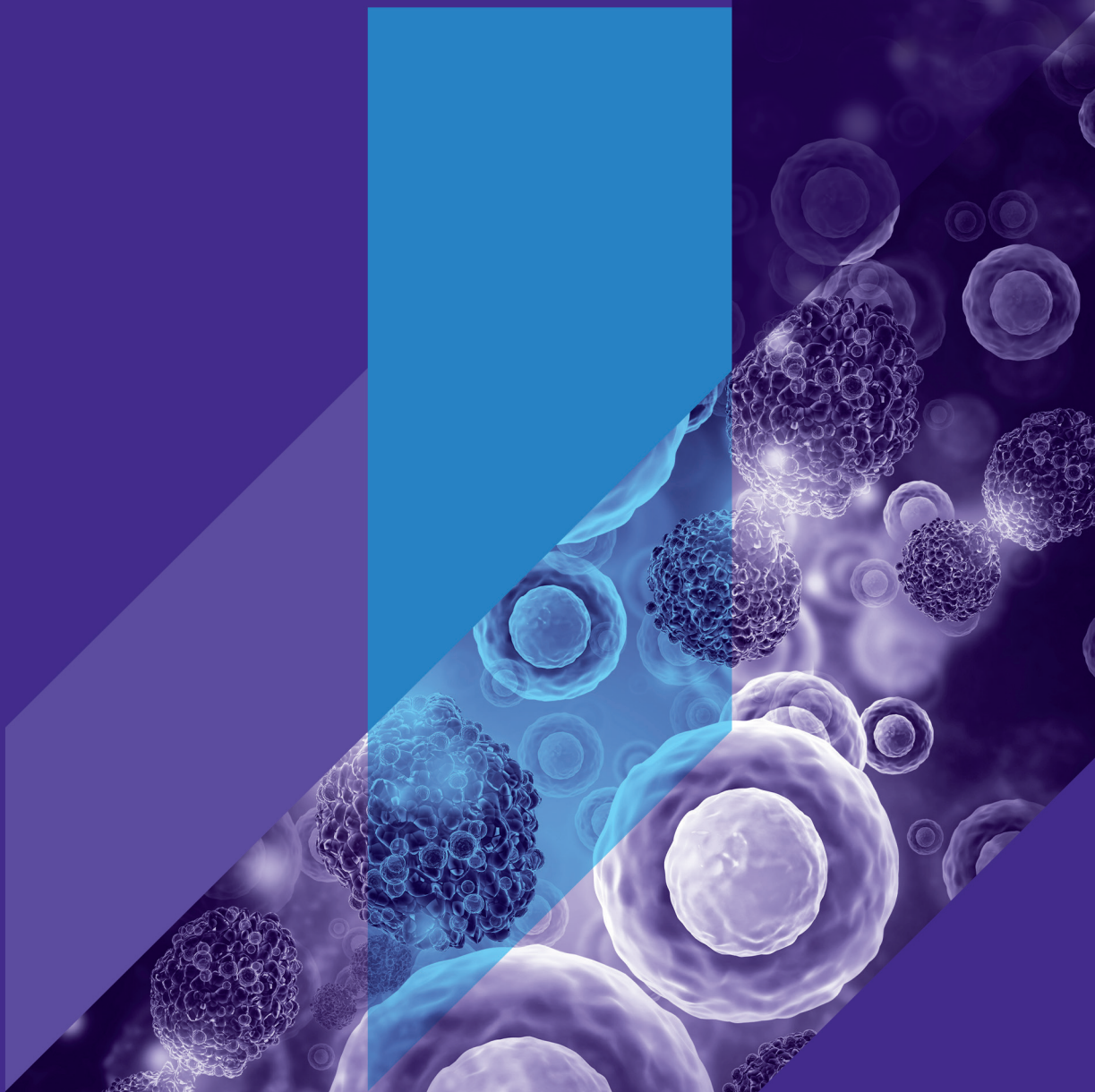
Ardigen

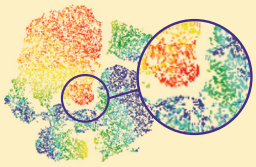
# TCR suite of computational tools



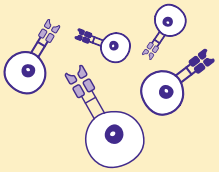
# From concept to TCR discovery
## design → explore → select → optimize

