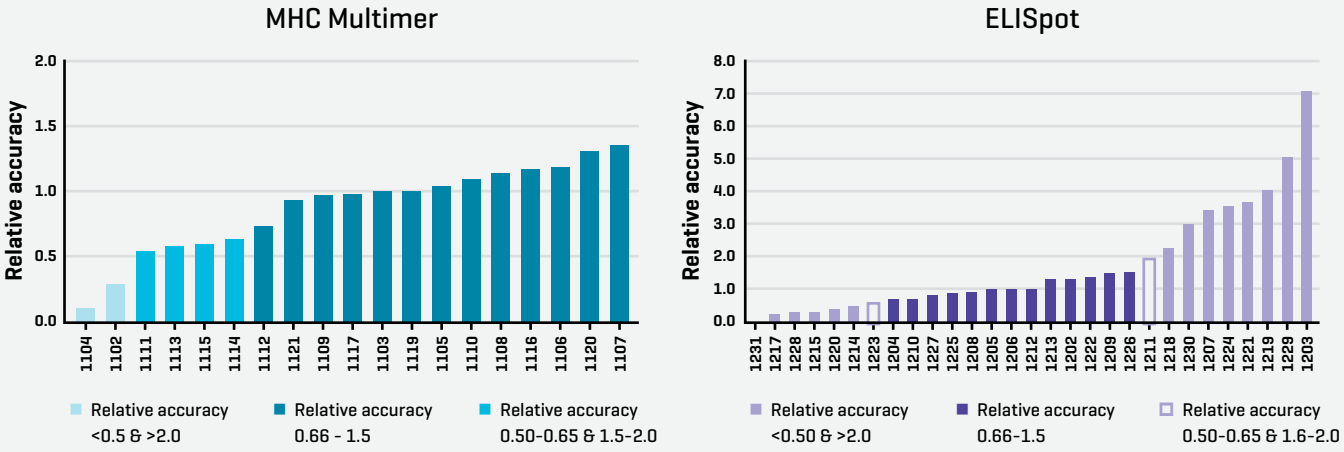


Fig. 3: MHC Multimer technology such as Dextramer® reagents are more consistent and reproducible compared to ELISpot, when tested by multiple different laboratories in a Proficiency Panel Study. Immudex offers Proficiency Panels to help researchers and clinicians worldwide evaluate their immune monitoring performance with the MHC Multimer and T-cell ELISpot assays. Using laboratory-specific protocols, participants determined the number of IFN-ɣ secreting antigen-specific T cells in a CMV positive human PBMC sample using ELISpot. In the MHC Multimer proficiency panel an EBV positive sample is stained with EBV-specific and Negative Control MHC Multimers. All laboratories received identical PBMC samples, and results were collated to assess the relative accuracy of results across the different laboratories. In the ELISpot study, 13 of the 29 participants (44,8%) had a relative accuracy between 0.66-1.5 and are considered "in the average range" (dark purple columns). In the MHC Multimer study, 13 out of 19 participants (68,4%) had a relative accuracy between 0.66 – 1.5 and are considered "the average range" (dark blue columns).

Methods

- SARS-CoV-2 MHC Class I Dextramer® Panels (**Table 2**) displaying epitopes from Spike (PE labelled) and Non Spike (APC labelled) proteins
- Positive control Dextramer® reagents displaying influenza, EBV or CMV epitopes (PE labelled)
- Negative control Dextramer® reagents (APC labelled)
- Antibodies for analysis of cell surface markers

Sample Collection and Analysis

- Peripheral blood mononuclear cells (PBMC) samples were isolated from blood collected from an individual 3 months after COVID-19 infection, and 4 weeks after each dose of the Pfizer mRNA vaccine administered in 2021.
- A PBMC sample from the same individual collected around 8 years before the pandemic was used as a reference.
- Whole blood was collected in heparin blood collection tubes and peripheral blood mononuclear cell (PBMC) isolation was performed immediately after blood collection, using the SepMate™ - Hassle-Free PBMCs Isolation technique of STEMCELL Technologies (Vancouver, Canada). The isolated PBMCs were aliquoted in 100 µL vials and stored at -150°C until use.
- HLA typing was conducted by genomic DNA isolation and subsequent high-resolution typing of HLA –A, -B, -C, -DRB1, -DQB1 and -DPB1 using next generation sequencing (IMGM Laboratories). Human antigen-specific T-cell monitoring requires that the HLA alleles of the Dextramer® reagents used for monitoring match those of the biological samples being analyzed.

