WHITE PAPER

# TargetScape®

## Identifying and Profiling the Cellular Immune Response

ImmunoScape

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T-cell recognition of antigens or peptides presented on major histocompatibility complex (pMHC) molecules occurs through T-cell receptors (TCRs). This pMHC–TCR interaction represents a critical step in the initiation of most adaptive immune responses. Still the complete understanding of human antigen-specific T-cell responses remains challenging, due to the many factors that influence the range and identity of the antigens targeted by T cells during infection and disease.[1]

System-level approaches to identify antigen-specific T cells are useful given the heterogeneity seen in the cellular profiles determined for each individual and the diverse responses observed between individuals.[2] Currently, high-dimensional flow cytometry, mass cytometry and various forms of single cell sequencing-based analysis methods are being widely adopted to expose the staggering heterogeneity of immune cells in many contexts.[3] In this white paper we will discuss the unique TargetScape technology offered by ImmunoScape® for identifying and profiling the cellular immune response.

## The Need for High-Dimensional Profiling

Immunotherapy modulates the immune response. To further develop these therapies for cancer, infectious diseases, autoimmune diseases, neurodegeneration and other disorders, a need exists for high-dimensional profiling to identify and better understand the role of the key effectors in many diseases, antigen-specific T cells (Figure 1). This knowledge can then be applied to answer a myriad of questions to design better clinical trials and to prioritize pipeline candidates.

High-dimensional, antigen-specific T-cell analysis can identify parameters that relate response to treatment leading to the identification of better biomarkers for patient stratification, thereby increasing the likelihood of targeting the correct patient cohorts during clinical trials. Additionally, it is impossible to clinically evaluate all potential immunotherapy combinations. Technologies that can identify surrogate markers of treatment efficacy, reading the effect of combination treatments in early clinical development, can provide information on prolonged responses and help design better combinations.

And, of course, the need to discover new therapeutic targets is pressing. These include

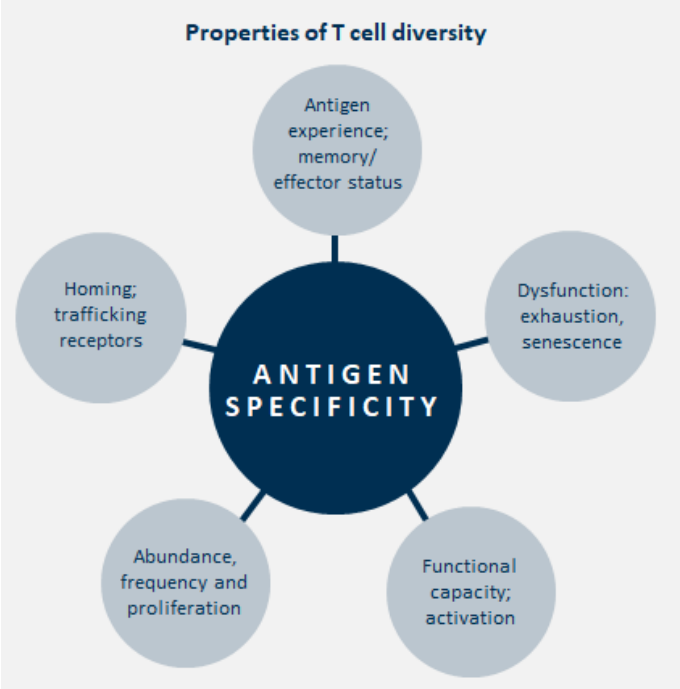


Figure 1 - Measurable categories of T-cell diversity in the context of antigen specificity. High-dimensional profiling can help identify and better understand this diversity within antigen-specific T cells, the key effectors in many diseases, and determine how their function changes during immunotherapy treatment.

"I consider ImmunoScape a strong partner for understanding immune response. I am impressed with the data from their high throughput screening of antigen specificity." Mahesh Yadav, Ph.D., Genentech

antigen targets for vaccines or novel druggable immune molecules that may be interesting to target in patients who do not respond to current therapies.

