INTRODUCTION

Recent advances in the field of cancer immunotherapy have significantly changed the landscape of available treatments, thus improving the prognosis for many patients with various hematological and solid malignancies. Leveraging the capacity of the immune system to differentiate tumor antigens from normal tissue has already shown to lead to sustained clinical responses [1]. Cell-based approaches for boosting natural defenses include strategies that are targeted against MHC (HLA) class I (ECM) or T-cell receptor (TCR) modified cells [2]. Adoptive cell therapies have a high potential for revolutionizing cancer treatment; however, despite their immense potential, they are not free of side effects. The immune system, while being revved up to fight the disease, might also act against healthy cells and tissues, even more so when T cells are genetically modified (see fig. 1). The development of such off-target immunoactivity can have serious health consequences for patients undergoing therapy and is a major clinical concern, as fatalities have been reported [3].

Antigen processing & presentation

The list of possible protein substructures is enormous, but only a tiny fraction of them can actually be presented on the cell surface. Such peptides can safely be responsible for the side effects of immunotherapy. To account for the sequence of biological events responsible for the process of the presented presentation, we use Artificial Intelligence models trained on mass spectrometry data (see fig. 4).

COMPUTATIONAL WORKFLOW WITH ARDIMUNE TOX

METHODS

Putative off-target epitopes

Given a target epitope and its associated HLA type, a large collection of putative off-target epitopes, tracing up to a predefined maximum number of amino acid differences with respect to the original epitope, is generated computationally. The analysis mimics an experimental approach (see fig. 2) by performing systematic substitution of amino acids in the target peptide, but allowing for simultaneous changes of several positions, thus resulting in orders of magnitude larger space of putative off-target epitopes [5].

Subsequently, computational methods (see fig. 2) are used to restrict the list of potential off-targets to the epitopes that (i) naturally occur in the human proteome, (ii) are highly probable to be presented on the cell surface. Finally, TCR-facing residues are compared with those from the target peptide (see fig. 3).

Determination of TCR-faced aAs

The workflow of ArdImmune Tox (see fig. 2) includes the following steps:

1. Calculation of similarity score between target epitope and putative off-target epitope or off-target sequences, since probing the entire space experimentally is practically unfeasible. Leveraging the recent advances in computational immunology and AI can augment these efforts, ultimately leading to an increase in the number and safety of available treatments. To this end, we have introduced ArdImmune Tox - a novel method for predicting and analyzing off-target toxicity. (bottom) Expression levels of mRNA (left) and protein that respective peptide originates from (right) for chosen types of human body tissues. (middle) TCR motifs assessment. (top) The table screenshot shows part of the list of putative off-target epitopes including ECM-A12 that causes mucosal damage in the skin (in phase 2). Some of the epitope signatures are scored based on the comparison with negative case (V4) or positive case (V2). (bottom) Targets requiring further validation.

RESULTS

Analyzed cases

Table 1. Composition of the dataset used for ArdImmune Tox evaluation [5, 6-8]. The dataset consists of (i) Two T-cell targets used in clinical trials that led to off-target toxicity and death of patients (case 1 and 2); (ii) One T-cell target never used in clinical trials with a potential off-target epitope identified using X-scan (case 3); (iii) One T-cell target used in clinical trials with no sign of off-target epitope (case 4).

Interactive dashboard of ArdImmune Tox

Visualization of outcomes. The table screenshot shows part of the list of putative off-target epitopes including ECM-A12 that causes mucosal damage in the skin (in phase 2). Some of the epitope signatures are scored based on the comparison with negative case (V4) or positive case (V2). (See fig. 7) for the cases with low safety score (9 peptides below 3.0). (See fig. 8) for the cases with high safety score (8 peptides below 1.0). Therefore, both cases 2 and 3 might lead to off-target toxicity, which stands in line with findings from preclinical and clinical stages.

DISCUSSION

Herein, we introduced ArdImmune Tox - a novel method for analyzing antigen cross-reactivity including identification of off-target epitopes that differ significantly from the targeted epitope. Our method pinpoints putative off-target epitopes that should be comprehensively verified.

We tested ArdImmune Tox in 10 different cases [3, 4]. Two of them have a defined clinical outcome where immunotoxicity was reported; (i) one was tested using X-scan, and (ii) in one no cross-reactive epitopes were identified in the clinic.

