71 WHOLE EXOME SEQUENCING OF INDIVIDUALS PRESENTING EXTREME PHENOTYPES OF HIGH AND LOW-RISK OF DEVELOPING TOBACCO-INDUCED LUNG ADENOCARCINOMA: RELEVANCE OF IMMUNE AND DNA-REPAIR RELATED PATHWAYS

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Background Individual susceptibility to carcinogens may depend on genetic background. We performed for the first-time Whole Exome Sequencing (WES) of germline DNA from individuals presenting phenotypes of extreme sensitivity and resistance to developing tobacco-induced lung adenocarcinoma, in order to characterize the genetic background associated with these relevant phenotypes.

Methods We performed WES of germline DNA from heavy smokers (≥15 pack-years) who either developed lung adenocarcinoma at an early age (≤55 years, extreme cases, n=50) or did not present lung adenocarcinoma or other tumors at an advanced age (≥72 years, extreme controls, n=50). We selected non-synonymous variants (missense and non-sense) located in the coding regions and consensus splice sites of the genes showing significantly different allelic frequencies between both cohorts. We validated our results in germline data from 52 additional extreme cases selected from TCGA using the same criteria (diagnosis of lung adenocarcinoma at ≤55 years, tobacco consumption ≥15 pack-years).

Results The mean age for the extreme cases and controls was respectively 49.7 and 77.5 years. Mean tobacco consumption was 43.5 and 54.4 pack-years. We identified 619 significantly different variants between both cohorts, and we validated 107 of these in 52 extreme cases selected from TCGA (mean age 49.3 years, mean tobacco consumption 37 pack-years). Nine validated variants, located in relevant cancer related genes, such as PARP4 (DNA repair), HLA-A (antigen presentation) or NQO1 (detoxification) among others, achieved statistical significance in the False Discovery Rate test (FDR) (table 1). The most significant validated variant (p=4.48 × 10-5) was located in the tumor-suppressor gene ALPK2. The Reactome Pathway Database analysis showed that the genes harboring the most significant validated variants were significantly related to antigen processing and presentation, interferon and cytokine signaling and immune regulation, also achieving statistical significance in the FDR test (table 2).

Conclusions We describe for the first time genetic variants associated with extreme phenotypes of high and low-risk for the development of tobacco-induced lung adenocarcinoma, assessed with WES. The most significant validated variants were related with antigen presentation, immune regulation and DNA repair. Our results and our strategy warrant further development to characterize these clinically relevant phenotypes.

Ethics Approval The study was approved by the Investigational Review Board of Clinica Universidad de Navarra, approval number 021/2009.

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Abstract 71 Table 1 Most significant validated variants.

Gene	Gene family and function	Variant	p identification	p validation	FDR p validation
PARP4 (NM_006437)	Poly-ADP-ribose polymerases. Maintenance of genomic stability	c.T3194C/p.V1065A	0.044238228	0.000229805	0.017957651
GSDMB (NM_018530)	Gasdermins. Regulation of cell proliferation and differentiation, and programmed cell death	c.C865T/p.P289S	0.04946443	0.000294914	0.020164734
ZNF761 (NM_001289951)	Zinc fingers. Transcriptional regulation, ubiquitin mediated protein degradation, signal transduction, actin targeting, DNA repair, cell migration, etc.	c.G1582A/p.G528S	0.014883284	0.000523869	0.028640922
VWA3A (NM_173615)	Von Willebrand factor. Regulation of hemostasis and thrombosis.	c.G1637A/p.C546Y	0.003787218	0.000574843	0.028640922
ZNF717 (NM_001128223)	Zinc fingers. Transcriptional regulation, ubiquitin mediated protein degradation, signal transduction, actin targeting, DNA repair, cell migration, etc.	c.1298T/p.S433I	0.048581632	0.00061091	0.028640922
ISCU (NM_001301140)	Iron-sulfur cluster assembly enzyme). p53 regulated maintenance of iron homeostasis	c.C35T/p.A12V	0.00374117	0.00062832	0.028640922
NQO1 (NM_001286137)	NAD(P)H:Quinone Oxidoreductase. Detoxification and bioactivation of quinones and reactive oxygen species	c.C343T/p.P115S	0.030727149	0.000696929	0.029324634
ZNF761 (NM_001289951)	Zinc fingers. Transcriptional regulation, ubiquitin mediated protein degradation, signal transduction, actin targeting, DNA repair, cell migration, etc.	c.G1807C/p.E603Q	0.017176972	0.000867587	0.033897847
HLA-A (NM_001242758)	Human leukocyte antigen. HLA mediated antigen presentation.	c.C453A/p.N151K	0.009672224	0.001077238	0.039283293