**DESIGN**

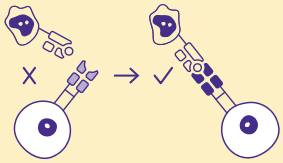
**EXPLORE**



**SELECT**



**OPTIMIZE**



## From highly diverse T-cell repertoire to target-specific T-cell receptors (TCRs)

T-cells are an essential component of adaptive immunity that **recognize aberrant and foreign antigens** through highly differentiating surface receptors, or TCRs. These receptors can bind some of short peptides processed inside the host cell and displayed on its surface via HLA-I or HLA-II molecules[1]. After recognizing peptide from pHLA complex as abnormal, T-cells can **trigger an immunological response** that eliminates pathogens or leads to the eradication of whole tumor cells. However, the targeted pHLA must have **adequate binding strength and sufficient interaction duration** for the T-cell activation to be successful.

To tackle the enormous variety of TCRs, Ardigen offers its expertise in experimental design and advanced bioinformatics analysis. Our interdisciplinary team of immunologists, bioinformaticians, and data scientists can create solutions tailored to your needs. We are now working on **Ardigen's TCRopt module, an optimization module for TCRs**, that maximizes the therapeutic potential of previously found hits.

**By working with us, you can lower the risk of off-target toxicities while boosting the likelihood of a successful treatment outcome.**

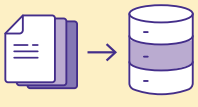
## Design the best experimental strategy to reach your goals

Reliable experiments start with optimal design. Use our expertise and design your experimental strategy starting from scratch and finishing on advanced bioinformatic analysis.

**INPUT**
- clinical data
- healthy donors
- animal models

**TREATMENT**
- stimulation
- pHLA multimers
- longitudinal data

**DATA COLLECTION**
- bulk
- single-cell[2]
- VDJ-enriched
- multi-omic



### Applications

- Monitoring of the response to therapy.
- Development of TCR-based therapies.
- Identification of antigen-binding TCRs.
- Comprehensive phenotyping of T-cells.

[1] Learn more about **Ardigen's ARDisplay models** that outperform standard *in silico* approaches over twofold in Average Precision.
[2] Different platforms e.g. 10x Genomics, BD Rhapsody, plate-based, split-pool barcoding, VASA-seq.

# Explore TCR clonotypes and get full insight into your data

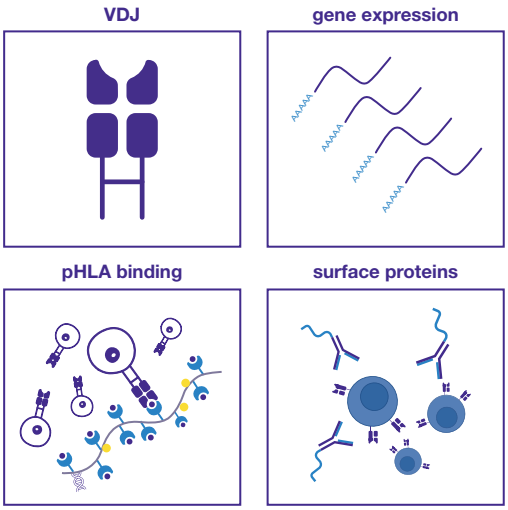
**At Ardigen, we aim to derive comprehensive insights from the data we obtain. Our team provides specialized TCR analysis for exploratory purposes, among others:**

- Reconstruction of full-length TCRs.
- Identification of shared and expanded clonotypes.
- Monitoring clonotypes at different timepoints.

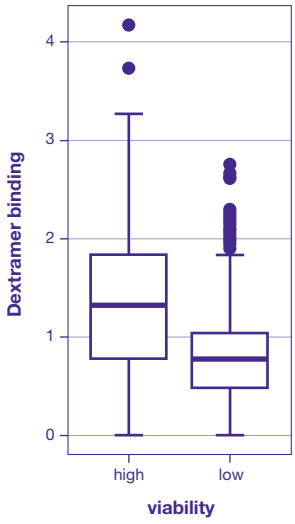
**We utilize single-cell sequencing and multi-modal data, and conduct integration studies that go beyond the TCR repertoire's primary analysis.**

- Assess the binding of your candidate targets.
- Using surface protein levels and transcriptomic profiles to perform phenotyping.
- Confirm the condition of the cell (activation, exhaustion, etc.) using transcriptome and surface markers.

