The oral microbiome and immune checkpoint inhibitor response in head and neck squamous cell carcinoma

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Background

Immune checkpoint inhibition has changed the treatment landscape for patients with head and neck squamous cell carcinoma (HNSCC) in both the localised and metastatic setting.[1] However, at best, responses are seen in approximately 35% of patients.[1] The contributing factors as to why some patients respond and some are resistant to immunotherapy are not fully understood.

Specific bacterial populations in the gut or the "gut microbiome" have been associated with increased response rates to immunotherapy in other cancers such as melanoma and lung cancer in humans and mouse models.[2-6] Interestingly, patients with melanoma who are not responding to ICI, who undergo a faecal transplant from patients who *are* responding to ICI (thus creating a new gut microbiome) subsequently respond to treatment.[7,8] This suggests that the microbiome has a significant role in ICI response and its function is not well understood.

Oral or *salivary* microbiome research in relation to treatment response is less advanced. Published literature has established associations with an increased risk of HNSCC with the presence or absence of certain bacterial species.[9-11] There is also data suggesting that periodontal disease, a chronic infective inflammatory process associated with specific bacterial species is also associated with increased risk of HNSCC.[12]

The oral microbiome is thus relevant to the inflammatory tumour microenvironment and may translate to a much-needed avenue to increase response to ICI through identifying bacterial species associated with responders and non-responders to ICI. There are currently no studies examining the association between the salivary microbiome and response to ICI.

A pilot study was conducted to (1) compare the microbial sequencing reads between different saliva sampling methods to identify the optimal collection method for larger scale studies; (2) examine potential differences in the pre-treatment and post treatment oral microbiome between responders and non-responders to ICI treatment

Methods

Free saliva and oral washes were collected from 5 patients with head and neck squamous cell carcinoma between 1st of January 2023 to 31st of December 2024. Demographic and oncological characteristics (age, sex, cancer type and stage, treatment and response to treatment) were collected through the electronic medical records. All patients received pembrolizumab for the treatment of their HNSCC.

Samples were taken before or during treatment with ICI and at tumour progression. Total DNA was extracted from oral biofluid samples before 16S rRNA gene amplification and sequencing with Ion Torrent PGM. The Human Oral Microbiome Database (HOMD) was used as the reference database for 16S rRNA identification of amplicon sequence variants. Levels of taxonomy was

defined to species-genus-phylum. Associations between species and genus and response and other clinical features were analysed.

Results

In the 2-year enrolment period samples from 15 patients, 5 of whom were analysed. Table 1 shows patient characteristics and immunotherapy response. All patients had either pre-treatment or during treatment samples as baseline. Patient 5 only had pre-treatment samples available for analysis, all other patients had both saliva samples and oral washes post treatment samples available. Median age of participants was 67 and all patients had metastatic disease at enrolment. All patients received pembrolizumab in combination with carboplatin doublet for the management of metastatic disease.

Table 1: Summary of patients enrolled

	Age (at first sample collection)	Sex	Primary HNSCC	Tumour present at time of sampling	Responder vs non responder
Patient 005	67	Male	Tonsillar	No	Responder
Patient 018	56	Male	Base of tongue	No	Non-responder
Patient 020	67	Male	Base of tongue	No	Responder
Patient 021	73	Male	hypopharynx	Yes	Non-responder
Patient 023	62	Female	Tonsillar	No	Non-responder

Sequencing was performed on the 9 saliva and 9 oral wash samples collected from the patients. **Figure 1** shows host versus microbial sequencing read counts. “S” and “W” denote saliva samples and oral washes respectively. From left to right the first two samples for each patient represent pre or during treatment samples and the last two samples represent post treatment collections. All samples were able to able to sequence both human and microbial sequences. Sample 18/02 W had small but detectable reads. **Figure 2** shows the fraction of microbial reads by either saliva or oral wash samples. There was a similar read fraction of microbial to human between the two different sampling strategies.

