Accounting for immune escape mechanisms in personalized and shared neoantigen cancer vaccine design

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INTRODUCTION

Immune escape mechanisms (IEMs) represent examples of microevolutionary processes. The high cancer mutability drives the emergence of tumor neoantigens, which might be exploited by the immune system to eliminate cancer cells. On the other hand, immunosurveillance exerts selective pressure on tumor cells, contributing to immune escape strategies of profound clinical relevance.

In particular, the presence of IEMs related to HLA class I antigen processing and presentation (APP) pathway can have an important impact on cancer treatment. This is particularly significant for treatments targeting shared neoantigens, such as adoptive T-cell therapies and off-the-shelf cancer vaccines. In the presence of such antigens IEMs might be more frequent by the following rationale: (i) these antigens are frequently occurring, hence they might be more often coming from oncogenes (than non-shared antigens); (ii) as such, they might be clonal and important for cancer progression; (iii) when recognized by the immune system, they are not easily dispensable and one way to "hide" them is via IEMs disrupting the antigen presentation processes.

Using a collection of data from five diverse tumor types from The Cancer Genome Atlas (TCGA), we investigate the associations between (i) quantity, quality, and sharedness of tumor-derived neoantigens; (ii) IEMs related to the HLA allele and to the APP pathway; (iii) composition of the tumor microenvironment (TME), and (iv) patients' survival. We draw conclusions affecting treatment strategies, in particular, for shared vs personalized approaches.

DATA

The results shown here are based on data generated by TCGA Research Network [1]. Based on the high number of patients and relative diversity in the tumor mutational burden (TMB) and in the immune microenvironment, the following five TCGA cohorts were chosen: COAD (colon adenocarcinoma), GBM (glioblastoma), LUAD (lung adenocarcinoma), LUSC (lung squamous cell carcinoma), SKCM (skin cutaneous melanoma) with 323, 146, 402, 291, and 103 patients, respectively. Only patients with complete NGS information (both WES and RNA-seq data) were selected. In the survival analysis, we use clinical data available via The Cancer Immunome Atlas (TCIA) [5] for 1234 out of 1265 considered patients. In addition, we use TCIA quanTIseq data for assessing TME.

COMPUTATIONAL WORKFLOW WITH ARDIMMUNE VAX

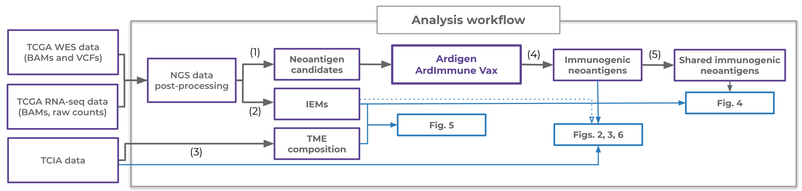

Fig. 1. Computational workflow using Ardigen's ArdImmune Vax (an AI-driven neoantigen selection platform). The following computational tools are used: (1) our customized NGS post-processing pipeline to determine candidate neoantigens, (2) Polysolver [2], LOHHLA [3], and our in-house methods to determine IEMs, (3) quanTIseq [4] to estimate the composition of TME based on TCIA data [5], (4) the ArdImmune Vax platform [6] to determine immunogenic neoantigens and (5) their shared/non-shared status. The immunogenicity prediction in (4) is performed based on selected properties of neoantigens (including features reflecting expression and clonality/heterogeneity), as well as AI models predicting the probability of binding to HLA, presentation on the cell surface, and recognition by the immune system leading to the immunogenic reaction. For better readability, figure associations are included.