## Multi-modal view at T-cells at the single-cell level




## The binding of the TCR cognate antigen decreases with cell viability



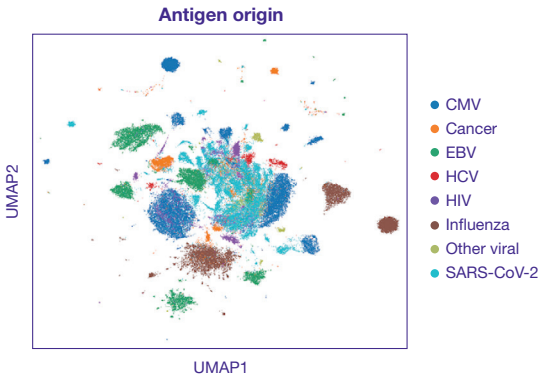

Lowering the binding capacity in dying cells might lead to false negative results.

Exclude poor-quality cells from your single-cell sequencing data using the **gene expression module**.

**Cell viability** is estimated based on the fraction of mitochondrial genes overexpressed in dying cells.

## Target antigen origin affects the TCR repertoire

TCRs demonstrated to bind a given cognate antigen tend to group by the antigen origin. Explore the **TCR repertoire diversity** specifically for the given antigen and its origin.




The UMAP embedding of TCR representations (~70k observations) was obtained from a deep-learning model. All TCRs are experimentally shown to bind specific antigens. Different colors represent various origin groups.

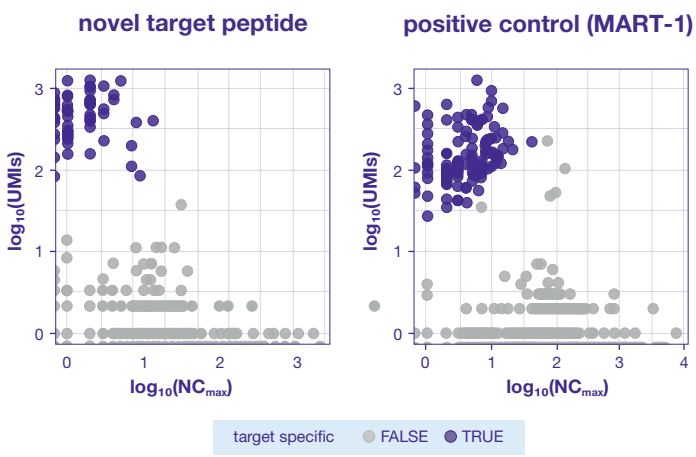
## Did you know...?

Activated T-cells show higher avidity due to the membrane reorganization of TCRs, whereas memory T-cells exhibit higher average affinity compared with naïve T-cells. Perform immuno-phenotyping with the **surface protein module** to delineate the cell type specific effects.

# Reveal complex multi-modal data to select the best TCR candidate

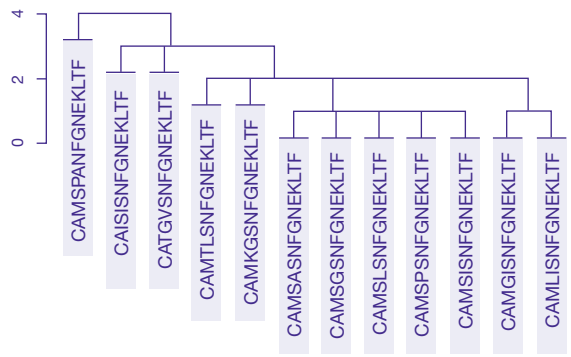
## Detect TCRs against novel tumor targets in naïve repertoires

The study included CD8+ T-cells derived from 13 healthy donors. Those T-cells were stained with 35 peptide:HLA (pHLA) complexes on a dextran backbone. Peptides were derived from tumor-associated (TAAs) and tumor-specific antigens (TSAs), and tested in a complex with 4 common HLA class I alleles.




