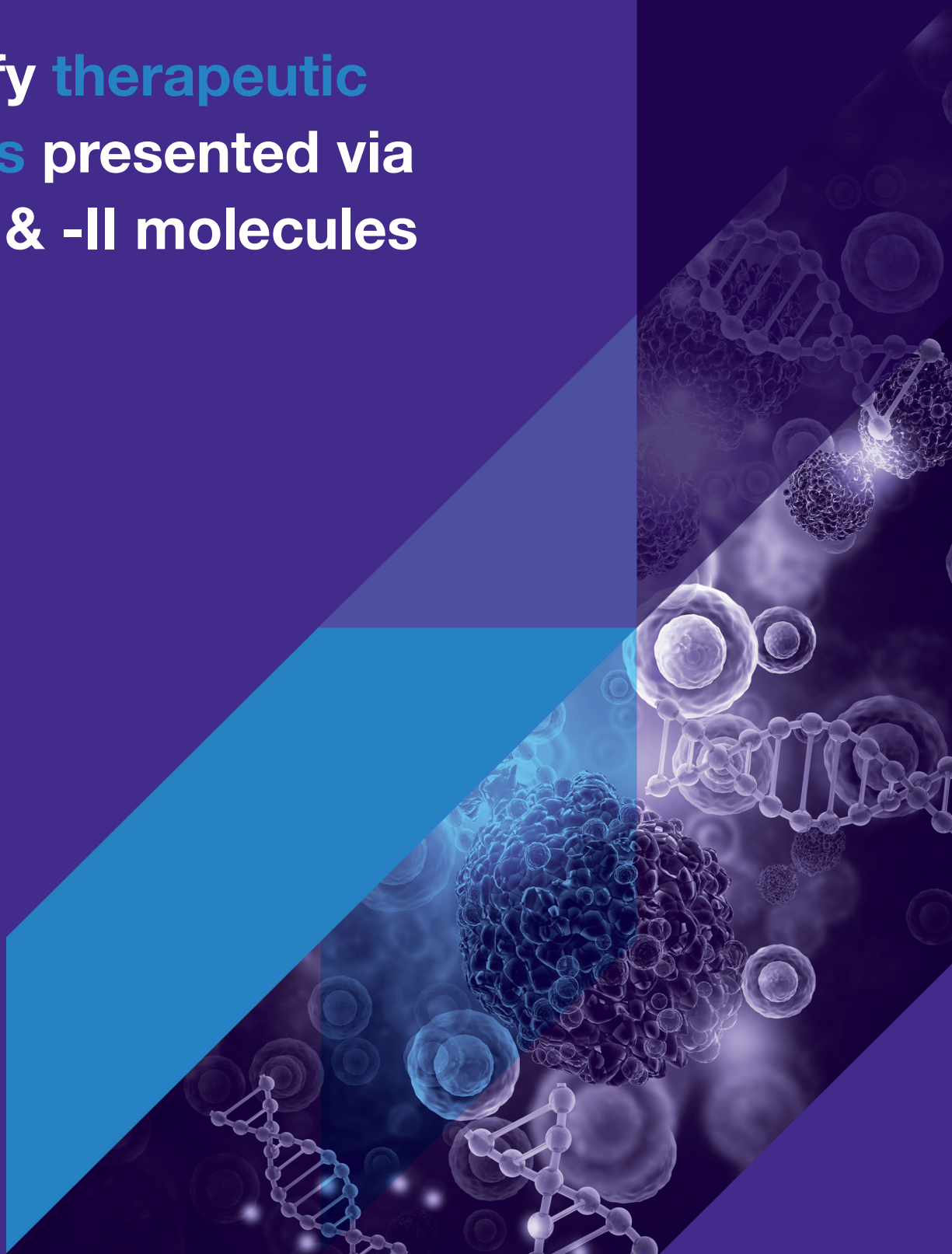
Ardigen

Identify therapeutic targets presented via HLA-I & -II molecules

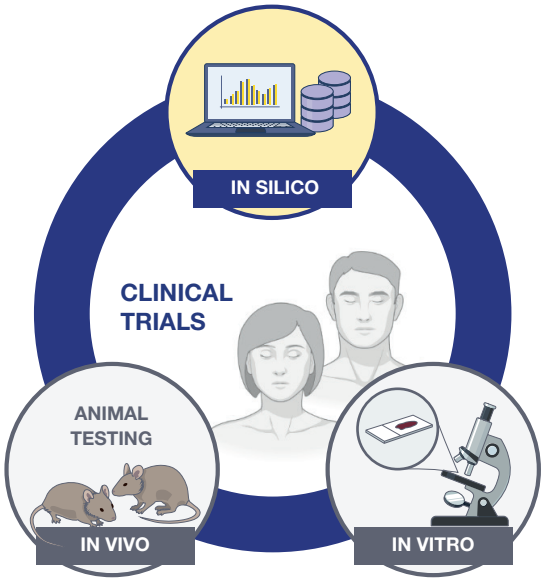


Identify the right therapeutic targets presented via HLA-I & -II molecules

Enhance your experimental approach with high accuracy *in silico* predictions to select the right target epitopes

Whether you are developing **gene and cell therapies** or aiming to **stimulate a patient's immune system**, you need to be sure that your therapeutics will have a chance to reach the selected target and will not affect healthy tissues.

Combine experimental methods with Ardigen's ARDentify platform - a computational approach to increase your chance of success.



Work with us to overcome the following challenges

Function-based screening assays tend to depict **limited fragments of biology**, thus bypassing other events like antigen processing and presentation or T-cell activation.	In some cases, affinity-based methods yield **high-affinity epitopes that are non-immunogenic** while omitting immunogenic ones with mild-affinity.	Pooling strategies for laboratory testing increase the peptide search space but have to undergo **labor-intensive deconvolution.**