Abstract 71 Table 2 Reactome pathway database analysis of pathways related to the genes that harbor the validated variants

Pathway name	p value	FDR
Antigen Presentation: Folding, assembly and peptide loading of class I MHC	1.11E-16	3.44E-15
Endosomal/Vacuolar pathway	1.11E-16	3.44E-15
ER-Phagosome pathway	1.11E-16	3.44E-15
Antigen processing-Cross presentation	1.11E-16	3.44E-15
Interferon gamma signaling	1.11E-16	3.44E-15
Interferon alpha/beta signaling	1.11E-16	3.44E-15
Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell	4.3E-14	1.12E-12
Interferon Signaling	3.46E-13	7.95E-12
Class I MHC mediated antigen processing & presentation	9.95E-12	1.99E-11
Adaptive Immune System	1.72E-6	3.09E-5
Cytokine Signaling in Immune system	1.31E-4	2E-3

72 ROUTINE USE OF COMPREHENSIVE GENOMIC PROFILING TO ASSESS TUMOR MUTATIONAL BURDEN ACROSS A COMMUNITY HEALTH SYSTEM

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Background Tumor mutational burden (TMB), defined as the average number of somatic mutations per megabase (mut/Mb) of DNA in tumor cells, has emerged as a predictive biomarker for response to immune checkpoint inhibitor (ICI) therapy. With more widespread adoption of comprehensive genomic profiling (CGP) assays in the clinic, it is now possible to routinely assess TMB across a wide variety of advanced cancers. Here we performed a retrospective study of routine TMB results assessed from CGP testing across a large community health system to reveal novel insights into the proportion of patients that may benefit from ICI treatment.

Methods Patients in the Providence St. Joseph Healthcare system diagnosed with advanced or metastatic solid tumors and tested for TMB using CGP tests (TruSight Oncology 500, research use only) between July 2019 and July 2020 were considered in this study. Deidentified electronic medical record data and CGP results were abstracted for downstream study.

Results A total of 1300 patients had one or more CGP tests with a TMB calculation. The median age of patients was 66 years, 51% were female, and 59% were white. TMB values ranged from 0–536 mutations per mut/Mb. Across tumor types, the proportion of patients with TMB ≥10 mut/Mb was 26% (n=341) and with TMB 5–9 mut/Mb was 27% (n=353). The proportion of patients with TMB ≥10 mut/Mb varied by tumor type: Melanoma (60%), NSCLC (42%), CRC (24%), pancreatic (5%). Of all the TMB-tested patients, 90 (7%) received IO therapy post testing. IO therapy use was highest among patients with TMB ≥10 mut/Mb (12%), followed by 7% with TMB of 5–9 mut/Mb, and 4% with TMB of 0–5 mut/Mb. Twenty-nine percent of TMB ≥10 also had high PD-L1 expression by IHC as compared to 8% of TMB <10. ICI therapy choice in this retrospective cohort appeared to be largely driven by other considerations (PD-L1 immunohistochemistry etc.) independent of TMB.

Conclusions A minority of TMB ≥10 patients assessed in this study received an ICI therapy, a result that is likely reflective of the lack of definitive guidelines for this emerging biomarker. As the adoption of TMB increases as a biomarker of

immunotherapy response, there is a greater need to expedite the standardization of sample collection, processing, and bioinformatics in TMB assessment.

Ethics Approval This study was approved by the Providence St. Joseph Health Institutional Review Board, approval number STUDY2019000048.

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73 ORTHOGONALLY AND FUNCTIONALLY VALIDATED ALGORITHM FOR DETECTING HLA LOSS OF HETEROZYGOSITY

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Background Human leukocyte antigen (HLA) genes facilitate communication between tumor cells and the immune system through the cell surface presentation of a diverse set of peptides. HLA loss of heterozygosity (LOH) has been associated with reduced immune pressure on neoantigens and impaired response to checkpoint blockade immunotherapy. Although HLA LOH is emerging as a key biomarker for response to immunotherapy, few tools exist to detect HLA LOH. Moreover, the accuracy of these tools is not well understood due to lack of orthogonal validation approaches. Here, we briefly describe DASH (Deletion of Allele-Specific HLAs), an algorithm to detect HLA LOH from exome sequencing data, and present a three-pronged validation approach to assess its performance.