The scatterplots showing relation of number of unique molecular identifier (UMI) associated with the target dextramer (UMI_count) andthe dextramer of the negative control (max. of any of the 4 negative controls - NC_max). Each dot represents a single cell. UMI threshold for TRUE T-cells that specifically bound target pHLA were identified based on UMI_count >15, and UMI count > 5 x NC_max.

**High homology among rare clonotypes of the antigen-binding TCRs**




Naïve repertoires contain a large number of rare clonotypes that may show specificity toward a selected target. As a verification step of binding specificity, we determine high-homology TCR clusters (CDR3 regions identical in at least 80%). The plot above shows a representative cluster and Levenshtein distance between individual CDR3 sequences.

## pHLA:TCR binding at the single-cell level

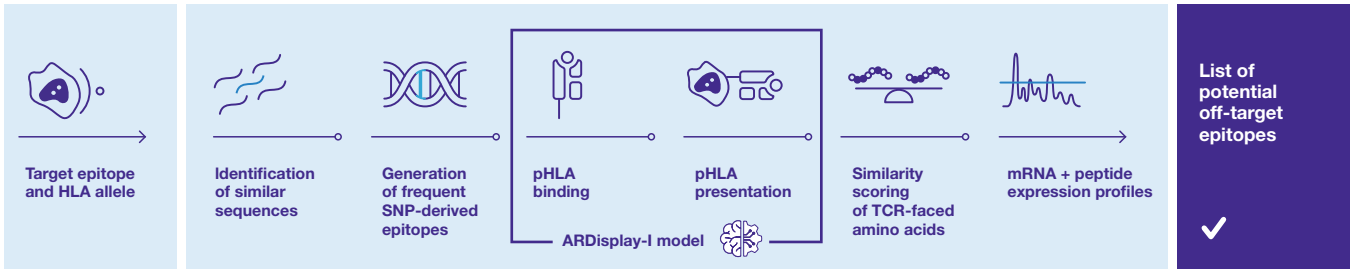
- pHLA:TCR pair identification success rate: 83%
- Tens to hundreds of full-length TCR sequences per donor
- High sensitivity enabling identification of rare clonotypes
- Identification of high-homology rare clonotypes

## Choose targets with low risk of side effects pinpointed by Ardigen's ARDitox platform

ARDitox platform

**Off-target toxicity**

Identify potential **off-target toxicities in cancer immunotherapies with Artificial Inteligence** to improve safety, and speed up therapy development



# Boost T-cell activity maintaining low toxicity

## Time is all you need, did you know...

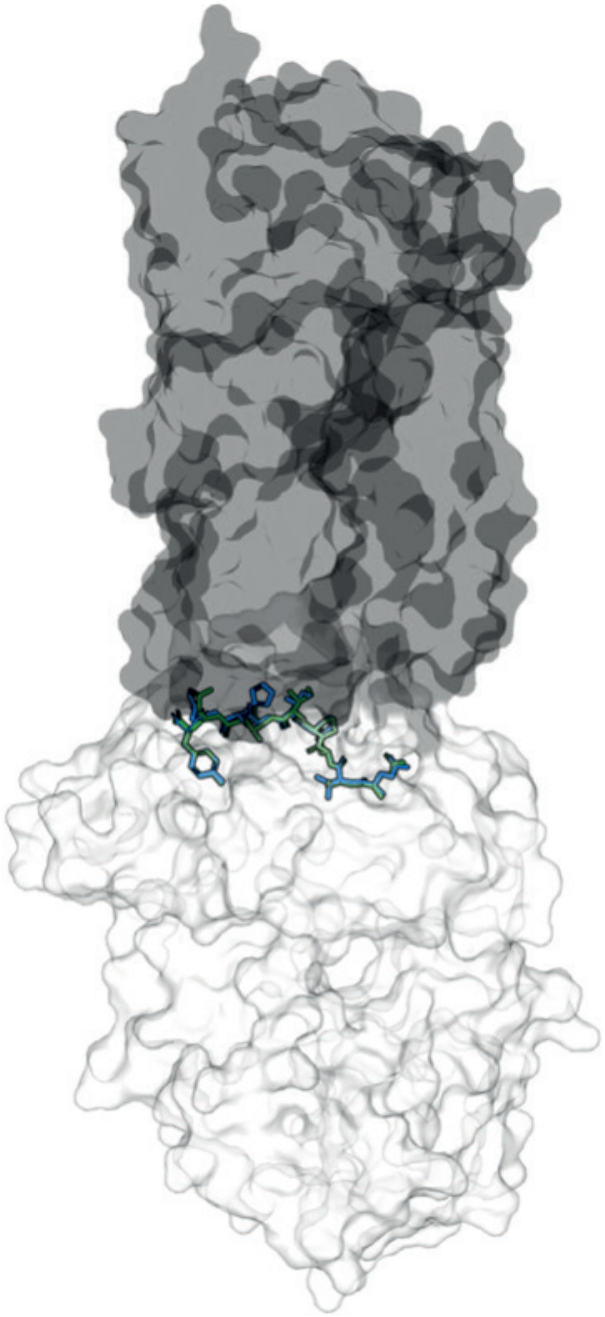
- pHLA-specific TCRs are often inactive.
- Increased pHLA:TCR binding affinity often results in dangerous cross-reactivities.
- Interaction between pHLA & TCR sustained over time drives T-cell activation.

