Background
Typical liquid biopsy panels offer a limited understanding of tumor biology, potentially under-representing the heterogeneity of resistance in late-stage cancers. Here, diminished scope can result in undetected, therapeutically-relevant biomarkers which respond dynamically to treatment, as well as potentially missed resistance mechanisms and pathway-level events. To address the challenges associated with identifying multiple concurrent heterogeneous resistance mechanisms in individual patients, we evaluated longitudinal exome-scale tumor-informed cell-free DNA (cfDNA) data from head and neck squamous cell carcinoma (HNSCC) patients receiving anti-PD1 therapy.

Methods
Pre- and post-intervention matched tumor, normal and plasma samples were retrospectively obtained from 15 stage II-IV HNSCC patients. Following baseline sample collection, all patients received a single dose of nivolumab or pembrolizumab. The primary tumor was then resected approximately one month later when possible, or a second biopsy collected where resection was impractical. Paired tumor and normal samples were then profiled using ImmunoID NeXT Platform®, an augmented exome/transcriptome platform and analysis pipeline. Exome-scale cfDNA profiling of matched plasma samples was performed using the NeXT Liquid BiopsyTM platform to detect somatic variants.

Results
Patient neoantigen presentation score (NEOPSTM) rapidly and significantly contracted following therapy (p=.00098). Novel neoantigens arising post-treatment which were predicted to be presented on lost HLA alleles were significantly higher in patients with longer overall survival (p=.019). Variant detection across same-patient serial cfDNA samples revealed significantly correlated VAFs (R=.62, p<.0001) despite significant contraction of mutational burden in solid tumor (p=.0039), suggesting complex clonal/subclonal dynamics. Investigation of the evolving tumor and cfDNA subclonal architecture revealed significant association between decreasing cellular prevalence and NOTCH signaling (q=.001) and the innate immune system (q=.002), while increasing cellular prevalence was associated with p53 signalling (q=.02) and hypoxia (q=.02). These findings were complimented by transcriptomic data which showed significant enrichment of multiple immune pathways across treatment.

Conclusions
We found that immune checkpoint blockade precipitates rapid evolution of the HNSCC tumor microenvironment. By leveraging comprehensive, tumor-informed liquid biopsy data we were able to identify contracting cellular populations enriched for NOTCH pathway mutations. Longer OS following either intervention was associated with an expansion of novel neoantigens predicted to be presented by lost HLA alleles. Our results suggest that tumor-informed liquid biopsy provides a more robust understanding of therapeutic response and resistance mechanisms than that attainable with typical liquid biopsy panels alone.

Ethics Approval
This study obtained ethics approval from Human Subjects Research at Stanford University. ID number is 40425. All participants gave informed consent prior to enrollment.

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