In recent years, high-dimensional profiling of antigen-specific T cells using the TargetScape technology has provided deeper insight into their role in disease. A 2017 study demonstrated that the TargetScape assay is a powerful tool for the detection and profiling of tumor-mutation-derived neoantigen-specific T cells that can provide deeper insight into the nature of tumor-specific T cells and the effects of cancer immunotherapy.[4]

Another investigation showed that human lung and colorectal cancer CD8+ TILs (tumor infiltrating lymphocytes) can not only be specific for tumor antigens, such as neoantigens, but also recognize a wide range of epitopes unrelated to cancer, such as those from Epstein–Barr virus, human cytomegalovirus or influenza virus. The study results demonstrated that not all TILs are specific for tumor antigens, and suggest that measuring CD39 expression could be a straightforward way to quantify or isolate tumor-specific T cells.[5]

**TargetScape Development**

In 2009, a method was published that allowed the simultaneous use of large numbers of different pMHC tetramers on small numbers of cells using standard flow cytometry and reagents. The method made use of fluorescently-tagged streptavidin backbones to create pMHC tetramers that utilized all possible combinations of fluorophores. [6]

Due to the limitations of flow cytometry a decade ago and the crosstalk between the limited number of available fluorophores, the tetramer multiplexing method was extended to a mass cytometry platform (CyTOF®) to enable broader and deeper profiling of antigen-specific T cells.[7,8] Multiplexing also solved the background noise and non-specific tetramer staining resulting from only using one channel. When looking for cells that are stained with unique combinations of heavy metals, the chances of non-specific detection decrease exponentially as the number of channels dedicated to a given antigen specificity increases.

Figure 2 - ImmunoScape co-founders. From right to left, Evan Newell, Ph.D., Choon-Peng Ng, Alessandra Nardin, DVM, and Michael Fehlings, Ph.D.

Using ten metal labels with combinatorial pMHC-tetramer staining in unique combinations of three labels per MHC multimer, 120 antigen specificities can be screened per sample, with a substantial number of labels, i.e. 30 or more, still left available for parallel phenotyping and functional analysis of the T cells.[1]

The tetramer-multiplexing technology was further developed at Singapore Immunology Network (SIgN), established in 2006 by the Agency for Science, Technology and Research (A*STAR), to include algorithms and scripts for automatic experimental set-up and data analysis. In 2016, ImmunoScape spun out the technology as the TargetScape platform and started operations with a unique focus on identifying and profiling antigen-specific T cells (Figure 2). The company was global from day 1; Genentech was the first collaborator.

**An Ex Vivo Approach**

"Tetramers allow you to look at the profile or characteristics of the antigen-specific T cells ex vivo without manipulating them. Other assays require cell stimulation that perturbs the cells and changes their profile," says Evan Newell, Ph.D., Associate Member of the Vaccine and Infectious Disease Division at Fred Hutchinson Cancer Research Center, and a co-founder and Scientific Advisor to ImmunoScape.

"Characterizing cells ex vivo is an advantage. Tetramer staining does not perturb the cells. They are stained for a very short period of time at low temperature so they retain their phenotypes and can even be completely dysfunctional. They do not have to be able to do something in response to antigen; they are detected through their binding characteristics."

Detecting and profiling cells in this meaningful way can lead to lower candidate attrition rate in clinical development, mitigate clinical trial failure risk, support overall drug development strategy by providing mechanistic understandings and replenish a drug pipeline with new targets and drug candidates.

**A Tailored Approach**
For high-dimensional analysis of antigen-specific T cells, flow and mass cytometry are complementary technologies. Depending on the scientific question and number and type of available samples, one approach may have advantages over the other.

Flow cytometry is about 10 times faster than mass cytometry (CyTOF) and more efficient in terms of recovery of the cells; mass cytometry needs a larger number of cells. If only the T-cell target is of interest and not the phenotypic characteristics of the T cells, or if cell numbers are low, then flow cytometry might be more suitable. Flow cytometry also allows downstream applications, such as cell sorting; cells are destroyed during mass cytometry.