METHODS

Immune Escape Mechanisms (IEMs)

We consider the following indicators of IEMs related to HLA class I antigen processing and presentation processes:

- **Loss of Heterozygosity (LOH) of HLA alleles (as returned by LOHHLA [3])**
  Loss of one of HLA alleles was found to be a common escape mechanism in up to 40% of patients in certain cancer types [3] resulting in a complete loss of antigen presentation for this allele.
- **Mutations in HLA (as returned by Polysolver [2])**
  Mutations in HLA were found to be a common IEM leading to an impairment of antigen presentation [2].
- **Low HLA expression (below 10th percentile per cohort)**
  One of the possible mechanisms leading to cancer immune escape is the downregulation or even loss of expression of classical HLA class I [7] (i.e. A, B, and C loci).
- **Non-synonymous mutations in the APP gene set:** Genes with well-characterized roles [7] in APP and T-cell activation, were selected: HLA-A/B/C, MIC-A/B, B2M, TAPBP, TAP1/2, NLRC5, ERAP1/2, CALR, PDIA3, PSME1/2/3, TPP2.
- **Expression of a signature composed of stimulatory genes in the APP gene set (using ssGSEA [8])**
  We perform single-sample Gene Set Enrichment Analysis (ssGSEA) [8] and consider signature values in the first quintile (calculated per cohort) as indicative of an IEM via downregulation of the following APP-related [7] genes: HLA-A/B/C, B2M, TAPBP, TAP1/2, NLRC5, ERAP1/2, CALR.

Tumor Microenvironment (TME)

TME consists of stroma, blood vessels, and tumor-infiltrating immune cells (TIICs). The composition of TME can effectively change the evolution of cancer and in certain cases lead to IEMs. To estimate fractions of immune cells in TME we use quanTIseq [4], which is based on RNA-seq data deconvolution. QuanTIseq returns the TME composition: fractions of certain immune cell types within the cancer sample. Such fractions can be compared between samples.

RESULTS

Number of immunogenic neoantigens vs. IEMs

Immunogenic neoantigens make the tumor detectable by the immune system. Such immunosurveillance can provide selective pressure, which in turn can trigger IEMs via microevolution. In particular, IEMs related to APP can "hide" immunogenic antigens, e.g. by downregulating the expression of the related HLA alleles. The antigens affected in such a way are no longer viable as therapy targets.

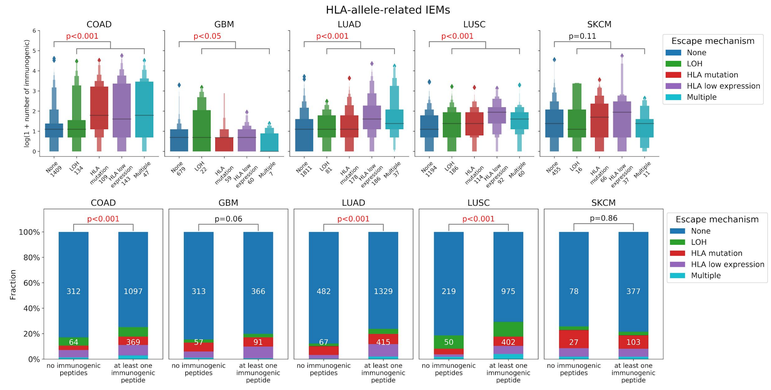

Fig. 2. ***(top)*** The number of immunogenic neoantigens per HLA class I allele under selected HLA-allele-related IEMs. The numbers below the ticks on x-axes correspond to the size of each group. The p-values describe the results of the one-sided Mann-Whitney-Wilcoxon (MWW) test. ***(bottom)*** The patient's HLA types are divided into those with/without immunogenic peptides (x-axis) and based on the presence of the selected (see legend) HLA-allele-related IEMs (y-axis). The numbers on bars correspond to the sizes of 4 groups (here we consider presence/absence of IEMs for y-axis) and the p-values are computed based on them using Fisher's exact test.