- Each PBMC sample (3 x 107 cells) was stained in a tube containing SARS-CoV-2 Dextramer® reagents spanning the human leukocyte antigen (HLA) alleles A*01:01, B*07:02 and B*44:02, displaying epitopes from Spike (PE labelled) and Non-Spike (APC labelled) proteins. In a second tube, cells were labelled with Dextramer® reagents consisting of same HLA alleles displaying influenza, EBV or CMV epitopes (PE labelled), as well as negative control Dextramer reagents (APC labelled).
- The stained cell samples were acquired by flow cytometry. Identification of the MHC-Dextramer-positive cell population was obtained by serial gating of the flow data. The lymphocyte population was gated using side scatter (SSC) versus forward scatter (FSC) and doublets (SSC-Area, versus SSC lin plot) and dead cells were excluded. Plotting CD8/BV421 against the MHC Dextramer/PE and MHC Dextramer/APC, respectively, identified the Dextramer-specific CD8+ T cells.
- Flow cytometry data were analyzed using FlowJo, version 10.8 (FlowJo LLC, Ashland, OR, USA). Statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software, San Diego, California, USA).

Detection of Longitudinal T-Cell Responses

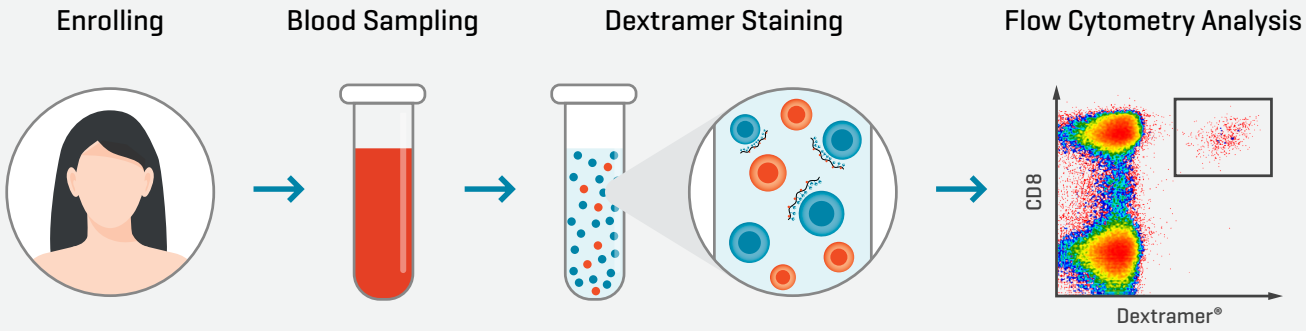


Fig. 4: PBMC samples collected from an individual pre-pandemic, after COVID-19 infection and after vaccination (approximately 9 months after infection) were analyzed using the Dextramer® reagents to determine the frequency of SARS-CoV-2-reactive CD8+ T cells.

Design of SARS-CoV-2 Dextramer® Panels

- The SARS-CoV-2 MHC class I Dextramer® Panel was designed to cover eight of the most common class I HLA alleles including A*01:01, A*02:01, A*03:01, A*11:01, A*24:02, B*07:02, B*35:01 and B*44:02 complexed to epitopes from Spike and Non-Spike (Nucleocapsid, ORF1ab and ORF3a) proteins of the SARS-CoV-2 reference strain (Lineage B, NCBI RefSeq reference genome NC_045512).
- Immunodominant epitopes were selected that were known to be present in large populations and to be recognized by T cells in COVID-19 patients, based a comprehensive evaluation of epitopes in published literature (**Tables 2** and **3**).
- The SARS-CoV-2 MHC class I Dextramer® Panel is predicted to have a population coverage of 87% in US and 94% in EU (population coverage was predicted using the IEDB.org tool at http://tools.iedb.org/population/) (Bui et al. 2006).

Analysis of Epitope Conservation

- Protein sequences from the SARS-CoV-2 reference strain (Lineage B, NC_045512), Delta (B.1.617.2), and Omicron (B.1.1.529) variants were retrieved in FASTA format from the National Center for Biotechnology Information (NCBI) database (**Table 1**).
- A multiple sequence alignment analysis was performed for Spike, Nucleocapsid, ORF3a, and ORF1ab using CLC Sequence Viewer 8.0 (Qiagen).
- The multiple alignment was overlayed with the specific epitopes included in the SARS-CoV-2 Dextramer® Panel (**Table 2**).