Figure 1: Host versus microbial sequencing read counts

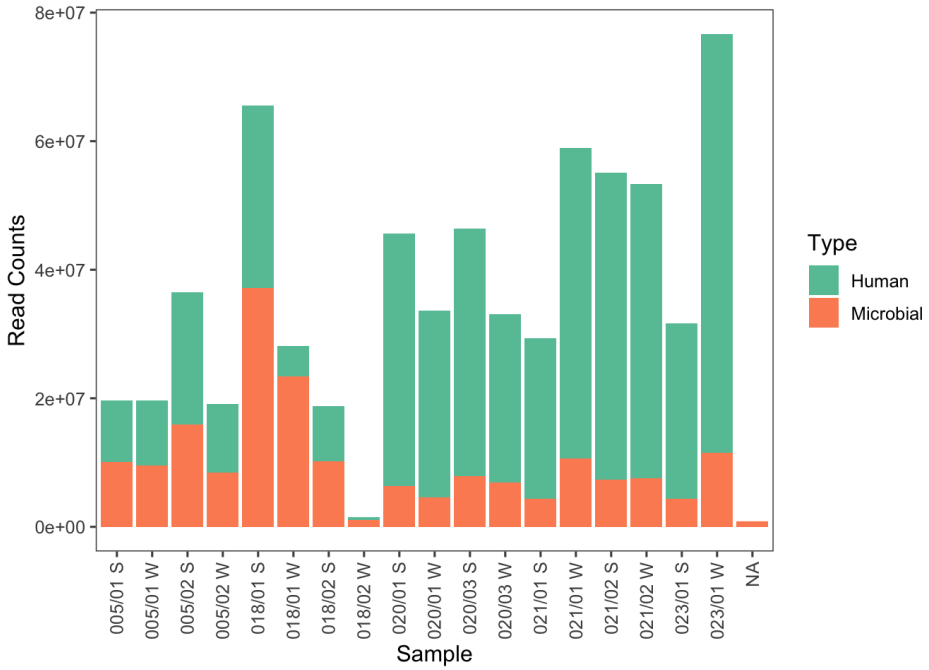
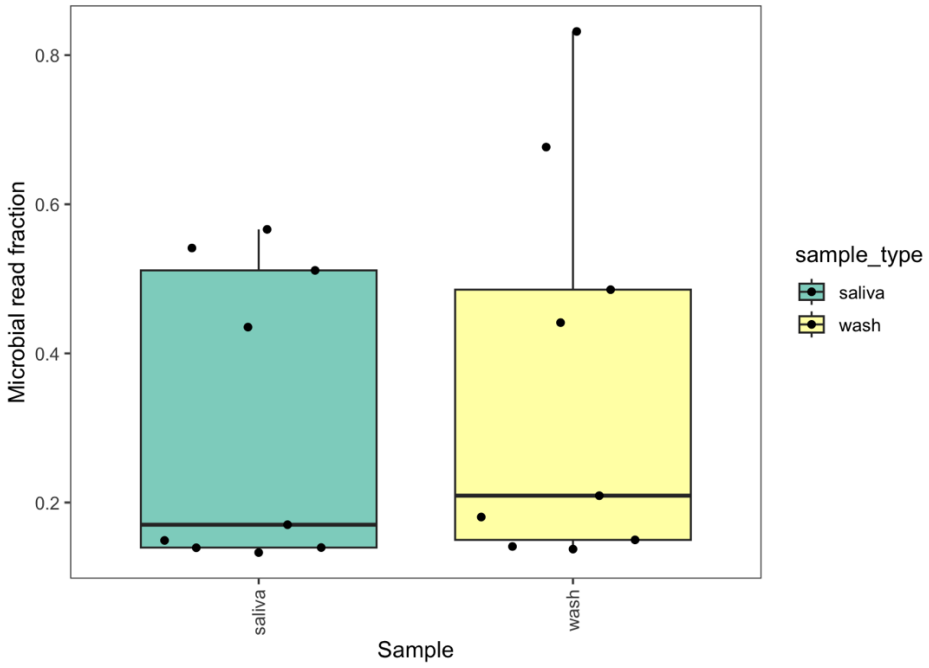


Figure 2 shows the fraction of microbial reads by sampling strategy



When looking for microbial difference between different groups within the participants, there was no strong signal between the following comparisons: responders (pre-treatment) versus non-responders (pre-treatment), and responders (pre-treatment) versus responders (post treatment) however, sample numbers were low. There was a signal for differences in the oral microbiome and the presence of an active tumour **(Figure 3).** To increase power, all samples

were combined, and the significant species after comparing responders vs non-responders are shown in **Figure 4.**

Figure 3. Oral microbiome differences based on tumour present at time of sampling

