Tumor-informed liquid biopsy monitoring of evolving therapeutic resistance mechanisms in head and neck squamous cell carcinoma patients receiving anti-PD-1 therapy

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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.

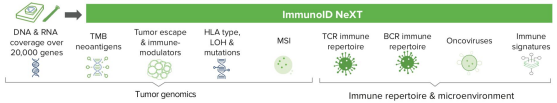
Methods

Cohort

Pre- and post-intervention matched tumor, normal and plasma samples were obtained from a cohort of 15 patients with stage II-IV HNSCC. After baseline sample collection, all patients received a single dose of nivolumab. The primary tumor was then resected, approximately one month later when possible, or a second biopsy was collected where resection was impractical.

Solid tumor

Paired tumor and normal samples were profiled using the ImmunoID NeXT Platform®, an augmented exome/transcriptome platform and analysis pipeline which produces comprehensive tumor mutation information, gene expression profiling, neoantigen characterization including our composite neoantigen burden score NEOPS™, HLA typing and allele-specific loss of heterozygosity (HLA LOH), TCR repertoire profiling, and tumor microenvironment profiling as outlined in the plot below.



Whole exome sequencing from plasma

cfDNA profiling of matched plasma samples was performed using the NeXT Liquid Biopsy™ platform. The enhanced exome assay and chemistry augment difficult to sequence genomic regions yielding more uniform, high average depth coverage (2000X) across the exome, with boosted coverage (5000X) for 247 clinically relevant genes. Sensitive variant detection across the exome was achieved using tools which incorporate an error correction model based on a panel of normal individual plasma samples, and well-characterized filters including a dedicated blacklist.

Results

Patient demographics

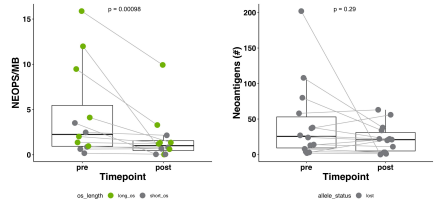
Characteristic	N	Overall, N = 14[1]	Non_responder, N = 9[1]	Responder, N = 5[1]	p-value[2]
Age	14	62.21 (13.66)	62.75 (16.68)	61.50 (9.71)	0.7
Sex	14				>0.9
F		6 (43%)	3 (38%)	3 (50%)	
M		8 (57%)	5 (62%)	3 (50%)	
Tumor site	14				0.5
Alveolar Ridge		2 (14%)	2 (25%)	0 (0%)	
Hard Palate		1 (7.1%)	0 (0%)	1 (17%)	
Maxillary Sinus		1 (7.1%)	1 (12%)	0 (0%)	
Oropharynx		1 (7.1%)	1 (12%)	0 (0%)	
Retromolar Trigone		1 (7.1%)	1 (12%)	0 (0%)	
Skin		4 (29%)	1 (12%)	3 (50%)	
Tongue		4 (29%)	2 (25%)	2 (33%)	
Stage	14				0.7
II		2 (14%)	1 (12%)	1 (17%)	
III		1 (7.1%)	0 (0%)	1 (17%)	
IV		11 (79%)	7 (88%)	4 (67%)	
p16 status	14				0.2
Negative		6 (43%)	5 (62%)	1 (17%)	
Positive		2 (14%)	1 (12%)	1 (17%)	
Unknown		6 (43%)	2 (25%)	4 (67%)	
Intervention	14				>0.9
Nivolumab		7 (50%)	4 (50%)	3 (50%)	
Nivolumab, resection		7 (50%)	4 (50%)	3 (50%)	

[1] Mean (SD); n (%)
[2] Wilcoxon rank sum test; Fisher's exact test

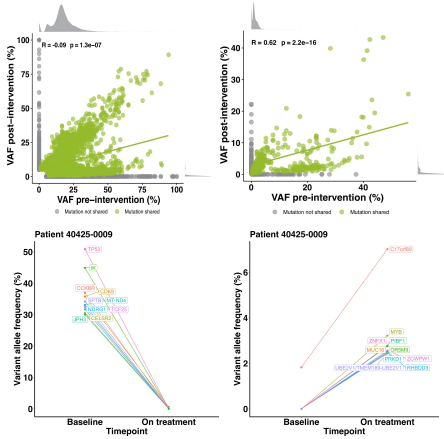
A total of 14 patients were classified into two groups, responder and non-responder, based on responses defined by RECIST criteria or histological assessment. Baseline characteristics including age, sex, tumor site, tumor stage, p16 status and intervention method were summarized for each group and presented as frequency distributions. Between groups p-value statistics were calculated. Baseline data for all comparisons were obtained before immunotherapy, at the time of initial biopsy. One patient stopped early due to biopsy-proven metastatic disease. A second patient with early recurrence and metastatic disease received subsequent chemoradiation (Cisplatin and 66Gy). Demographic data was unavailable for one patient.