Synthesis of pHLA multimers is often **restricted to peptides predicted as strong binders** (based on standard tools like MHCflurry or netMHCpan) and can lead to omitting valuable targets.	Selecting promising binders (short peptides) out of genetically-encoded longer sequences is **labor-intensive** because they need to be sequenced.	Adverse toxic effects of the therapy might remain **undetected in the *in vitro* testing phase** because they are caused by off-target proteins expressed only in living tissues.
Time-consuming validation of epitope targets that are not even presented on the cell surface via HLA molecules.	Epitopes displayed on the cell surface **lack immunogenicity**.	Laboratory tests may lead to **inconsistent results**, e.g. mass-spectrometry tests repeated on the same samples reach reproducibility of 60%.

Did you know…?

As many as 70% of first-in-class drugs, which target an until-then unknown target or biological pathway, are identified through a target-based drug discovery strategy. At the same time, inappropriate target selection is often indicated as a **major cause of experimental drug failures.**

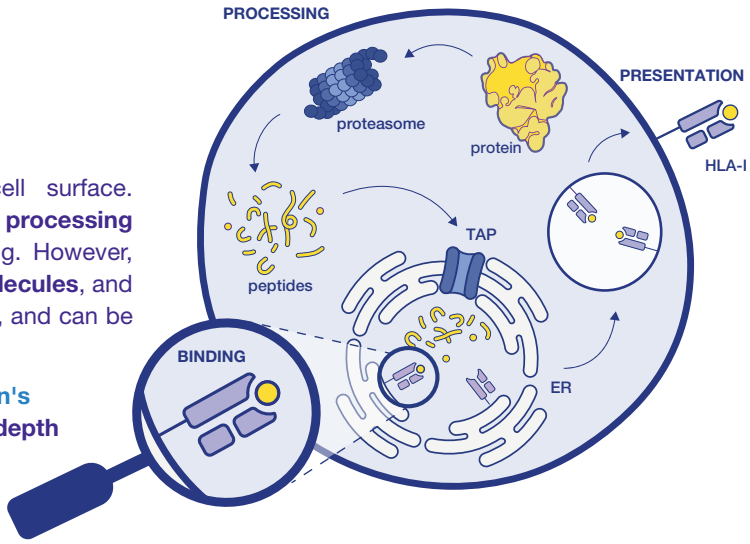
Before you invest additional resources in a target, it is important to provide as much evidence as possible in support of your choice, which can be approached by **experimental methods or computational inference**. Often a combination of those approaches is required to fully identify and understand the mechanisms of on-target and off-target effects.