Multiplexing is well established on CyTOF. Yet today with the advent of spectral flow instruments and the advances in fluorochrome technology the ability to multiplex has increased. Although still not at the level of mass cytometry due to unpredictable non-specific staining and crosstalk between channels, flow cytometry provides a certain amount of parallel profiling.

"The channel separation in flow cytometry is not as clean as it is for mass cytometry. When you look for very rare cells, like antigen-specific T cells, accurate detection depends on the number of parameters that are being measured," says Dr. Fehlings. "We have a platform that allows us to do this by flow cytometry in addition to our well established CyTOF platform. There are reasons that speak for one or the other approach and we carefully evaluate these for each project, including sample availability, time, the requirement for downstream applications, cost, etc."

"Mass cytometry offers a decent throughput while assessing a high number of parameters, which cannot be achieved by flow cytometry. Flow cytometry is the highest throughput methodology and now with the new instruments and technologies you can assess a larger number of parameters, 20 to 30, which was not imaginable a couple of years ago," says Michael Fehlings, Ph.D., co-founder and Director, Scientific Affairs, ImmunoScape.

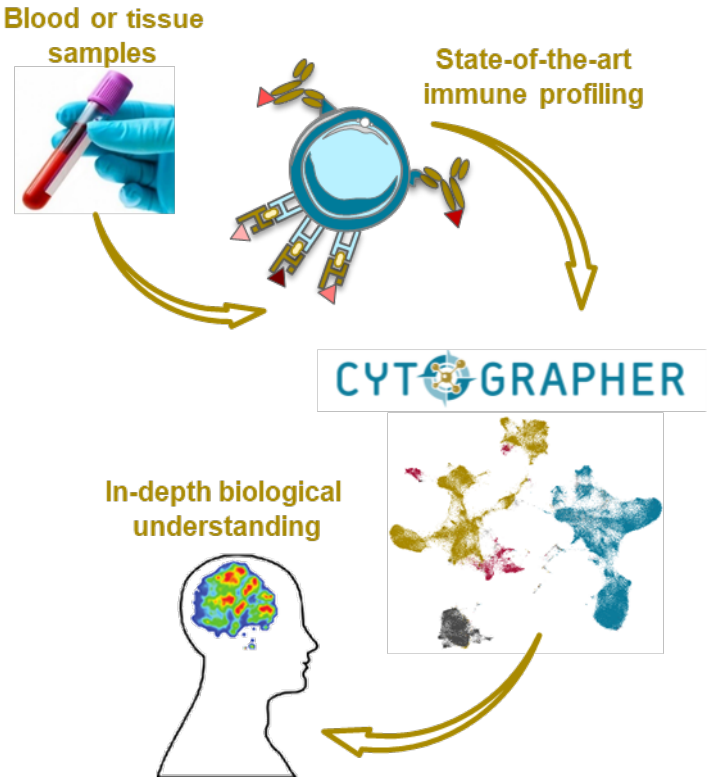


Figure 3 - ImmunoScape T-cell profiling workflow: from sample processing to immunological interpretation of data. Clinical samples are used for high-dimensional profiling then data analyzed, visualized and interpreted by a team of immunologists to provide deep biological understanding of T-cell responses.

"For example, for very large number of patient samples CyTOF would be more expensive because of the low acquisition rate. The speed of flow cytometry is an advantage but the smaller amount of multiplexing allows a less deep characterization of the detected cells limiting the range of candidate antigens that can be screened."

**A Common Question**

Clinical samples are precious and often scarce, and since they are frozen quality can be sometimes affected. The number of cells needed for the TargetScape assay is a simple number game. Antigen-specific T cells are rare cells that are typically between 0.001 to 1% of the CD8+ T cell population, itself a subset of circulating or tissue immune cells. If there is 1 cell of interest in 100 cells then when you acquire 1000 total cells you can acquire 10 cells of interest. But if you only have 80 cells then the one cell of interest will not be found. Generally ImmunoScape works with a few million total immune cells; the more the better due to the increase in sensitivity (Figure 3).

**Data Analysis**

High-dimensional antigen-specific T cell profiling delivers a lot of data. In a standard analytical workflow the raw data are preprocessed and normalized to select for good events. Then the multiplex tetramer staining is deconvoluted to identify the antigen-specific T-cell populations, followed by a deep analysis of the cell phenotypes.

A proprietary HitEC scoring system determines an aggregate score that quantifies the confidence level of any hit. "As the technology was developed, it was formalized. ImmunoScape has made a science out of using tetramers to detect antigen-specific

Figure 4 - UMAP dimensionality reduction showing T-cell subsets. The data analysis is tailored to apply the right approach based on the biological questions. High-dimensional reduction tools, high-dimensional cluster analysis or discriminant analysis methods may be used.

T cells in contrast to the typical use which involves a qualitative decision by sight," says Dr. Newell.

High-dimensional reduction tools, such as t-SNE, UMAP or oneSENSE, are used to condense the data to two dimensions for visualization; a perspective that is needed when working with 40 plus original dimensions (Figure 4). High-dimensional cluster analysis can be performed and the clusters superimposed to compare characteristics, sample compositions and phenotypes from different patients and/or time points. Cluster visualization tools can indicate if different clusters are enriched or diminished in specific samples, especially those from patients who have undergone therapy. Individual clusters can be further processed to identify the markers that describe the cells.

Discriminant analysis methods are also being adapted to compare samples to find distinguishing characteristics. The analytic approach ultimately depends on the biological question.

"The analysis is tailored to apply the right approach based on the question. We help formulate the questions and design the experiment to frame it for analysis. Taking all project-specific parameters into account we make statistical comparisons and correlate the study's findings with the clinical parameters to develop a hypothesis or conclusion depending on the sample size. As immunologists we do an interpretation that makes sense," states Dr. Fehlings.

**Customization**

The TargetScape assay is well developed, the work flow and quality control optimized. For instance, the proper mixing of the streptavidins is controlled by a robotic system using proprietary software to ensure reproducibility.

The assay can screen for antigen-specific T cells using several hundred candidate antigens simultaneously. This takes advantage of the sample; every cell is stained with all of the antigens. Not only can cells be screened to find potential targets but also the cells that might be reactive with the perspective targets can be phenotypically characterized (Figure 5).

"Everyone is interested in finding the T-cell target but to what extent depends on the project specifics and the available sample material," explains Dr. Fehlings.

