ARDiTox: Safer pHLA-Targeted Immunotherapies Through AI and Computational Immunology

Victor Murcia Pienkowski, Bartłomiej Król-Józaga, Piotr Skoczylas, Anna Sanecka-Duin, Joanna Marczynska-Grzelak, Maciej Jasiński, Jan Kaczmarczyk

Ardigen, Krakow, Poland

BACKGROUND

Adoptive therapies with engineered TCRs, including TCR-T cells and TCR mimics, hold strong promise in oncology but are associated with the risk of severe off-target toxicity when healthy tissues are also recognized [1–3]. Comprehensive experimental mapping of cross-reactivity is infeasible, and purely sequence-based approaches or exhaustive TCR–pHLA modeling have important limitations. To address this gap, we developed **ARDiTox**, an AI-powered pipeline for systematic off-target risk assessment. ARDiTox integrates multiple layers of evidence - ranging from sequence similarity and experimental X-scan data to structural analysis of pHLA complexes. In particular, its structural module enables comparison of pHLA surface properties, including electrostatics, hydrophobicity, and interaction propensities, derived from generated pHLA structures. This multi-pronged approach provides a practical and scalable framework to prioritize potential off-targets for TCR and TCR-mimic therapies.

METHODS

The ARDiTox pipeline was developed to systematically identify and prioritize potential off-target epitopes for TCR and TCR-mimic therapies. The workflow proceeds as follows (Figure 1):

Input: Target peptide (8–11 aa) and HLA type; X-scan or alanine scan data can be used if available.

Candidate search: Off-targets identified in the reference proteome via sequence similarity or using the X-scan-derived sequence patterns.

Structural modeling: pHLA 3D structures and 2D surface projections [4] of target and off-targets are compared.

Presentation prediction: peptide - HLA binding and presentation probability are estimated using Ardigen's proprietary **ARDisplay** model [5].

Safety scoring: Putative off-targets are scored based on (i) physicochemical properties of TCR-facing residues, (ii) X-scan data, or (iii) 2D surface projections of modeled pHLA structures (Figure 2).

Expression integration: mRNA and peptide expression data are included in final report.

Output: Ranked list of cross-reactive antigens.

RESULTS

ARDiTox reduces the search space by extracting, from the whole proteome, sequences that are sufficiently similar to the target. The method focuses on a subset of plausible epitopes, rather than the entire pool of potentially presented peptides (Figure 3).

To evaluate the efficiency of this approach, we tested ARDiTox in several settings:

- **Literature benchmarks:** Three TCRs with known off-targets (Figure 4, plots #1-3): $MAGEA3_{112–120}$ [6], $MAGEA3_{168–176}$ [7], and $AFP_{158–166}$ [3].
- **Therapeutic candidates design**
  - **a.** T cell therapy: we investigated potential off-targets of a TCR specific to a pHLA complex expressed by glioblastoma cells. ARDiTox predicted a single off-target epitope, which was subsequently validated *in vitro* (Figure 4, plot #4) [8].
  - **b.** TCR-mimic therapeutic: we evaluated a candidate TCR-mimic molecule. ARDiTox identified numerous peptides with potential cross-reactivity, several of which were validated *in vitro* (Figure 4, plots #5–6) and further derisked in functional assays.

DISCUSSION

Ardigen's ARDiTox demonstrates strong and consistent performance in identifying putative off-target epitopes using computational approach. Its predictive power is further enhanced when experimental data (X-scan or A-scan) are available, highlighting the value of combining *in silico* and experimental methods.

ARDiTox key features:

- Substantially reduces the search space, making it easier to **identify true off-target** epitopes.
- Computationally efficient and adaptable to specific targets and **HLA types**.
- Enables **comparison of off-target epitopes** of different lengths **presented by the same HLA.**
- Significantly **accelerates the drug discovery process** by narrowing down number of molecules for wet-lab validation.