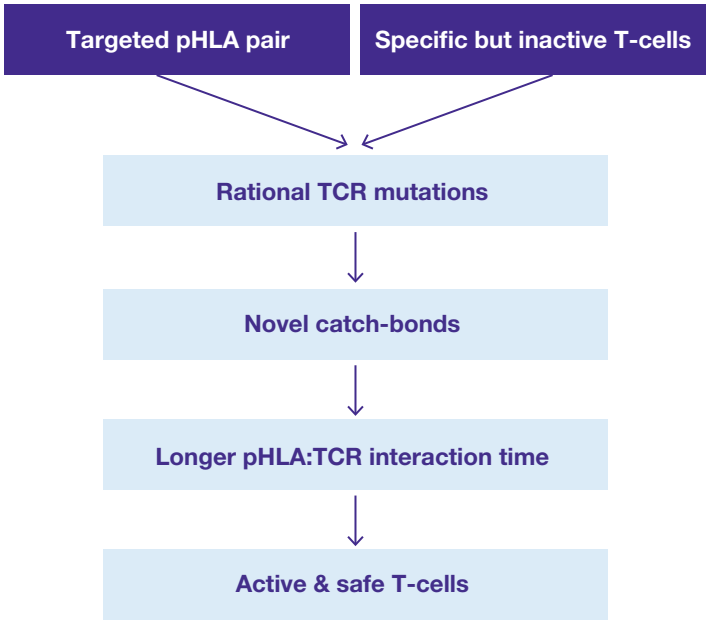
Catch bonds are **non-covalent interactions** whose lifetime increases with an external force applied to the whole pHLA:TCR complex. Typically, catch bonds **do not change the binding affinity** between TCR and its target and can lead to a higher level of T-cell activation by **increased binding avidity**.

## How can you increase the interaction time with Ardigen's TCRopt module?

- Predict an exact **structure of pHLA:TCR complex** to feature correct interactions between TCR and pHLA.
- Optimize T-cells activation by **introducing modifications in TCR sequences** that result in the presence of additional catch bonds.
- Analyze interactions between TCR and cognate pHLA complex **at the atomic level** for a rational design of mutations that induce the formation of new catch bonds.