Treatment-induced evolutionary pressure on neoantigens presented by lost vs kept alleles

Neoantigen presentation score (NEOPS), a neoantigen burden-based composite biomarker which incorporates damaging APM events and HLA LOH to more accurately model neoantigen presentation to the immune system, was calculated. NEOPS rapidly and significantly contracted following therapy (plot at left, p=0.00098), attaining greater significance than TMB (p=0.004). This increase can be understood when considering the change in neoantigens predicted to be presented on lost alleles, which do not significantly contract during therapy (plot at right, p=0.029). Novel neoantigens arising post intervention were significantly expanded in lost vs kept HLA alleles in patients with long OS (p=0.05), but not those with poor OS (p=0.70), suggesting impaired immune surveillance, and varied evolutionary pressure arising from immune checkpoint blockade.

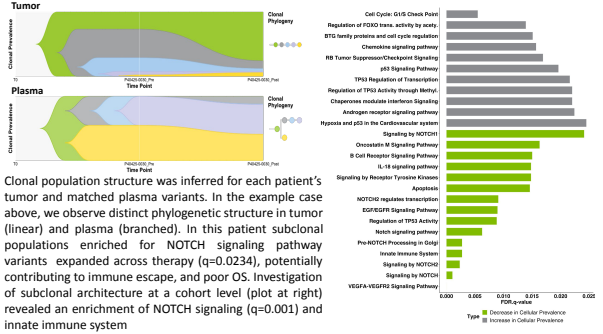


Treatment-induced temporal heterogeneity in solid and liquid biopsy



Variant detection across same-patient solid tumor pre- and post-treatment samples revealed two distinct populations; one that contracted in response to therapy, and another that persisted across intervention. This phenomenon is largely reduced when evaluating variants detected in the plasma compartment. When considering the most dynamic variants across intervention in a patient with long OS, we observe consistently decreasing VAFs in solid tumor, while In plasma the most dynamic variants are expanding. Additionally, we detected outgrowth of a new clone harboring a PSG6 variant, potentially preventing this patient from achieving complete response. Taken together, these findings suggest HNSCC tumors posses a complex subclonal architecture that is capable of rapid evolution in response to immune checkpoint blockade (ICB).

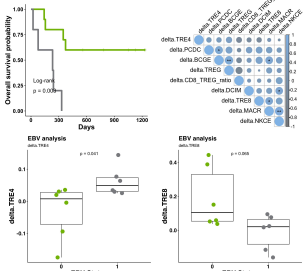
Clonality profiling in tumor and matched plasma



Clonal population structure was inferred for each patient's tumor and matched plasma variants. In the example case above, we observe distinct phylogenetic structure in tumor (linear) and plasma (branched). In this patient subclonal populations enriched for NOTCH signaling pathway variants expanded across therapy (q=0.0234), potentially contributing to immune escape, and poor OS. Investigation of subclonal architecture at a cohort level (plot at right) revealed an enrichment of NOTCH signaling (q=0.001) and innate immune system function (q=0.002) in contracting populations, and significant enrichment of p53 signaling (q=0.02) and hypoxia (q=0.02) pathway variants in clonal populations expanding during therapy.

Evolving immune landscape in response to intervention

Next, we investigated the tumor immune landscape and found that elevated pre-intervention TREG was significantly associated with longer patient survival (p=0.003), consistent with findings from previous studies. Numerous correlations were observed in therapy-induced changes to immune cell populations, including correlation between TREGs and B-cells, which were also significantly associated with longer OS (p=0.003). EBV infection status significantly influences response of CD4- (p=0.041) and CD8-positive T cell (p=0.065) populations to ICB, likely influencing immune surveillance and response in these patients.



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.

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