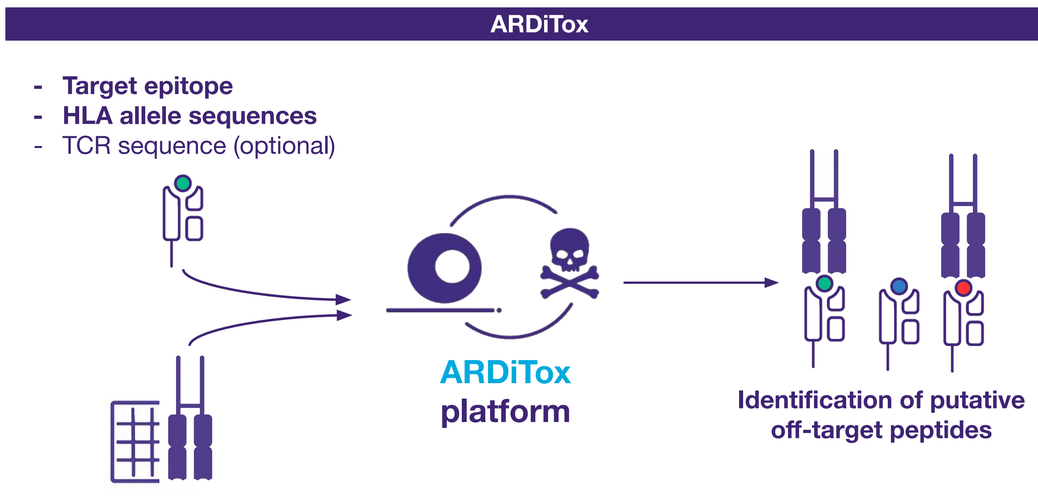

Figure 1: *Overview of the ARDiTox pipeline for systematic identification and prioritization of potential off-target epitopes in TCR and TCR mimic therapies, from input peptide and HLA type through candidate search, structural modeling, cell surface presentation prediction, safety scoring, protein expression patterns evaluation, and ranked output of cross-reactive antigens.*

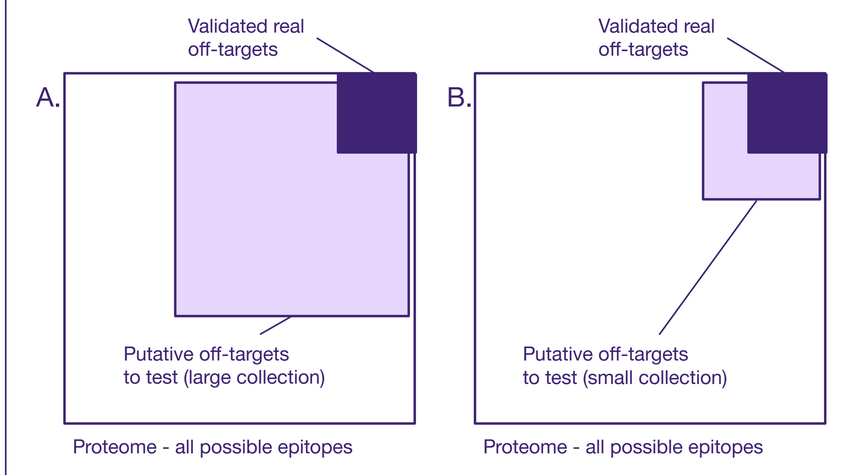

Figure 3: *ARDiTox reduces the number of putative off-target epitopes that need to be tested experimentally while still capturing the truly TCR-binding off-target epitopes. A. A large set of putative off-targets (purple area) represents what would need to be tested without ARDiTox filtering. Only a small subset of these are true TCR-binding off-targets (blue box). B. ARDiTox dramatically narrows the search space (smaller purple area), retaining the same true off-targets while excluding many irrelevant epitopes. This significantly reduces experimental workload without missing critical safety-relevant off-targets.*