**With laboratory experiments and in silico simulations, verify the mutations proposed by Ardigen's TCRopt module.**





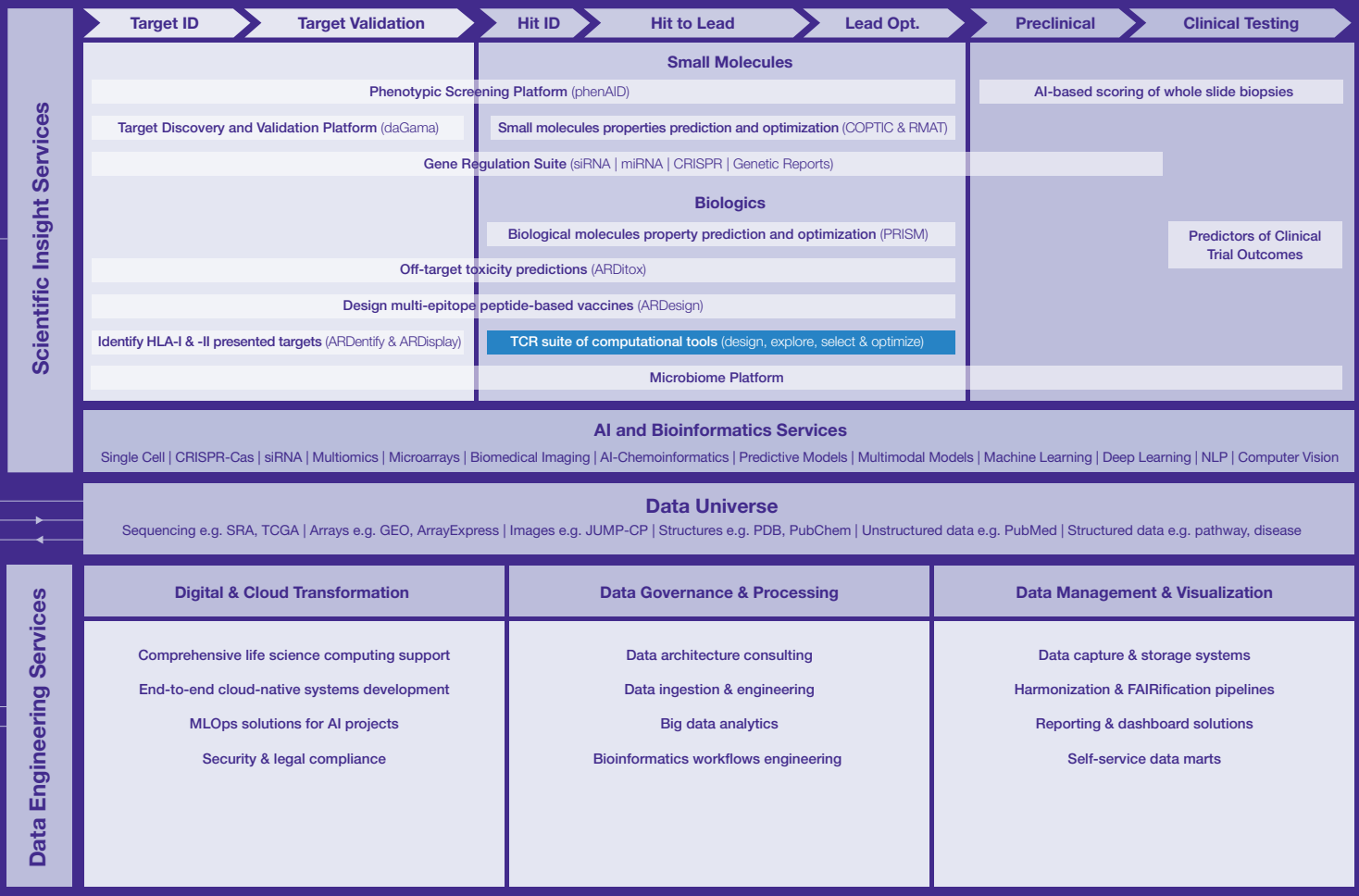

## How can we help?

- We provide solutions for the **prediction of pHLA:TCR structures** at experimental accuracy, based solely on the amino-acid sequences.
- We propose initial **mutations to introduce novel catch bonds** to your pHLA:TCR and potentially lead to a higher T-cells activity.
- We specify, rank, and confirm which of the mutations are most promising with our **in-house optimized *in silico* simulations**.
- We prepare a **detailed plan of AI-lab loop experiments** that further determine the best mutations.

Ardigen

# Artificial Intelligence & Bioinformatics for Precision Medicine

## Discover Our Cutting-Edge Services and Accelerate Your Drug Discovery Process





**United States**

**West Coast**
611 Gateway Boulevard South
San Francisco, CA 94080
+1 (628) 200 09 14

**East Coast**
One Broadway, 14th Floor
Cambridge, MA 02142
+1 (628) 200 09 14

**Europe**

Podole 76
30-394 Kraków, Poland
+48 12 340 94 94

**Contact us**

Agnieszka Blum, PhD MD
agnieszka.blum@ardigen.com

Maciej Jasiński, PhD
maciej.jasinski@ardigen.com


ardigen.com

Scan to get to know us better: