Sensitive HLA loss of heterozygosity detection reveals allele-specific neoantigen expansion as resistance mechanism to anti-PD-1 therapy

#6678

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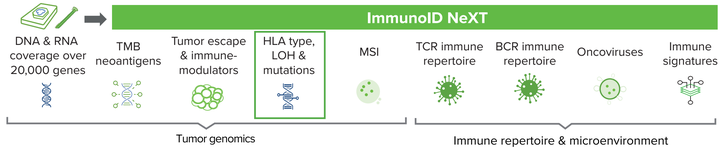
I. Background

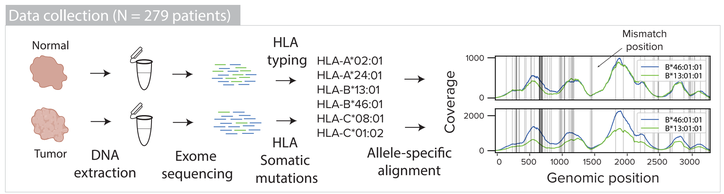
Loss of heterozygosity (LOH) in the HLA locus is increasingly being recognized as an important mechanism of immune escape and a proposed biomarker for immunotherapy response. Neoantigens that bind to a deleted HLA allele will no longer be presented to the immune system, potentially allowing subclones with these deletions to escape immune surveillance. Despite interest in the field, few methods exist to detect HLA LOH, and their sensitivity is not well understood. Moreover, the mechanistic impact of HLA LOH in response to immune checkpoint inhibitors (ICI) remains unexplored.

II. Augmented exome capture with ImmunoID NeXT

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



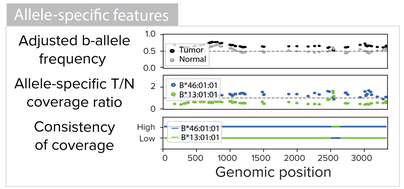
III. Alignment of reads to patient-specific reference



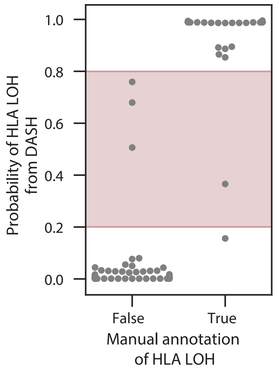
We performed exome sequencing with the ImmunoID NeXT Platform on tumor and normal samples from 279 patients to create a training dataset for our model. For each patient, we detected germline HLA types and somatic HLA mutations. Then, we mapped their tumor and normal reads to their patient-specific HLA reference and used this information as input for our machine learning features. Finally, we visualized each allele pair and manually annotated LOH.

IV. Feature development and model performance

A. Explanation of features



B. Performance of DASH

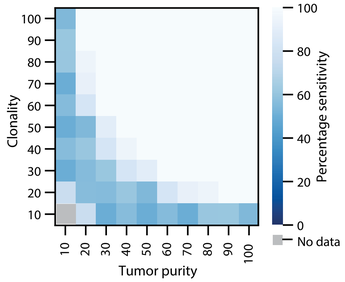


As input to our machine learning model, we collected six features for each pair of heterozygous genes: adjusted b-allele frequency, allele-specific tumor-normal ratio, consistency of coverage, tumor purity, tumor ploidy and deletion of flanking regions. Then, we trained an XGBoost model, **D**eletion of **A**llele-**S**pecific **H**LAs (DASH), that could accurately discriminate between genes with and without HLA LOH. Ambiguous calls (shown in red) are enriched for samples with low tumor purity.

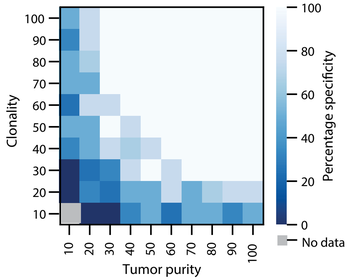
V. Assessing sensitivity and specificity with cell line mixtures

We assessed the limit of detection of DASH in both low clonality and low tumor purity settings using a tumor-normal paired lymphoblast cell line that has HLA LOH. After deeply sequencing both cell lines, we mixed the reads across a range of ratios to simulate the potential spectrum of purities and clonalities. Across the ranges, we found 100% sensitivity when at least 18% of the reads came from the clone with HLA LOH, highlighting the accurate and low limit of detection of DASH.

A. Sensitivity of DASH



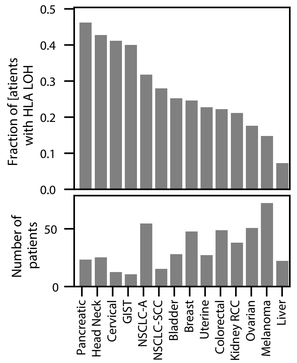
B. Specificity of DASH



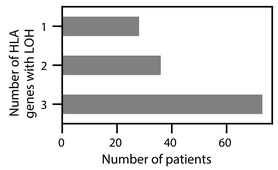
VI. Prevalence of HLA LOH across tumor types

To assess the pervasiveness of HLA LOH as an immune escape mechanism, we applied DASH to 504 tumors across 14 tumor types (described in #2512). The fraction of patients with at least one incidence of HLA LOH ranged from 48% of patients in pancreatic cancer to 8% of patients in liver cancer. We found the same incidence of HLA LOH in non-small cell lung cancer squamous cell carcinoma (NSCLC-SCC) as compared to a previous study (29%, McGranahan et al.) but found a much lower incidence in non-small cell lung cancer adenocarcinoma (NSCLC-A) (33% and 61%, respectively).

A. Prevalence of HLA LOH



B. Number of genes with LOH

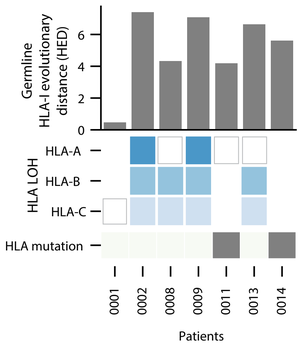


Moreover, we show that patients with HLA LOH most often lose all three genes and least commonly lose only one gene.

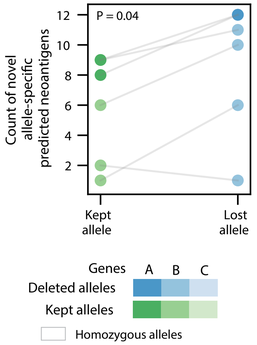
VII. Neoantigen expansion in response to checkpoint therapy

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 #2085), suggesting the evolutionary force of HLA LOH as a resistance mechanism during ICI therapy.

A. HLA variation in cohort



B. Novel neoantigens



VIII. Conclusion

We developed a sensitive method to detect HLA LOH and observed neoantigen expansion to deleted HLA alleles in response to ICI therapy, emphasizing the limitations of deleted alleles to ignite an immune response. Moreover, we found widespread occurrences of HLA LOH across tumor types, highlighting the importance of accurate HLA LOH detection as a pan-cancer biomarker.

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