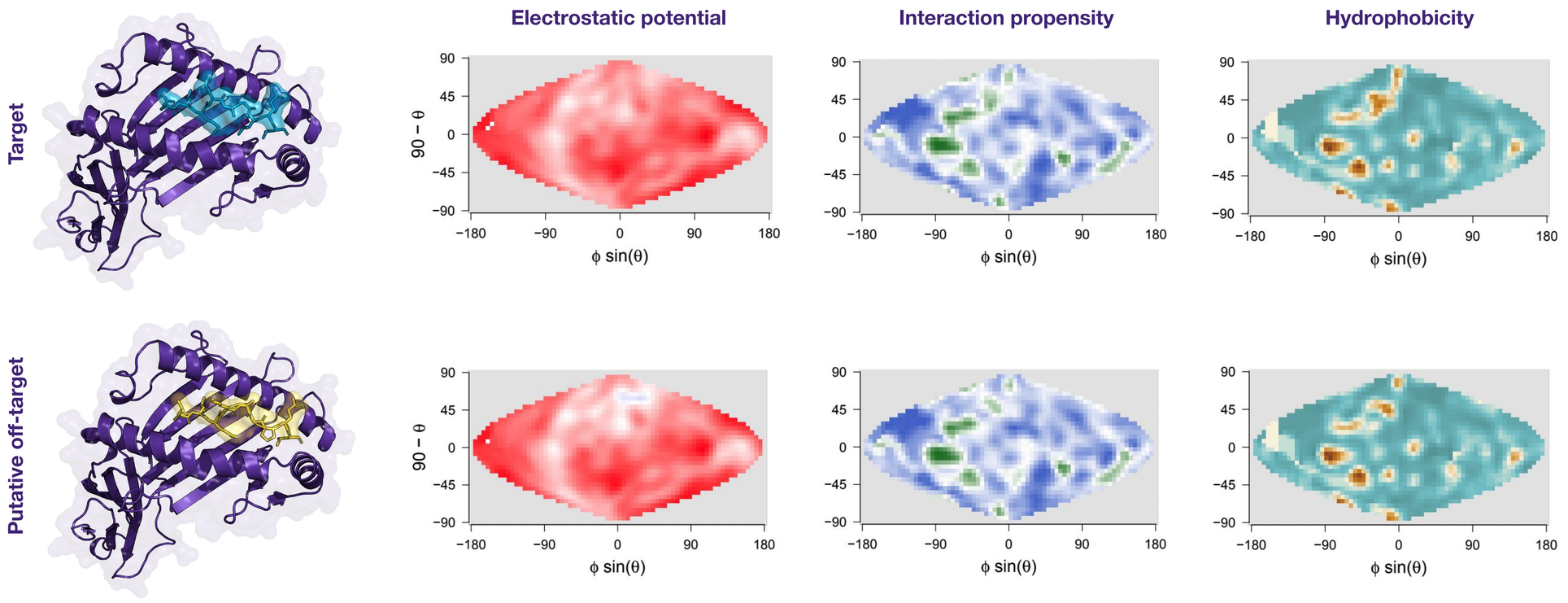

Figure 2: *To identify potential off-target epitopes, 3D structures of the target and putative off-target epitopes are first generated. Then, 2D projections of these structures are created for all pHLAs, capturing key properties such as electrostatic surface potential, interaction propensity and hydrophobicity. Finally, each off-target epitope's properties are compared to the corresponding properties of the target. The more similar these properties are between the target and off-target epitopes, the higher the likelihood of off-target epitope interaction with the TCR.*