SARS-CoV-2 sequences used in multiple sequence alignment

Protein	Reference Strain	Delta	Omicron
Spike	YP_009724390.1	BCX29369.1	UFP04971.1
Nucleocapsid	YP_009724397.2	BCX29377.1	UFO69287.1
ORF3a	YP_009724391.1	BCX29370.1	UFO69280.1
ORF1ab	YP_009724389.1	BCX29367.1	UFO69277.1

Table 1: NCBI accession numbers for protein sequences of SARS-CoV-2 variants used for multiple sequence alignment.

Results

Magnitude and Kinetics of SARS-COV-2-Specific CD8+ T-Cell Immunity

A SARS-CoV-2 CD8+ T cell response was detected in all samples after infection and vaccination [**Fig. 5**]. CD8+ T cells specific to the Spike epitopes increase after infection, but their numbers increase even further after each dose of vaccine [**Fig. 5B**]. This could indicate that vaccination boosted the initial immune response to a higher level than that following the naturally acquired infection, in agreement with studies that have reported convalescent individuals show a greater response to the vaccine compared to naïve individuals[13,16,17,18]. However, other studies have reported similar levels of Spike-specific T cells in vaccinated and convalescent individuals[14].

The quantity of Spike-specific T cells has been reported to vary depending on factors such as disease severity and time after infection or vaccination[1]. Different subpopulations of T cells can also differ in longevity[19], so the magnitude and kinetics of the T cell response needs further investigation.

CD8+ T cells specific to Non-Spike epitopes were elevated to a similar level in samples after infection, 1st and 2nd vaccination, compared to the pre-pandemic sample. This may be because the vaccine is based on the Spike protein, hence the Non-Spike response is not boosted by the vaccine.

There was no detectable CD8+ T-cell response against Spike or Non-Spike in the pre-pandemic sample [**Fig. 5B**], which suggests the absence of pre-existing immunity towards COVID-19 in this individual. SARS-CoV-2 epitopes may be cross-reactive with common cold coronaviruses[19,20], raising the intriguing possibility that prior infection with the common cold could lead to cross-reactive memory T cells that may potentially provide a level of protection against SARS-CoV-2[21].