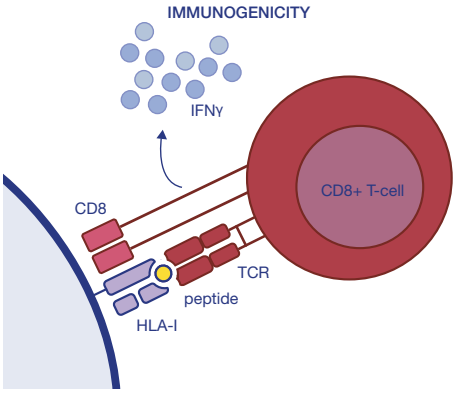
Identify the right intracellular protein targets by modeling the HLA-I presentation pathway

Identify peptide sequences that have a potential to trigger an immune system response

Almost every self-protein is represented on the cell surface. Endogenous peptides coming from **proteolytic processing & degradation** can escape further lysosomal processing. However, only a tiny fraction of protein fragments **binds to HLA molecules**, and an even smaller subset is **presented on the cell surface**, and can be reached by T-cells.

Enable the detection of such peptides by using Ardigen's ARDisplay-I model - our methodology based on an in-depth understanding of the antigen processing and presentation pathways.





Check how we stand out from other solutions

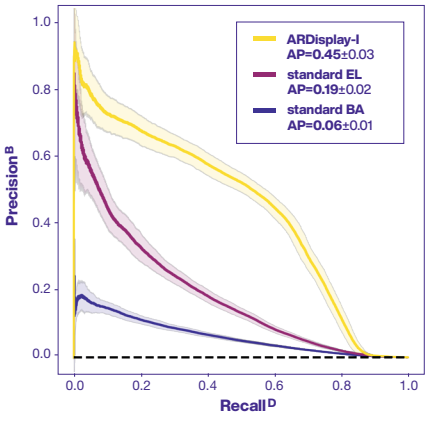
Integrating data from various sources is essential to achieving accurate predictions and high-performance machine learning models.

- We combine non-presented artificially-generated samples, results from binding affinity (BA) assays, and information about mass spectrometry eluted ligands (MS EL).
- We incorporate MS EL from **multiple high-quality sources** (with over 2M unique peptides presented via 182 HLA alleles) **collaborating with an academic expert in immuno-peptidomics**.
- Our data collection, comprised of single-allelic and multi-allelic samples, originates from **EBV-transformed cell lines, cancer patients, and healthy donors**.

Additionally, we provide a **customized approach to data processing** that includes filtering of positive observations, hard-examples mining, and biologically-aware generation of negative examples.

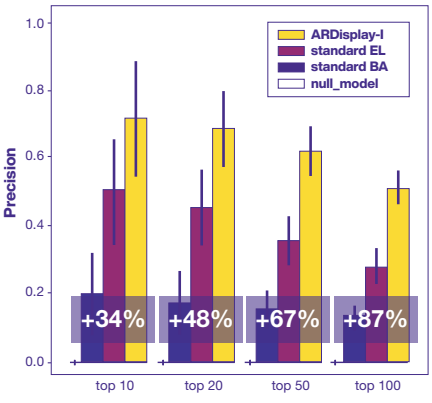
Ardigen's ARDisplay-I model enables prediction of HLA-I presented peptides with over 2 times higher Average Precision[A] than standard solutions[1]

The study cohort[2] includes 22 patients with CRC (colorectal cancer, adenocarcinomas) and represents a wide range of HLA alleles. It is comprised of 49 distinct alleles (HLA-A: 13, B: 20, C: 16), and almost 90% of the world population[3] has at least 3 HLA alleles in common with these patients.



Comparison of precision-recall (PR) curves[C]

Our model achieves higher results at each point of the PR curves. The regions with standard deviation do not overlap, which indicates a high statistical significance of the performance difference between the methods.