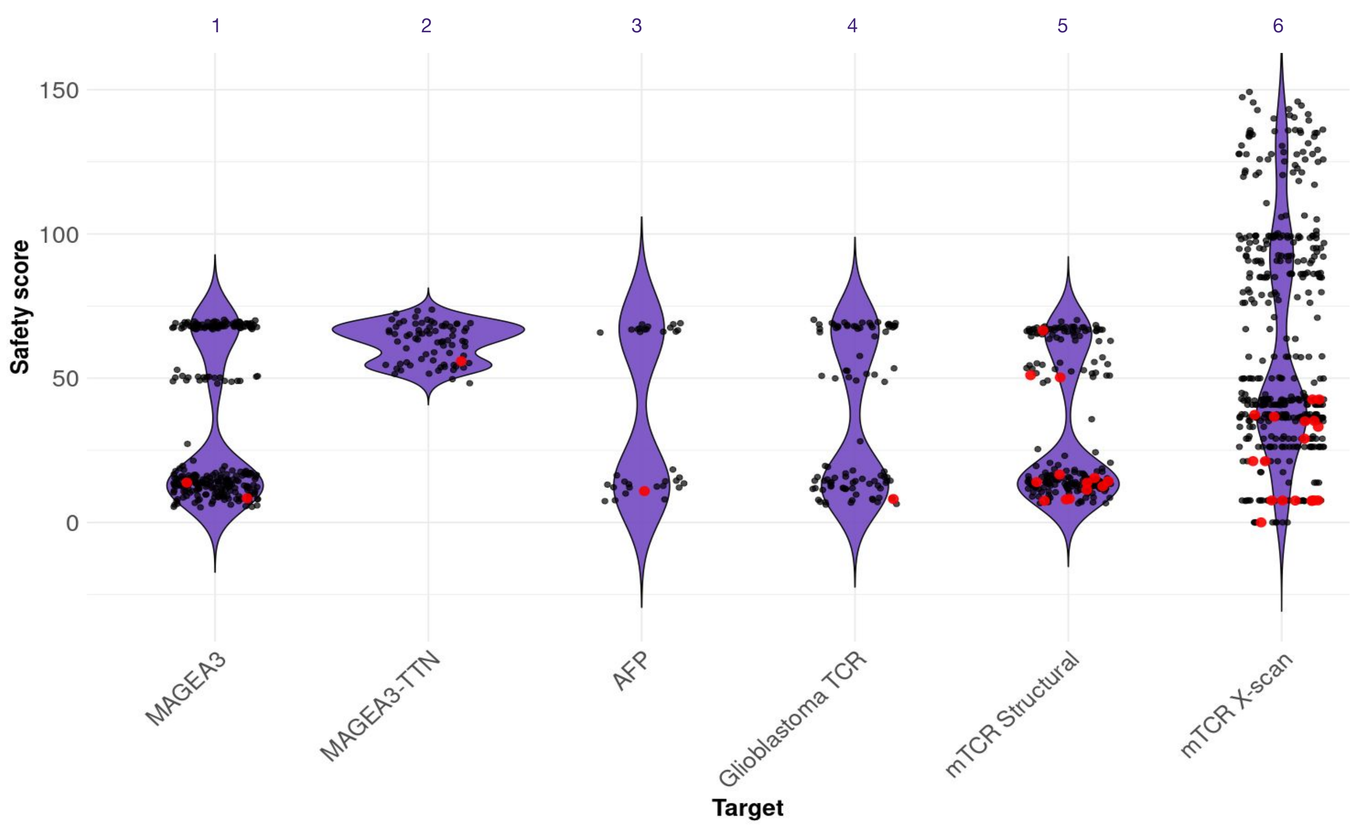

Figure 4: *ARDiTox reduces the number of putative off-target epitopes that need to be tested experimentally while still capturing the truly TCR-binding off-target epitopes.*
The violin plots show the distribution of safety scores for analyzed epitopes. The red dots represent true off-target epitopes, identified by ARDiTox and confirmed in vitro. The black dots represent putative off-target epitopes that were identified but did not bind the given TCR.

MAGEA3, MAGEA3-TNN, AFP: *In all cases, the structural approach identified true off-target epitopes. These peptides were consistently ranked among those with the lowest safety scores (indicating the highest risk of off-target toxicity).*

Glioblastoma TCR: *The structural approach identified a true off-target epitope, which ranked among the lowest safety scores (indicating the highest risk of off-target toxicity).*

mTCR Structural and mTCR X-scan: *Both the X-scan and the structural approach rank the true off-targets within the group of putative peptides with the lowest safety scores.*

REFERENCES

[1] Coebel CM, et al. Adaptive immunity maintains occult cancer in an equilibrium state. Nature. 2007
[2] Farkona S, et al. Cancer immunotherapy: the beginning of the end of cancer? BMC medicine. 2016
[3] Linette GP, et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. Blood. 2013
[4] Schweke H, et al. SURFMAP: A Software for Mapping in Two Dimensions Protein Surface Features. Journal of Chemical Information and Modeling 2022
[5] Mecklenbräuker S, et al. Identification of tumor-specific MHC ligands through improved biochemical isolation and incorporation of machine learning. bioRxiv 2023
[6] Coebel CM, et al. Adaptive immunity maintains occult cancer in an equilibrium state. Nature. 2007
[7] Farkona S, et al. Cancer immunotherapy: the beginning of the end of cancer? BMC medicine. 2016
[8] Chich Y, et al. Vaccine-induced T cell receptor T cell therapy targeting a glioblastoma stemness antigen. Nature Communications 2025
[9] Murcia-Pienkowski V, et al. Computational identification of cross-reactive TCR epitopes with ARDiTox. Journal of Cancer Research and Clinical Oncology. In press

IMPACT

A validated solution for mitigating off-target toxicity

ARDiTox directly addresses the critical challenge of off-target toxicity in TCR and TCR-mimic therapies. Our AI-powered platform provides a scalable solution for identifying and prioritizing cross-reactivity risks, accelerating the development of safer, more effective treatments. The impact is validated by two peer-reviewed publications [8, 9] and its successful use in an IND filing. This demonstrates the platform's real-world applicability in making crucial go/no-go decisions and streamlining the therapeutic development pipelines.