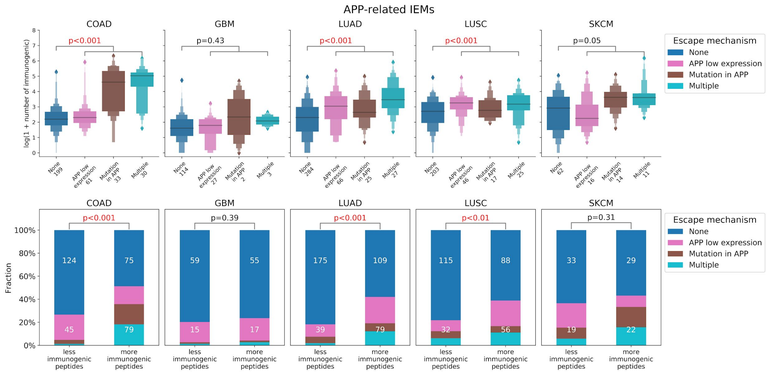

Fig. 3. ***(top)*** The number of immunogenic neoantigens per patient under selected APP-related IEMs. The numbers below the ticks on x-axes correspond to the size of each group. ***(bottom)*** The fraction of patients in the groups with less/more immunogenic peptides (x-axis; divided on median per cohort) and based on the presence of the selected APP-related IEMs (y-axis). The numbers on the bars correspond to the sizes of 4 groups (here we the consider presence/absence of IEMs for y-axis). All p-values are computed as in Fig. 2.

IEMs in personalized vs. shared cancer vaccines

We developed an algorithm for design of a shared-vaccine composition, which maximizes coverage of patients for a long-peptide vaccine including 20 peptides at most.

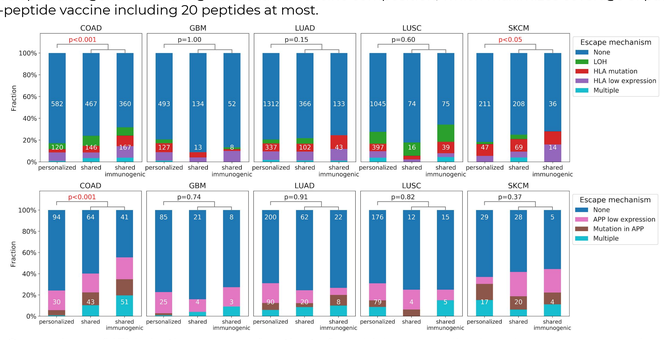

Fig. 4. Comparison of IEMs in the groups of patients for which personalized or shared cancer vaccines can be used. The latter is additionally divided into those with/without immunogenic antigens (labeled as "shared immunogenic" and "shared"). We show numbers of IEMs in the three groups for ***(top)*** HLA-allele-related IEMs (per HLA allele) and ***(bottom)*** APP-related IEMs (per patient). The p-values are computed based on Fisher's exact test.

Relation between Tumor Microenvironment (TME) and IEMs

The interplay between cancer cells and the host's immune system is complex, continuous, and of nonlinear dynamics. Both suppressive and effector cells in TME can have an important influence on tumor evolution. We analyze RNA-seq data with quanTIseq [4] to estimate the proportions of ten types of TIICs in TME. We investigate correlations between the presence of IEMs and the frequencies of important groups of TIICs.

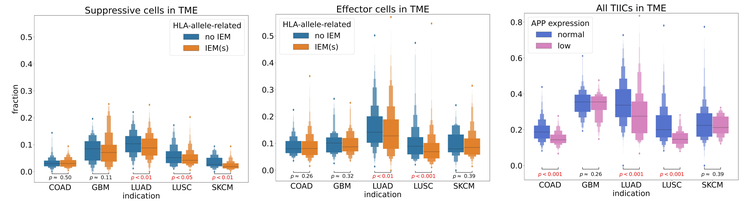

Fig. 5. ***(left/center)*** The relationships between total fractions of suppressive/effector TIICs and HLA-allele-related IEMs. The following immunosuppressive cells are included: regulatory T cells and M2 macrophages, whereas the considered effector cells are the following: CD4+ and CD8+ T cells, M1 macrophages, and NK cells. ***(right)*** The relationship between the fraction of suppressor and effector TIICs and signature level of APP genes (where *low* stands for the first quintile). The p-values correspond to the results of the one-sided MWW test.

Survival analysis

To check whether the presence of the considered IEMs in combination with features related to neoantigens can predict 5-year patient survival, we perform survival analysis using clinical data [5].