Monitoring SARS-CoV-2-specific CD8+ T cell responses over time

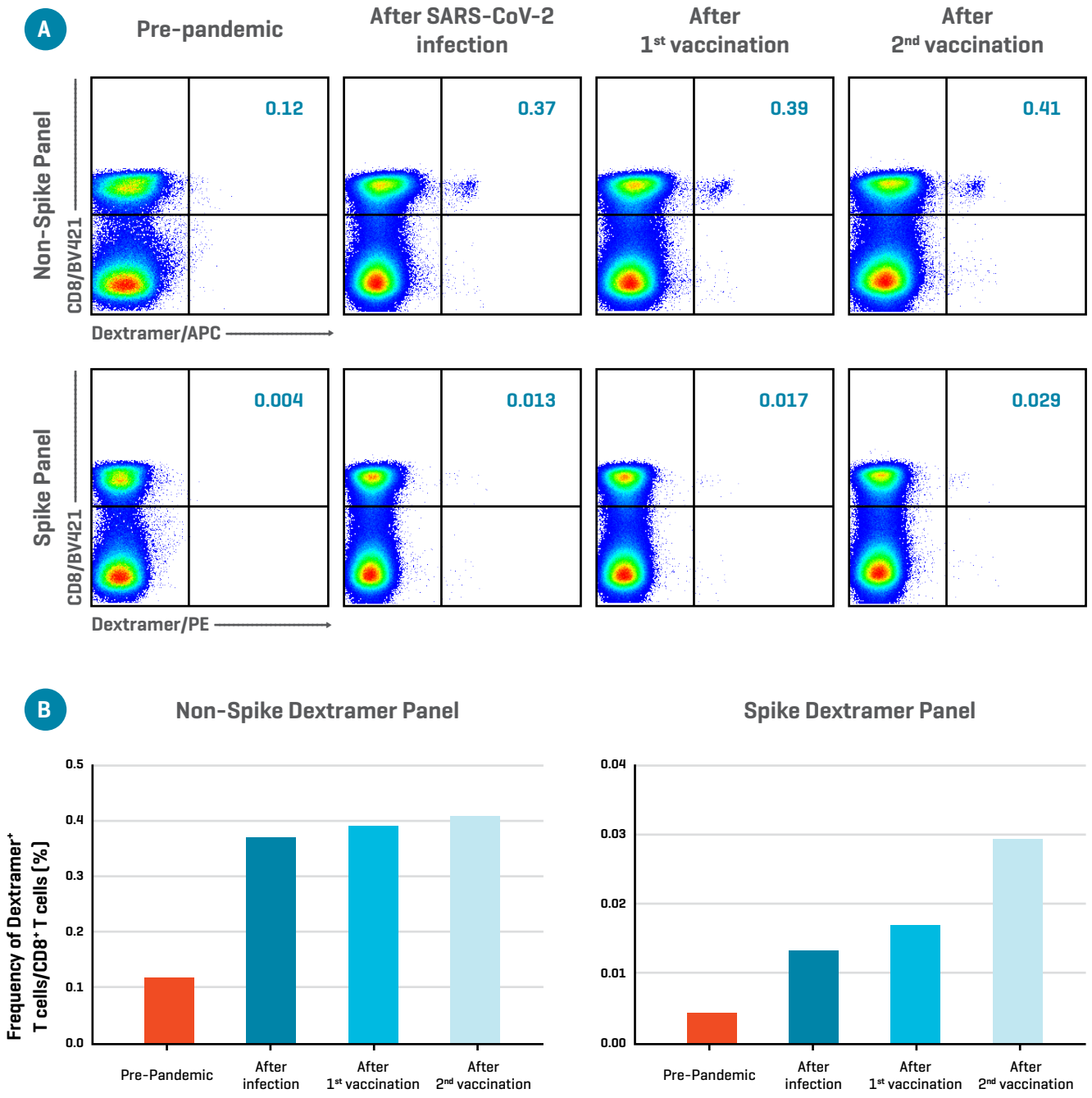


Fig. 5: Dextramer® Panels detect SARS-CoV-2-specific CD8+ T-cell responses displaying different magnitude and kinetics. (A) Flow cytometry plots showing SARS-CoV-2-specific CD8+ T cells reactive to Spike and Non-Spike epitopes detected in PBMCs from a vaccinated convalescent donor. The frequencies are defined as SARS-CoV-2-specific CD8+ T cells expressed as a % of total CD8+ T cells. (B) Bar plots summarizing change in the frequencies of Spike- and Non-Spike-specific CD8+ T cells across time points.

Conservation of T-Cell Epitopes in Delta and Omicron

To assess whether the SARS-CoV-2 Dextramer® Panel would be suitable for analysis of samples from individuals infected with the Delta or Omicron variants, a bioinformatics analysis was performed to understand whether the selected epitopes are conserved in Delta and Omicron.

The multiple alignment analysis of the selected CD8+ T-cell Spike epitopes across the SARS-CoV-2 variants Delta and Omicron showed a conservation of 15 and 14 out of 17 investigated CD8+ T-cell epitopes, respectively, corresponding to 88% and 82% [**Tables 2** and **4**]. Analysis of SARS-CoV-2-specific CD4+ T-cell epitopes also showed a high degree of conservation in Delta and Omicron variants [**Table 4**].

All but one of the selected Non-Spike CD8+ and CD4+ T-cell epitopes were conserved across the reference strain and both variants [**Tables 3** and **4**].

The high degree of conservation across SARS-CoV-2 T-cell epitopes [**Table 4**] verifies that the SARS-CoV-2 Dextramer® panels can be used to monitor T-cell immunity across variants. Furthermore, should new more divergent variants arise in the future, SARS CoV-2 Dextramer® reagents can be rapidly tailored to any new variant.

SARS-CoV-2 Dextramer® Panel of Spike-specific epitopes

Allele	Peptide	Antigen	Reference Strain	Delta	Omicron	Citation
A*0101	LTDEMIAQY	S				[22], [23], [24]
A*0101	WTAGAAAYY	S				[25]
A*0201	YLQPRTFLL	S				[24], [26], [27], [28], [29], [30], [31], [32], [33], [34]
A*0201	NLNESLIDL	S				[31]
A*0201	FIAGLIAIV	S				[25], [31], [33]
A*0301	KCYGVSPTK	S				[27]
A*0301	GVYFASTEK	S			GVYFAS|EK	[24]
A*1101	RLFRKSNLK	S				[24]
A*1101	KCYGVSPTK	S				[27]
A*1101	GVYFASTEK	S			GVYFAS|EK	[24], [25]
A*2402	QYIKWPWYI	S				[22], [27], [24], [29], [32], [34], [35]
A*2402	NYNYLYRLF	S		NYNYRYRLF		[24], [32], [34], [35]
B*0702	SPRRARSVA	S		SRRRARSVA	SHRRARSVA	[23], [28]
B*0702	APHGVVFL	S				[24]
B*3501	QPTESIVRF	S				[32]
B*3501	LPFNDGVYF	S				[32]
B*3501	IPFAMQMAY	S				[32]

Table 2: Spike-specific CD8+ T-cell epitopes in the SARS-CoV-2 Dextramer® Panels, and the conservation of these epitopes across the SARS-CoV-2 reference strain (NC_045512), Delta (B.1.617.2), and Omicron (B.1.1.529) variants. Green = no mutations in epitope, Red = mutations in epitope.