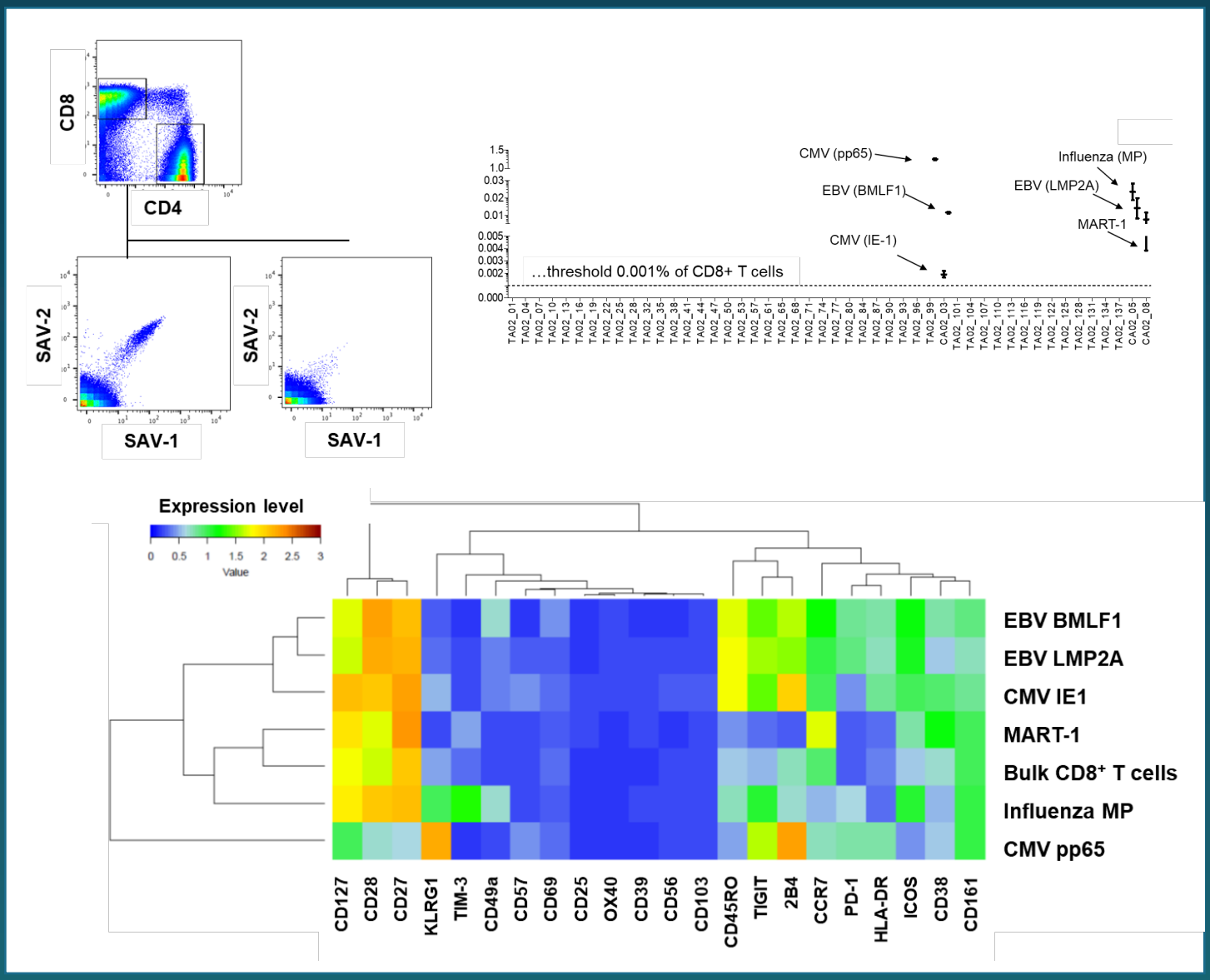


Figure 5 - The TargetScape assay provides sensitive identification and deep profiling of antigen-specific T cells. After T-cell subsets are gated within the sample (top left), rare T cells reactive against viral or tumor targets are accurately identified using proprietary algorithms (top right). Properties and functions of the viral and tumor-specific T cells can then be compared across antigen specificities and also in longitudinal samples, to assess the effect of treatment (bottom).

"The sample may already come together with information retrieved from predictive genomic or bioinformatics approaches, and just needs to be screened. Or we may help leverage the information and tools to predict targets, or perform literature searches and use our immunological expertise to identify targets that would make sense to screen in the context of a certain disease. In all cases, the TargetScape assay uses the information of the different alleles of the population."

"We are very deeply involved in the panel design and panels can be uniquely customized. If a marker is not in our standard repertoire we develop and validate it then add to our library. Our goal is to provide our collaborators with a deeper immunological understanding of what their specific compound or drug might do to the immune system."

**Summary**

The TargetScape assay is a unique proven technology utilizing tetramers to identify and profile antigen-specific T cells. Detecting and phenotypically characterizing cells in this meaningful way can lead to lower candidate attrition rate in clinical development, mitigate clinical trial failure risk, support overall drug development strategy by providing mechanistic understandings and replenish a drug pipeline with new targets and drug candidates.

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