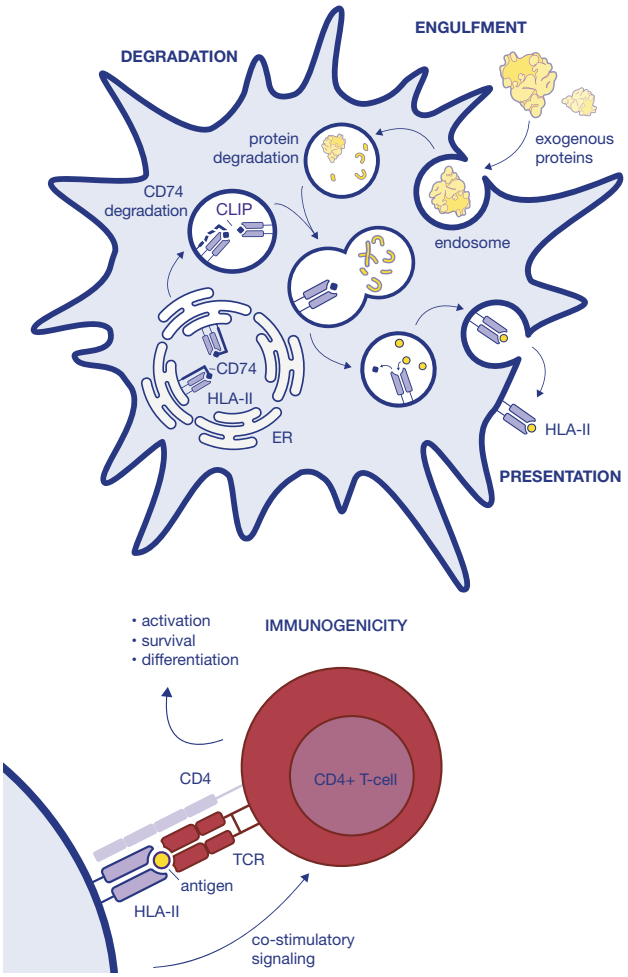
Positive predictive values (also PPV)[B] with four selected thresholds, i.e., top-10, 20, 50, & 100 pHLA pairs selected by each method. For example, the score for PPV at top-10 describes what fraction of hits can be expected by testing in the laboratory 10 pHLA pairs with the highest rank from each method.

Identify the right extracellular protein targets by modeling the HLA-II presentation pathway

Consider the presentation pathway of HLA-II restricted peptides in the development of cancer therapies

Important role of CD4+ T-cells...

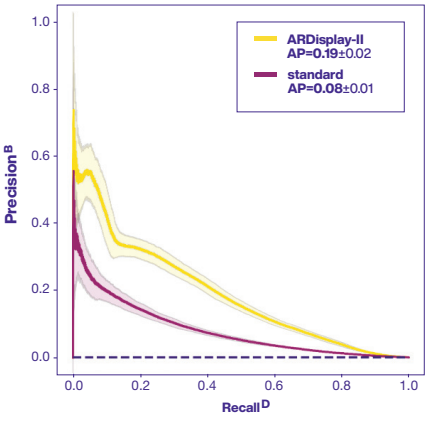
- **Tumor eradication** depends on the proper activation of both CD8+ and CD4+ T-cells.
- Anti-tumor CD4+ T-cells can directly eliminate tumor cells and **orchestrate local immune responses**, supporting the activity of other cells.
- When only CD8+ are activated, **escape of the immune response** by the tumor is more common than when both CD8+ and CD4+ are activated. Moreover, some cancer types (for instance, CRC) tend to **downregulate HLA-I expression** while upregulating it for HLA-II.
- The structure of HLA-II allows it to bind **longer and more diversified peptides** than HLA-I.
- The lack of immunogenic HLA-II antigens may cause **unresponsiveness to immunotherapy** in patients with immunogenic HLA-I antigens.



Ardigen's ARDisplay-II model enables prediction of HLA-II presented peptides with over 2 times higher Average Precision[A] than standard solutions[4]

The study cohort[5] includes patients with GBM (glioblastoma) and induced presentation.