Methods In-silico evaluation of the limit of detection (LOD) of DASH was performed by deeply sequencing a tumor-normal paired cell line with HLA LOH and mixing reads at different proportions to simulate variable tumor purity and clonality. Direct genomic validation was performed using digital PCR (dPCR) with allele-specific primers targeting both predicted kept and lost alleles in ten patient samples and one cell line. Quantitative immunopeptidomics was performed to compare peptides presented by HLA alleles in tumor cells and adjacent normal cells. The relative increase or decrease of peptide presentation per allele was estimated by predicting the binding of each peptide to the patient-specific alleles.

Results DASH is a machine learning model built upon the HLA-enhanced ImmunoID NeXT Platform®. We validated the performance of DASH using three orthogonal approaches to better understand the factors driving sensitivity and specificity of the algorithm. Evaluation using cell line mixtures that simulate LOH at various dilutions helped establish the LOD of DASH. For fully clonal tumors, DASH had 100% sensitivity at all tumor purity levels above 8% and 100% specificity at tumor purity levels higher than 24%. Patient-specific and allele-specific dPCR assays provided sensitive, direct evidence of HLA LOH. All samples predicted to have HLA LOH by DASH with high confidence were confirmed by dPCR. Finally, a quantitative immunopeptidomics experiment in one patient with HLA LOH revealed a large decrease in the peptides presented by deleted alleles, revealing the functional implications of HLA LOH.

Conclusions HLA LOH detection methods need to be rigorously validated in order to be used as a clinical biomarker. Here, we introduced three methods to assess performance, demonstrated the strong predictive power of DASH, and highlighted the need to consider tumor purity in such assessments.

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74 NOVEL APPROACH FOR PROFILING IMMUNE-TUMOR CELL INTERACTIONS AND MUTATIONS IN THE SAME TUMOR SECTION BY MULTIPLEX IMMUNOHISTOCHEMISTRY AND NGS IN IMMUNO-ONCOLOGY TRIALS

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Background Both proteins (e.g., PD-L1 IHC) and tumor mutation burden (NGS-based) are known to independently predict clinical response to anti-PD-1/PD-L1 therapies. In a meta-analysis of tumor specimens from 8135 patients treated with PD-1/PD-L1 blockers, multiplex fluorescence immunohistochemistry (mFIHC) had significantly higher diagnostic accuracy than PD-L1 IHC, tumor mutational burden (TMB), or gene expression profiling alone in predicting clinical response[1] or equivalent to a multimodality approach (e.g., PD-L1IHC + TMB). While the benefits of combining mFIHC (tumor-immune interplay) and NGS approaches in selection of patients for next generation immunotherapies is appealing, tumor tissue is a key limiting factor for multimodality analyses in clinical trials. To address this critical limitation, we developed a novel approach for sequential profiling of tumor and immune cell interactions by 7-parameter mFIHC assays, followed by analyses of nucleic acid extracted from same tissue sections.

Methods Formalin-fixed paraffin-embedded (FFPE) tumor tissue and cell line blocks were sectioned, and then stained using mFIHC followed by isolation of nucleic acids, or direct isolation of total nucleic acids. NanoString, qPCR, and NGS were performed on isolated nucleic acids. Nucleic acid quality, transcript abundance, and TMB scores were compared before and after mFIHC staining.

Results mFIHC revealed a broad range of immune cell phenotypes and spatial interactions, including T cells, B cells, NK cells, monocytes, neutrophils, and their functional status. Isolation of testable quantities of DNA from mFIHC treated slides was achieved when using a DNA-only isolation method, and TMB scores were robust across tested conditions. Cell phenotypes identified by mFIHC were compared to TMB scores across the tested samples. Following mFIHC treatment, RNA yields were reduced relative to the non-mFIHC treated replicates, but still sufficient for optimal input into a 770-target NanoString gene expression panel. However, for mFIHC treated samples, transcript levels were not distinguishable from background for the assessed targets.

Conclusions In summary, integrating mFIHC testing and TMB analysis on the same samples allows for comprehensive biomarker evaluation. The real world benefits of the combined approach will be described in upcoming clinical trials.

REFERENCE

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