SARS-CoV-2 Dextramer® Panel of Non-Spike epitopes

Allele	Peptide	Antigen	Reference Strain	Delta	Omicron	Citation
A*0201	LLLDRLNQL	N				[23], [24], [25], [27], [28], [31]
A*0301	KTFPPTEPK	N				[24], [27], [29], [36]
A*1101	ATEGALNTPK	N				[22], [27], [37]
A*1101	KTFPPTEPK	N				[24], [27], [36]
B*0702	KPRQKRTAT	N				[23], [28], [37]
B*0702	SPRWYFYYL	N				[23], [24], [27], [28], [36], [38]
A*0101	FTSDYYQLY	ORF3a				[23], [24], [37], [27], [36], [38]
A*0201	LLYDANYFL	ORF3a				[23], [24], [27], [28]
A*2402	VYFLQSINF	ORF3a				[22], [24], [27], [34], [39]
A*0101	CTDDNALAYY	ORF1ab				[23], [27], [29]
A*0101	TTDPSFLGRY	ORF1ab		TTDLSFLGRY		[22], [27], [29], [38]
A*0201	ALWEIQQVV	ORF1ab				[24], [27], [34]
A*0301	KTIQPRVEK	ORF1ab				[24], [27]
A*0301	VVYRGTTTYK	ORF1ab				[23], [24]
A*1101	ASMPTTIAK	ORF1ab				[22], [23], [34]
A*2402	VYIGDPAQL	ORF1ab				[22], [24], [27], [39], [40]
B*0702	IPRRNVATL	ORF1ab				[23], [24], [27]

Table 3: Non-Spike-specific CD8+ T-cell epitopes in the SARS-CoV-2 Dextramer® Panels, and the conservation of these epitopes across the SARS-CoV-2 reference strain (NC_045512), Delta (B.1.617.2), and Omicron (B.1.1.529) variants. Green = no mutations in epitope, Red = mutations in epitope.

SARS-CoV-2 sequences used in multiple sequence alignment

Protein	T cells	Reference strain	Delta	Omicron
Spike	CD8+	17/17 (100%)	15/17 (88%)	14/17 (82%)
Non-Spike	CD8+	17/17 (100%)	16/17 (94%)	17/17 (100%)
Spike	CD4+	17/17 (100%)	15/17 (88%)	13/17 (76%)
Non-Spike	CD4+	12/12 (100%)	12/12 (100%)	12/12 (100%)

Table 4: Conservation of selected CD8+ and CD4+ T-cell epitopes across the SARS-CoV-2 reference strain (NC_045512), Delta (B.1.617.2) and Omicron (B.1.1.529) variants.

Discussion

Population stratification based on cellular immune monitoring

Monitoring SARS-CoV-2-specific T cells using technologies such as the Dextramer® Panel opens the possibility to use long-term immune monitoring for population stratification. This would enable the identification of individuals which show low levels of memory T cells potentially indicating low or declining immunity, and suggesting the need for booster vaccination [**Fig. 6**].

