Pan-cancer survey of HLA loss of heterozygosity using a robustly validated NGS-based machine learning algorithm

#399

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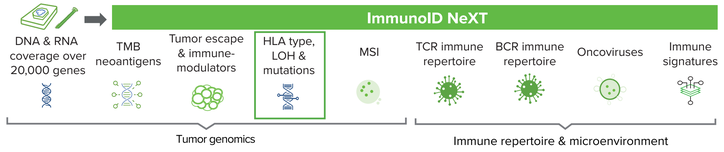
I. Background

HLA loss of heterozygosity (LOH) is increasingly being recognized as an important immune escape mechanism in response to checkpoint inhibitor therapy. HLA LOH reduces the repertoire of neoantigens displayed on the cell surface of cancer cells, limiting the efficacy of the immune system to detect and eliminate them. Though highly accurate HLA LOH detection algorithms are needed to allow clinical utility, the field lacks the robust, allele-specific validation approaches required to demonstrate performance. Moreover, algorithms of unknown sensitivity and specificity have led to significant discrepancies in the estimated occurrence of HLA LOH as an immune escape mechanism across tumor types. To address these challenges, we have developed a machine learning algorithm to detect HLA LOH called DASH (**D**eletion of **A**llele-**S**pecific **H**LAs) that is integrated into ImmunoID NeXT.

II. Augmented exome capture with ImmunoID NeXT

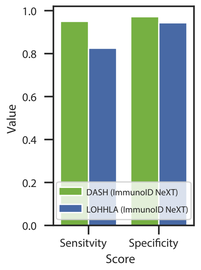
The ImmunoID NeXT platform provides joint tumor genomics and immune profiling from a paired tumor/normal sample. Through augmenting coverage of the HLA locus, ImmunoID NeXT also provides the data to accurately type HLA alleles, detect somatic mutations and probe copy number deletions in this highly polymorphic region.



III. Feature development and model performance

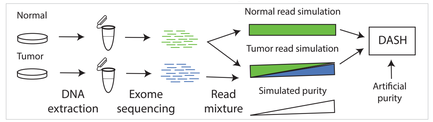
To build DASH, we profiled 279 patients on the ImmunoID NeXT Platform to create a training dataset. Our novel features, which account for allele-specific differences in exome probe capture and capitalize on our whole exome platform by including information about copy number alterations in the regions flanking the HLA genes, were used to train an XGBoost model. We benchmarked our performance on our held-out dataset against LOHHLA, a publicly available algorithm, and demonstrated superior sensitivity and specificity. Both algorithms were tested using the ImmunoID NeXT platform.

A. Performance

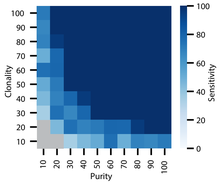


IV. Assessing sensitivity and specificity with cell line mixtures

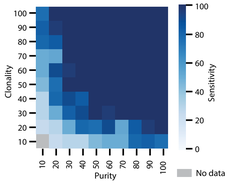
A. In silico cell line methodology



B. Cell line #1



C. Cell line #2

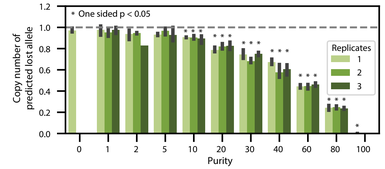


Orthogonal, allele-specific validation is required to accurately assess sensitivity and specificity for clinical utility. Thus, we profiled over 30 paired tumor-normal cell lines on the ImmunoID NeXT Platform® and identified four cell lines with HLA LOH. Using in silico mixtures for two of these cell lines, we found 100% specificity across all levels of tumor clonality and purity. Moreover, we found 100% sensitivity for tumors with at least 20% tumor content. In comparison, LOHHLA's sensitivity dropped dramatically across all tumor purities for events that were less than 80% clonal.

