EXCLUDING TREG EPITOPES AND INTEGRATING CD8 AND CD4 EFFECTOR NEOEPIPOSE CONTENT IMPROVES PROGNOSTIC BIOMARKER TOOL IN BLADDER CANCER

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Background Improvement of current prognostic biomarkers will enhance our ability to identify cancer patients at higher risk of recurrence and will further advance the personalization of patient monitoring and treatment. We hypothesized that the presence of a mutation alone is not sufficient to generate an immunogenic neoepitope, but that significant differences must exist between the Human Leukocyte Antigen (HLA)- and/or T Cell Receptor (TCR)-interfaces of the neoepitope and its non-mutated form, or with other self-epitopes, in order to be recognized as non-self by the immune system. As such, cancer patient clinical outcomes may be better understood by neoepitope analyses that integrate these considerations.

Methods We analyzed large scale (n=412) bladder cancer genomic data from The Cancer Genome Atlas (TCGA) using Ancer, an automated machine-learning-based pipeline we designed for neoantigen screening and vaccine design. Ancer shares components with other commercial-grade screening platforms used routinely in immunogenicity assessments of biologics and infectious disease antigens, such as the EpiMatrix algorithm for HLA-I and HLA-II neoepitope identification, and the JanusMatrix algorithm for tolerated, tolerogenic, and cross-reactive T cell epitope identification. Evaluation of patient survival with Ancer was compared to other analyses employing tumor mutational burden (TMB) or neoepitopes identified with the commonly used NetMHCpan-4.0 and NetMHCIIpan-3.1 T cell epitope prediction tools.

Results We stratified bladder patients based on their Ancer HLA-I and HLA-II neoepitope burdens and observed significantly prolonged disease free and overall survival in patients whose tumor contained both high numbers of HLA-I and HLA-II neoepitopes compared to other individuals. Stratifications performed with Ancer were superior to comparative analyses performed with TMB or with NetMHCPan and NetMHCIIpan. In addition, we showed that Ancer’s precise filtering and characterization of mutated epitopes contributed to the increased association with survival, as showcased by gradual improvements in survival analyses performed after each of its filtering step. Multivariate survival analyses indicated that Ancer neoepitope content remained a significant factor in patient overall survival even when adjusted for TMB, and other clinical covariates such as age at diagnosis and disease stage, unlike analyses involving NetMHCPan and NetMHCIIpan neoepitopes.

Conclusions Our analysis suggests that enhanced presence of CD8, CD4 T cell epitopes, and limited inclusion of Treg epitopes in the tumor genome plays a key role in cancer survival. Ancer scoring provides a predictive method for predicting patient outcomes, by defining the number of true neoepitopes and by identifying Treg epitopes that would interfere with T cell-based immune activation and response to the tumor.

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INTEGRATING DEEP PROTEOMICS PROFILING WITH SURVIVAL ANALYSIS TO IDENTIFY NOVEL BIOMARKERS OF RESPONSE TO PD-1 BLOCKADE IN NSCLC PATIENTS

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Background Immune checkpoint inhibitors have improved clinical responses and overall survival for patients with non-small cell lung cancer (NSCLC). However, the response is not equal and known NSCLC biomarkers are not sufficient in predicting therapy outcome. Deep proteomic analysis of NSCLC patient’s plasma treated with anti-PD-1-blockade using a state-of-the-art data independent acquisition mass spectrometry (DIA-MS) is a powerful and unbiased way of identifying protein signatures associated with disease stage or response to treatment. However, to unravel these associations large-scale omics data should be analyzed with respect to available clinical information. To achieve this goal, we have used an approach previously applied by Uhlen et al., 20171 for transcriptomic datasets. In this approach survival data is used to set the most optimal thresholds for candidate biomarkers.

Methods 125 plasma samples were analyzed by capillary flow liquid chromatography coupled to DIA-MS. Data were extracted with latest SpectronautTM and proteins were quantified. Each recorded protein intensity was used as a threshold for two groups of samples for which Kaplan-Meier estimates were generated using ‘survival’ package in R. Benjamini-Hochberg correction was applied and p-values with corresponding intensity cut-offs were extracted to generate panels of potential biomarkers.

Results 125 plasma samples (in total 75 baseline and 50 after 8-weeks treatment) from advanced NSCLC patients treated with an anti-PD-1 inhibitor following at least 1 prior line of treatment were analyzed. 727 unique proteins were quantified across all samples. Data analysis was performed separately for each line of treatment and treatment status resulting in more than 100,000 p-values. For each group, panels of proteins with best performance in separating progression free survivals were defined at FDR of 0.10, giving 64 unique proteins which were mapped to acute phase response, platelet degranulation and complement activation. Several of these proteins were listed in the Early Detection Research Network database of the National Cancer Institute, and one of them – LYPD3 – was a potential therapeutic target in a preclinical study for NSCL treatment.2 Selected proteins were then used to cluster patients into cohorts that showed association with the response to therapy.

Conclusions Deep proteomic profiling of plasma samples using DIA-MS in conjunction with clinical outcome enables a holistic and stringent analysis of potential circulating biomarkers. Such analysis generates functional insights into the plasma proteome that enable deeper understanding and comprehensive integration of clinical data with proteomics markers at different disease stages and treatment phases.

REFERENCES

Background Tumor mutational burden (TMB), as measured by exome or panel sequencing of tumor tissue (tTMB) or blood (bTMB), has been identified as a potential predictive biomarker for treatment benefit in patients with various cancer types receiving immunotherapy targeting checkpoint inhibitors (e.g. PD-1, PD-L1, CTLA-4). However, significant variability in TMB measurement has been reported due to differences in pre-analytical and laboratory methods, panel size, number of genes covered and bioinformatics pipelines. Reference standards have been proposed and evaluated for tTMB analysis by the Friends of Cancer Research (FoCR) to enable harmonization and standardization across different tTMB panel providers. Reference standards for bTMB are likely to be even more important given the unique challenges and higher sensitivity required for bTMB assays.

Methods Contrived bTMB reference materials with 0.5% and 2% tumor content were developed using RNA from tumor cell lines and donor-matched lymphoblastoid cell lines fragmented and size-selected to mimic cell-free DNA with TMB scores of 7, 9, 20 and 26 mut/Mb. Mutation coverage, mutant allele frequency (MAF) and bTMB scores were assessed using the PredicineATLAS and GuardantOMNI next-generation sequencing (NGS) platforms.

Results The DNA fragment size for the contrived samples was similar to naturally occurring circulating cell-free tumor DNA and mutation patterns were aligned with those from parental tumor lines. As anticipated, low frequency artefactual MAF variants were observed, requiring removal by bioinformatic filtration. For samples with 2% tumor content, standards for 7, 20 and 26 mut/Mb were found to have as-expected bTMB scores across both evaluation platforms, with good reproducibility, following removal of low frequency MAFs. Results for 0.5% tumour content were also promising, although with greater variability in post-filtration bTMB scores observed.

Conclusions The findings demonstrate it is feasible to produce bTMB reference standards across a range of bTMB levels. The data highlight the importance of data filtration to account for underlying low MAFs in such cell-line derived samples and that this reference material can control for variant sensitivity though not variant specificity. bTMB reference standards reported here could support the calibration and validation of bTMB platforms and help harmonization between panels and laboratories, thus improving the accuracy of testing to aid treatment decisions in oncology.

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