The dataset is comprised of 17 distinct alpha & beta chains of corresponding HLA class II canonical alleles (loci DQ, DP, and DR). The population coverage[3] shows that the study cohort is representative and shares at least three alpha/beta chains with almost 80% of the worldwide population.



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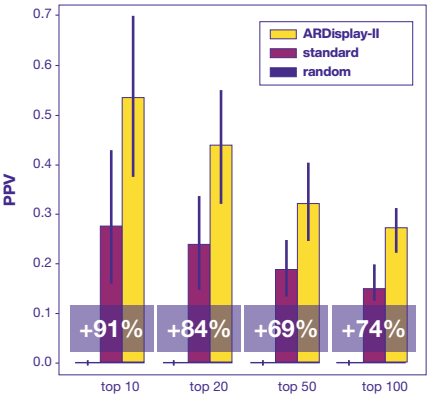
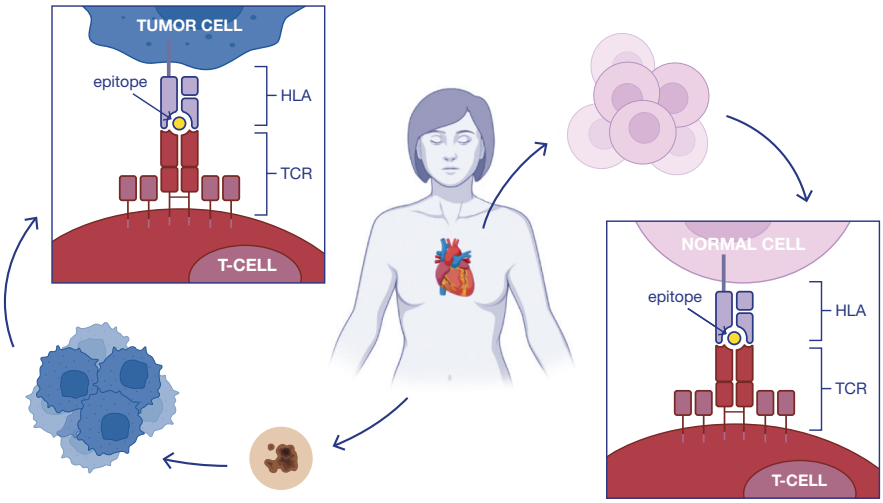


Positive predictive values (also PPV)[B] with four selected thresholds, i.e., top-10, 20, 50, & 100 pHLA pairs selected by each method. For example, the score for PPV at top-10 describes what fraction of hits can be expected by testing in the laboratory 10 pHLA pairs with the highest rank from each method.

Identify the right protein targets with cross-reactivity check

Address off-target toxicity in cancer immunotherapies long before it happens

There are different strategies of boosting the immune system to **find and destroy harmful germs or cells**, including cancer cells. Such immunosurveillance is possible due to T-lymphocytes' recognition of epitopes presented via HLA molecules. If you know which peptides are on the cell surface, you can use them as targets in **adoptive cell therapies** (TILs or TCR-engineered T-cells).



Typically, T-cells can naturally recognize more than one epitope as foreign. Therefore, **off-target toxicity** is a key issue to consider when developing immunotherapies. To select the safest peptide targets, support your research with **computational immunology and Ardigen's ARDitox platform (patent pending, see EP22461636).**

Did you know…?

Cross-presentation of exogenous peptides can lead to the presentation of epitopes of the **intestinal microbiota and other bacteria** on the cell surface. This can result in the development of **allergies** and autoimmune diseases.

Monitoring the cross-reactivity in patients based on **whole exome sequencing** results combined with **microbiome analysis** may help to explain these health problems and enable the design of specific therapies.

The development of **autoimmune diseases** can be related to **molecular mimicry**; a mechanism caused by T-cells interacting with both self-antigens and viral antigens.

Off-target toxicity leading to **side effects of variable intensity** - from mild reactions to severe ones - is a common cause of clinical trial failure in cancer immunotherapies.

The severity of these reactions strongly depends on the type of tissue affected. The most common side effects are skin reactions, flu-like symptoms, organ inflammation and changes in mental status. There are clinical trials reported where it has led to **coma or even death**.