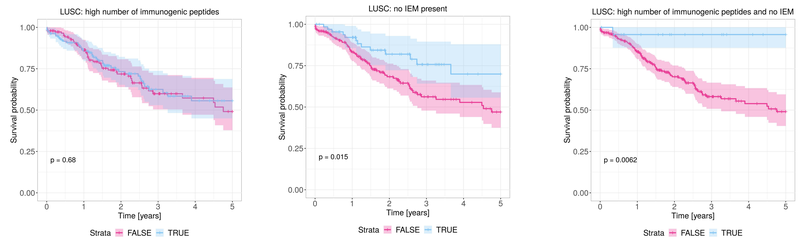

Fig. 6. Survival plots for the stratification of patients within the LUSC cohort. We split the cohort by the number of immunogenic neoantigens with threshold of median value per cohort - 16 ***(left)***, absence of IEMs ***(middle)*** and the two conditions combined ***(right)***. We use clinical data available via TCIA [5] for 284 out of 291 patients from the LUSC cohort. The numbers of patients within each group are the following: FALSE-146, TRUE-138 *(left)*, FALSE-197, TRUE-70 *(middle)*, FALSE-254, TRUE-25 *(right)*.

DISCUSSION

- A higher number of immunogenic neoantigens correlates with the presence of HLA-allele-related (Fig. 2) and APP-related (Fig. 3) IEMs in COAD, LUAD, and LUSC cohorts.
- Using our ArdImmune Vax platform we proposed a shared vaccine composition for the considered cohorts (for a long-peptide vaccine). For COAD the amount of shared neoantigens enables us to cover a large group of patients (Fig. 4). Shared immunogenic neoantigens for COAD are more frequently (than non-shared neoantigens) co-occurring with IEMs and more frequently originating from oncogenes (not shown), which might be the trigger for IEMs.
- The presence of HLA-allele-related IEMs can correlate with lower infiltration of immune cells in TME (Fig. 5), as exemplified by lower fractions of TIICs (both suppressive and effector) in the LUAD and LUSC cohorts. For SKCM it correlates only with a lower fraction of suppressive cells. Low expression signature of the APP-related genes correlates for the COAD, LUAD, and LUSC cohorts with lower fractions of TIICs (of all types combined).
- A high number of immunogenic neoantigens, which can be efficiently presented (i.e. in the absence of IEMs), is a strong predictor of survival for the LUSC cohort (Fig. 6). For other cohorts using the selected predictors (IEMs and neoantigen quality and/or quantity), we do not observe particularly good stratifications.

CONCLUSIONS

- Off-the-shelf treatments targeting shared neoantigens require particular attention to IEMs.
- A minimal delay between monitoring for IEMs and the administration of treatment should be made. For personalized approaches (e.g. personalized cancer vaccines) IEMs have less severe implications, but should also be accounted for (e.g. by not targeting neoantigens related to the patient's affected HLAs).
- As the next step, we will validate the shared vaccine design strategy used for COAD on a dataset with a large number of neoantigens validated for immunogenicity for colorectal cancer [9].

REFERENCES

1. TCGA, https://www.cancer.gov/tcga
2. S.A. Shukla, et al. *Comprehensive analysis of cancer-associated somatic mutations in class I HLA genes.* Nat Biotechnol. 2015;33(11):1152–1158.
3. N. McGranahan, et al. *Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution.* Cell 2017. 171(6):1259–1271.e11.
4. F. Finotello, et al. *Molecular and pharmacological modulators of the tumor immune contexture revealed by deconvolution of RNA-seq data.* Genome Medicine, 2019. 11(1):34.
5. P. Charoentong, et al. *Pan-cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade.* Cell Rep. 2017. 18:248-262.
6. G. Mazzocco, et al. *AI-augmented design of effective therapeutic cancer vaccines and adoptive cell therapies.* Poster presented at: Society for Immunotherapy of Cancer (SITC); 2019; National Harbor.
7. M.H. Pedersen, et al. *Downregulation of antigen presentation-associated pathway proteins is linked to poor outcome in triple-negative breast cancer patient tumors.* Oncoimmunology. 2017. 6(5):e1305531.
8. D.A. Barbie, et al. *Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1.* Nature. 2009. 462:108-112.
9. M. Parkhurst, et al. *Unique neoantigens arise from somatic mutations in patients with gastrointestinal cancers,* Cancer Discov. 2019. 9(8): 1022-1035.

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