Intelligent monitoring of long-term cellular immunity

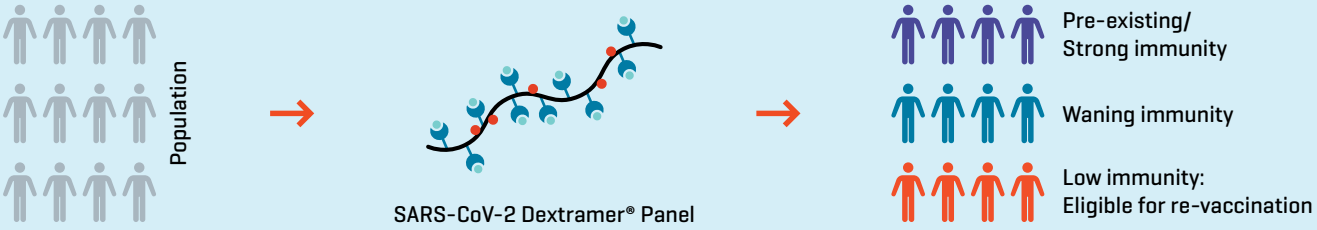


Fig. 6: Proposed model for intelligent monitoring of long-term cellular immunity using a SARS-CoV-2 Dextramer® Panel for population stratification based on SARS-CoV-2 immunity status or predicted disease prognosis. Grey color symbolizes unknown status of immunity in the population. Analysis of populations by Dextramer® Panel can reveal the immune status and stratify the population into sub-populations based on immunity towards SARS-CoV-2. Sub-populations showing low immunity are eligible for revaccination.

Impact of COVID-19 variants

The emergence of the highly transmissible SARS-CoV-2 B.1.1.529 (Omicron) variant has once again raised concerns about the effectiveness of vaccine-induced immunity. Omicron is recognized by Spike-specific CD4+ and CD8+ T cells induced by vaccines designed to the SARS-CoV-2 reference strain, demonstrating that the T cell response remains largely intact against Omicron[41,42], although there may be some reduced reactivity in some individuals[43]. Spike-specific T cells in vaccinated individuals were able to recognize Omicron more comprehensively compared with convalescent individuals[41].

This suggests that vaccine boosting may play an important role in generating protective cellular immunity in the context of emerging variants. When monitoring antigen-specific cellular immunity, it will be important to use accurate and robust techniques that take into consideration the different SARS-CoV-2 variants that are present and can be readily adapted as new variants emerge.

Future perspectives

We plan to analyze larger cohorts of vaccinated convalescent individuals in order to investigate the variation in durability and kinetics of T cell immunity between individuals.

Large-scale prospective studies monitoring both humoral and cellular immunity will be key to defining the correlates of long-term SARS-CoV-2 protection. Such studies will require precise measurements of antigen-specific T-cell responses, as well as in-depth functional phenotypic analysis.

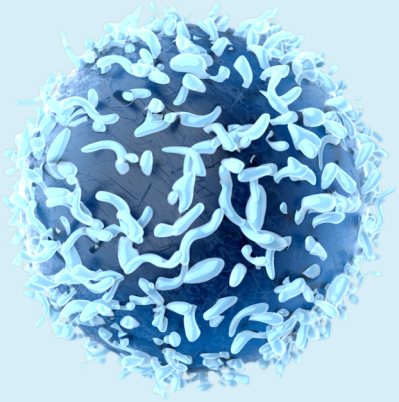
A detailed understanding of the long-term cellular immunity in COVID-19 is essential to guide future vaccination strategies and help to avoid a recurrence of the pandemic, as new variants emerge.

Conclusions

- We developed SARS-CoV-2 Dextramer® Panels to monitor the dynamics of SARS-CoV-2-specific CD8+ T cell responses over time.
- We analyzed PBMC samples collected from an individual pre-pandemic, post-infection and post-vaccination and demonstrated that Spike and Non-spike-specific CD8+ T-cell responses exhibited different patterns of magnitude & kinetics.
- SARS-CoV-2 Dextramer® panels can monitor long-term cellular immunity following infection or vaccination.
- SARS-CoV-2 T-cell epitopes included in the Dextramer® panels are largely conserved across Omicron and Delta, and new reagents can be rapidly developed for variants, supporting the utility of SARS-CoV-2 Dextramer® panels for immune monitoring across variants.
- This approach can be used to evaluate new vaccine candidates and support booster vaccination decision making by identifying sub-populations of individuals with low or declining immunity.

Resources

We are dedicated to helping you get the most out of your Dextramer® reagents by offering multiple helpful resources and support:



SARS-CoV-2 Dextramer® Panels

We provide Dextramer® reagents for the detection of SARS-CoV-2-specific CD8+ and CD4+ T cells by flow cytometry, *in-situ* staining, NGS, and single-cell multi-omics. Create your own SARS-CoV-2 Panel to monitor virus-specific T cells based on Immudex's curated epitope list.

Read more

Published COVID-19 T-Cell Epitopes

We want to share a curated list of validated epitopes shown to be recognized by T cells in COVID-19 patients.

Download the list (human)

Download the list (mouse)

Accelerate Vaccine Development

Explore how Dextramer® technology can support all phases of vaccine development from epitope discovery to monitoring antigen-specific T-cell responses in pre-clinical studies and clinical trials.

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