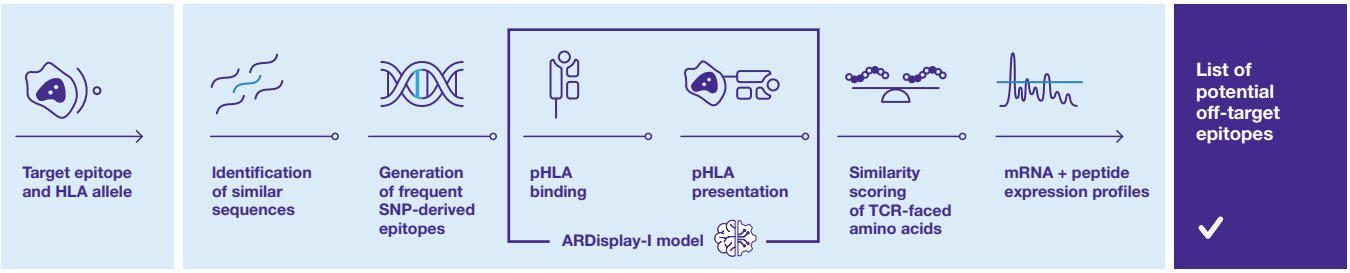
Identify the right protein targets with cross-reactivity check

Ardigen's ARDitox platform is a powerful tool for augmenting toxicity evaluation designed to improve cancer immunotherapy development. This computational approach is ideal for screening target epitopes to assess the risk of potential off-target toxicity.

ARDitox platform

Off-target toxicity

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



Check how we stand out from other solutions[6]

- We cover a larger space of peptides, as in other methods the **number of permitted mismatches** between the target peptide and potential off-target epitopes is limited to only a few amino acids. This factor is the most **important in the safety evaluation**.
- Extend the reference proteome (of the human population or your study cohort) with **frequently occuring mutations** to make sure you do not miss targets present in some human sub-population.
- Get a higher performance thanks to **ARDisplay model, our custom deep learning presentation model** trained on mass-spectrometry data that outperforms standard models like MHCflurry or netMHCpan.
- Recognize **TCR-facing amino acids** (epitope) from HLA-facing ones (agretope). Incorporate this information while comparing the target peptide with putative off-targets and **determine the risk of cross-reactivity** based on the physico-chemical properties of the selected amino acids.
- User-friendly dashboard for the inspection of the generated mRNA and protein **expression levels** of the identified cross-reactive peptides.

Exemplary results

The MAGE-A3 specific T-cells infused to patients during a clinical trial did recognize not only the targeted epitope, but also a Titin (TTN) peptide. The recognition of this peptide presented on healthy heart tissue has led to two fatalities.

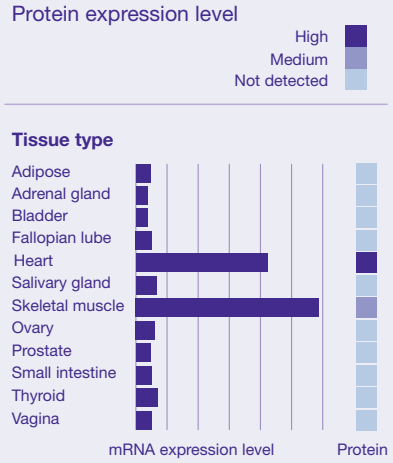
With ARDitox platform being tested retrospectively, we were able to anticipate the danger.

The list of putative off-targets detected with ARDitox platform for MAGE-A3

The safety score of 0 for TTN peptide indicates a high risk of immuno-toxicity

Gene	Off-target epitope	pHLA presentation with ARDisplay	Safety score
TTN	**ESDPIVAQY**	**0.88**	**0**
MAGEA6	EVDPIGHVY	0.86	0
MAGEB18	EVDPIRHYY	0.91	0
ALCAM	EMDPVTQLY	0.89	1.1
IGSF10	ESNPIAHLK	0.59	1.2
CPNE8	KSDPICVLY	0.99	1.5
FGD5	EVGPIFHLY	0.59	2.5