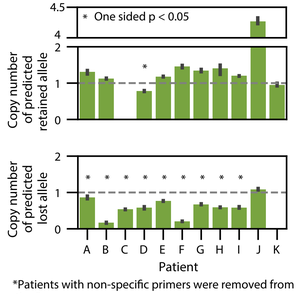
V. Digital PCR validation

A. Digital PCR methodology

B. Limit of detection assay



C. Tissue performance



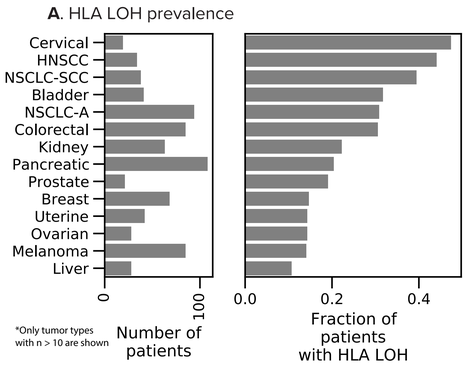
*Patients with non-specific primers were removed from the figure.

We designed a digital PCR (dPCR) assay using patient-specific and allele-specific primers that target a single HLA allele while avoiding all other HLA alleles. We tested the limit of detection of the assay using mixtures of 'Cell line #2' from above and found that the assay was sensitive to detect HLA LOH when 10% of the DNA was derived from the tumor with the event. Given the robustness of the analytical methods, we performed dPCR with patient-specific primers on 11 tumor and normal sample pairs. 20 out of 22 primers were found to be specific in the detection of the desired allele. Nine out of ten patients with predicted HLA LOH by DASH were confirmed to have HLA LOH with the digital PCR assay.

VI. Prevalence of HLA LOH across tumor types

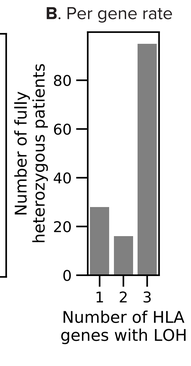
After establishing the high sensitivity and specificity of DASH, we profiled 766 patient samples spanning 23 tumor types on the ImmunoID NeXT Platform. The fraction of tumor samples with at least one incidence of HLA LOH ranged from 47% of patients in cervical cancer to 11% of patients in liver cancer, with 24% of patients pan-cancer having at least one HLA LOH event. Moreover, we show that patients with HLA LOH most often lose all three genes and least commonly lose only one gene.

A. HLA LOH prevalence



*Only tumor types with n > 10 are shown

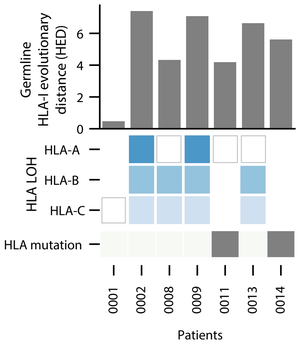
B. Per gene rate



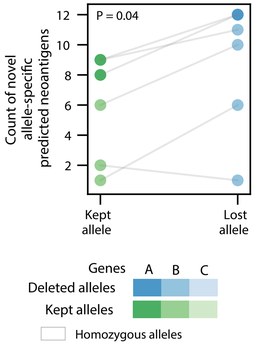
VII. Neoantigen Expansion

To explore the mechanistic impact of HLA LOH in response to ICIs, we studied a cohort of nine head and neck carcinoma patients who received a single dose of nivolumab, and sequenced pre- and post treatment tumor biopsies for each patient. With DASH, we detected HLA LOH in four of the patients, pre treatment. These patients had diverse HLAs and no somatic mutations in HLA genes. Moreover, we found that novel neoantigens post-treatment were more often predicted to bind to the deleted HLA alleles than the kept alleles (predicted with method described in #1898), suggesting the evolutionary force of HLA LOH as a resistance mechanism during ICI therapy.

A. HLA variation in cohort



B. Novel neoantigens



VIII. Conclusion

In summary, we developed an HLA LOH detection method and demonstrated its specificity and specificity using tumor cell line mixtures and patient-specific digital PCR. Using DASH on cancer patient tumor samples, we exposed widespread HLA LOH across tumor types and observed the mechanism of immune escape in response to immunotherapy.

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