We discovered that:

1. In three positive cases (cases 1&2) the off-target epitopes are correctly identified (see fig. 7 and 8).
2. In the negative case (case 4) we identify just a single epitope with low safety score (see fig. 7).
3. Two of the positive cases that were clinically validated show expression levels (see fig. 6) that are significantly lower than those of the positive cases.

In these two cases we obtain a considerable less number of off-target epitopes (22 peptides identified with a low safety score is high 8 peptides below 3.0 for each case). Therefore, both cases 2 and 3 might lead to off-target toxicity, which stands in line with findings from preclinical and clinical stages.

CONCLUSIONS

The workflow of ArdImmune Tox (see fig. 2) takes into account: (i) peptide cleavage, (ii) pHLA binding, (iii) pHLA presentation probability, (iv) determination and similarity of TCR-based aA, (v) frequent variants as a potential source of off-target epitopes, and (vi) gene mRNA & protein expression levels. By integrating the above aspects we developed a multi-step, accurate, and easy-to-use method for the evaluation of cross-reactivity. A computational approach for the screening of targeted epitopes towards potential off-target toxicity is a powerful tool that might augment toxicity evaluations and improve immunotherapy development. Other possible applications include the identification of cross-reactive antigens to leverage molecular mimicry, e.g., in the development of microbiome-based therapeutics.

REFERENCES


Fig. 1. Example of fatal toxicity in two patients after adoptive cell therapy. The MAGE-A4 specific T cells induced cardiac failure by not recognizing the targeted epitope, but also Thin peptide presented on host tissue.

Fig. 2. Identification of putative off-target epitopes using ArdImmune Tox. (i) Input consists of the target sequence (8-15 amino acids) and the HLA type (ii) Identification of off-target sequences in the reference proteome that are similar to the original peptide (iii) Prediction of putative off-target epitopes using ArdImmune Var (iv) Calculation of safety score based on similarity between target epitope and putative off-target epitope or off-target sequences, since probing the entire space experimentally is practically unfeasible. Leveraging the recent advances in computational immunology and AI can augment these efforts, ultimately leading to an increase in the number and safety of available treatments. To this end, we have introduced ArdImmune Tox - a novel method for predicting and analyzing off-target toxicity. (bottom) Expression levels of mRNA (left) and protein that respective peptide originates from (right) for chosen types of human body tissues. (middle) TCR motifs assessment. (top) The table screenshot shows part of the list of putative off-target epitopes including ECM-A12 that causes mucosal damage in the skin (in phase 2). Some of the epitope signatures are scored based on the comparison with negative case (V4) or positive case (V2). (bottom) Targets requiring further validation.

Fig. 3. Typical TCR motif assessment. Each amino acid position is assessed according with 8-15 alternatives naturally occurring amino acids (2177 possible epitopes). (in case of epitopes with 9 AAs)

Fig. 4. ArdImmune Tox dashboard presenting exemplary results from case 2 (the target epitope ESPF0K0 (MAE-A2) presented on HLA-A*01:01 [5,6]). (top) The table screenshot shows part of the list of putative off-target epitopes including ESPF0K0 that cause mucosal damage in the skin (in phase 2). Some of the epitope signatures are scored based on the comparison with negative case (V4) or positive case (V2). (middle) TCR motifs assessment. (bottom) Targets requiring further validation.

Fig. 5. Identification of TCR-based amino acids for the target epitope ESPF0K0 (MAE-A2) and its cross-reactive epitope ESPF0K0 (TOX).

Fig. 6. ArdImmune Tox dashboard presenting exemplary results from case 2 (the target epitope ESPF0K0 (MAE-A2) presented on HLA-A*01:01 [5,6]). (top) The table screenshot shows part of the list of putative off-target epitopes including ESPF0K0 that cause mucosal damage in the skin (in phase 2). Some of the epitope signatures are scored based on the comparison with negative case (V4) or positive case (V2). (middle) TCR motifs assessment. (bottom) Targets requiring further validation.

Fig. 7. Distribution of safety scores for putative off-target peptides for case 1 [3] and case 4 [6]. Case 1. Example of a peptide with high likelihood of inducing immunotoxicity due to a higher number of peptides with low safety score (8 peptides below 3.0). Case 4. Example of an epitope with low likelihood of off-target toxicity (relatively low number of putative off-target epitopes, only 1 peptide with safety score below 3.0).