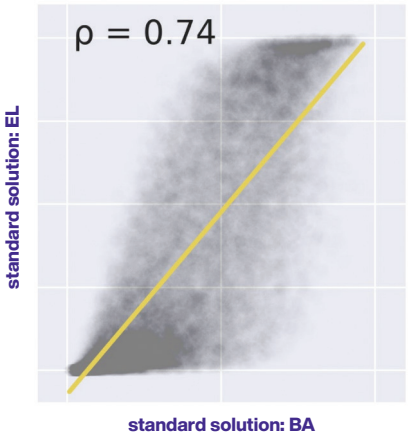
Tissue expression profile of TTN showing its abundance in heart and skeletal muscles



Identify the right protein targets
possible applications of our tools

Possible issues when using open-source tools

- Freely/academically available tools are not adjustable to **peptides from alternative sources**. There is no easy way to check the **model applicability nor adjust it** to your domain.
- There are almost 25k alleles known for canonical HLAs (HLA-A, -B, and -C). Most of them are not well studied, and standard models are not adapted to them and lack **problem-specific solutions**.
- Predictions of **binding affinity (BA) and presentation probability (EL) are highly correlated** with Pearson correlation coefficient ρ=0.74[7].



Our expertise & technology tailored to your needs

Explore targets from **alternative cancer germline** transcripts

Indicate short peptides from diverse **minigene-encoded or lentiviral-derived epitopes** that end up on the cell surface

Identify **patient's HLA alleles** presenting a given peptide

Predict whether your peptide can be presented on **multiple HLAs**

Determine **agretopic parts** for a peptide with a given HLA allele

Determine **binding cores of HLA-II** restricted peptides

Predict potential **off-target toxicity** long before it happens

Search for mimotopes to develop vaccines against viral diseases - find **peptides mimicking your target epitope** by comparing their structural & functional similarities

Identify mimotopes that can lead to **allergies** caused by patient epitome cross-reactivity

Use reliable predictions with **domain applicability check** and model confidence verification

Adjust & finetune our AI-based solutions to your specific needs

Integrate data from different types of experiments so that every relevant biological aspect is addressed

Examine the influence of different proteasome subunits and peptidases on **pools of truncated peptides**

Build *in silico* **peptide library** and detect only promising targets to validate experimentally, thus overcoming the problem of labor-intensive deconvolution

Overcome the **limitations of laboratory screening** by easily incorporating all scanning approaches at once[8]

Identify **unmet clinical needs** and make recommendations for treatment approaches

Glossary

[A] **Average precision (AP)** is a weighted mean of precision at each threshold, ie. proxy to AUC.

[B] **Positive predictive value (PPV) or precision**; the percentage of positively classified cases in binary classification that were true positives.

[C] **Precision-recall curve (PR curve)** is a standard curve for binary classification model evaluation. It is especially useful for very imbalanced data and provides a trade-off between precision and recall.

[D] **True Positive Rate (TRP), also recall or sensitivity**; the fraction of all positives detected by the model.

[1] Both models were trained on eluted ligands detected by MS experiments. ARDisplay-I model differs a lot from standard solutions for EL prediction; for top-100 pHLA pairs, on average, there are only 14-18 samples shared (25th & 75th percentile, respectively).

[2] Löffler et al., *Mapping the Ligandome of Colorectal Cancer Reveals Imprint of Malignant cell transformation*, Cancer Research 78.16 (2018): 4627-4641.

[3] HLA class I & II population coverage based on **http://tools.iedb.org/population/**

[4] Both models were trained on eluted ligands detected by MS experiments. ARDisplay-II model differs a lot from standard solutions for EL prediction; for top-100 pHLA pairs, on average, there were 12±3 samples shared.

[5] Forlani et al., *CIITA-Transduced Glioblastoma Cells Uncover a Rich Repertoire of Clinically Relevant Tumor-Associated HLA-II Antigens*, 2021.

[6] Expitope 2.0, iVax (JanusMatrix), Dhanik et al. (2016), or Lee et al. (2020).

[7] Mazzocco et al., *AI aided design of epitope-based vaccine for the induction of cellular immune responses against SARS-CoV-2*, 2021.

[8] for instance, alanine scanning, random replacements, scrambled, truncated, positional, overlapping.

Ardigen

Artificial Intelligence & Bioinformatics for Precision Medicine

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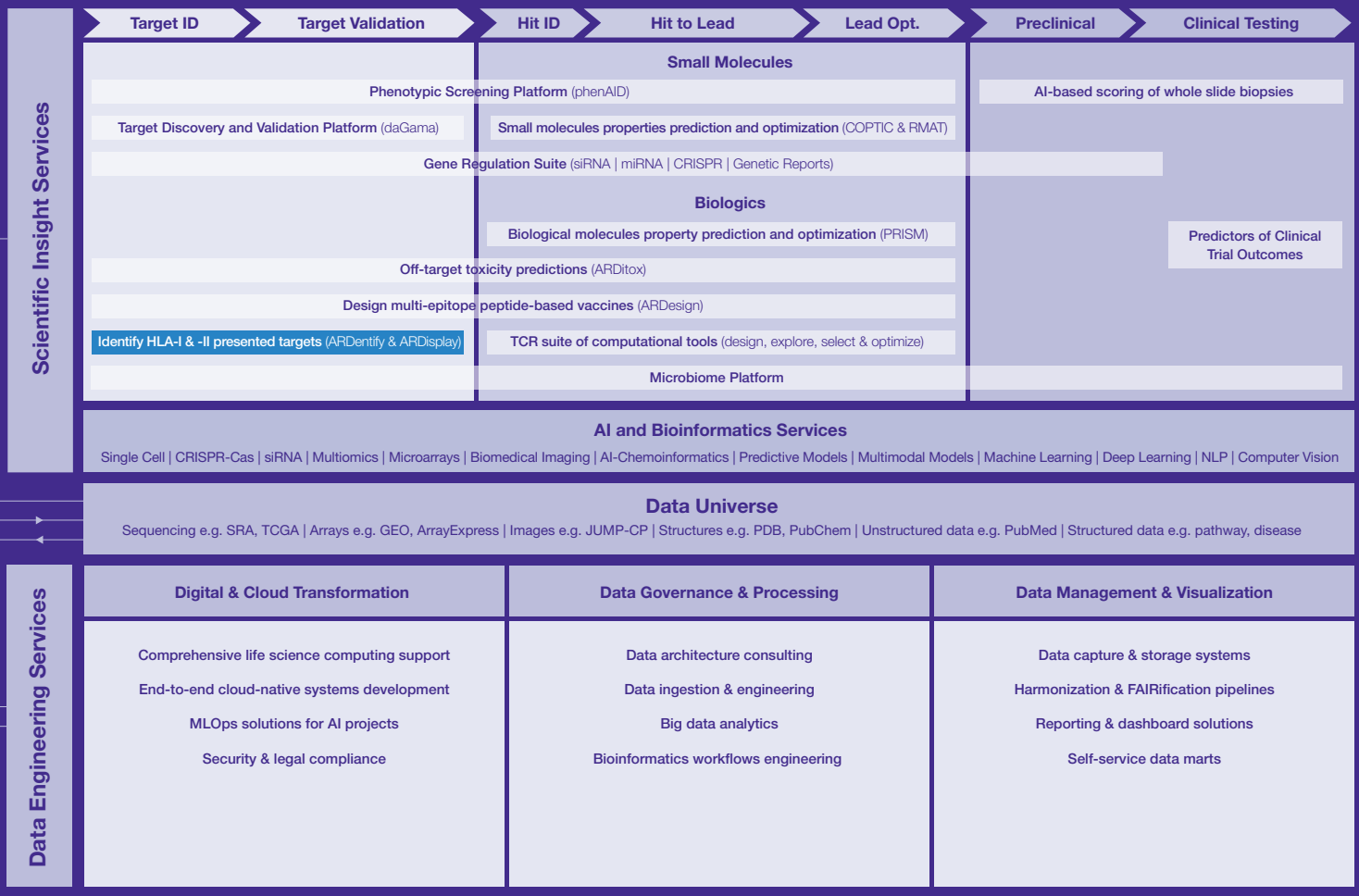

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