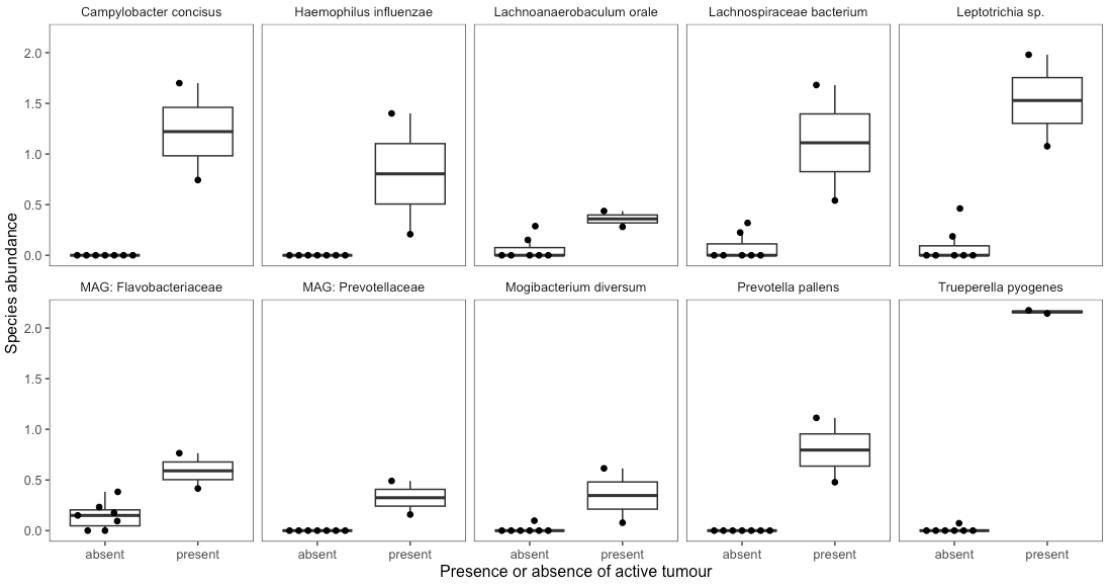
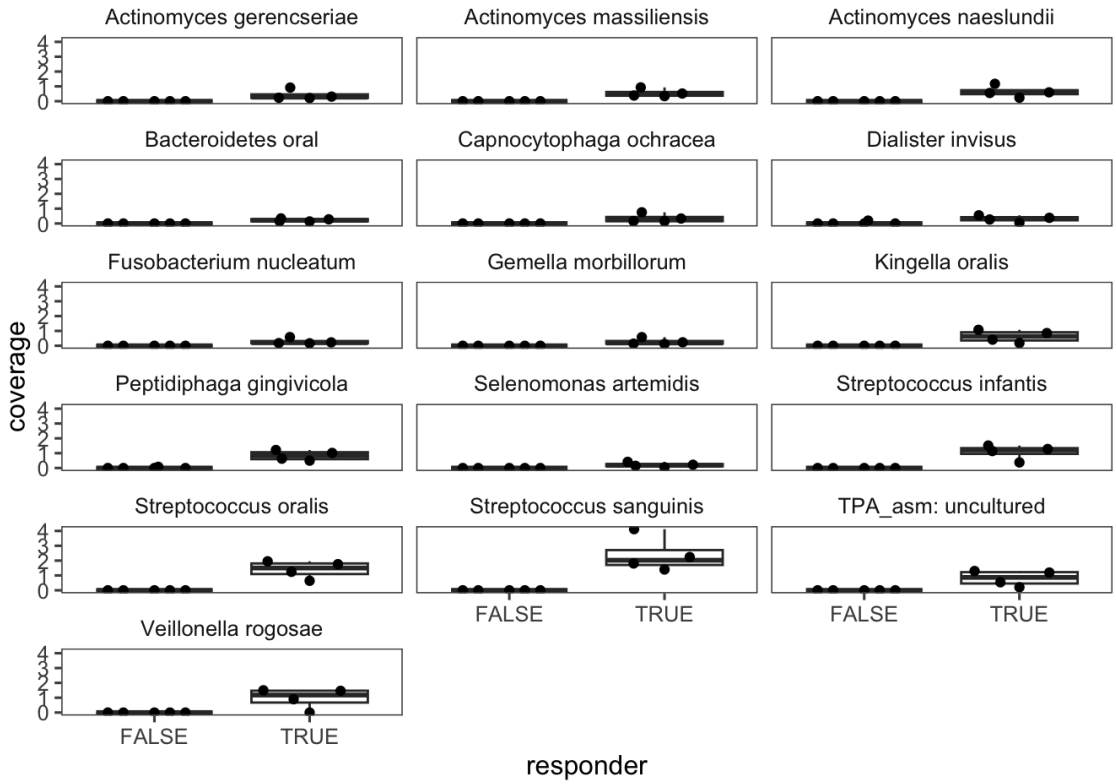


Figure 4. Oral microbiome differences in responders versus non-responders (all samples)



Discussion

This pilot study demonstrates that oral microbiome characterisation via 16S rRNA sequencing is feasible in HNSCC patients undergoing immunotherapy. All 18 samples (9 saliva, 9 oral wash) yielded both human and microbial DNA. Saliva and oral wash produced comparable microbial read fractions, suggesting the two collection methods are broadly interchangeable. Viable samples were obtainable even post-chemoradiotherapy, despite anticipated barriers such as xerostomia and trismus. This supports the feasibility of longitudinal salivary sampling across the treatment course.

The detection of a signal between oral microbiome composition and the presence of an active tumour (Figure 3) is biologically plausible and consistent with the broader literature linking oral dysbiosis to HNSCC. Combining all samples irrespective of timepoint did identify candidate differentially abundant species between responders and non-responders (Figure 4). However, this approach pools pre-treatment, on-treatment, and post-progression samples, limiting its interpretability. While exploratory, these findings support the rationale that the oral microbiome is not a passive bystander but may actively reflect, or interact with, the local tumour immune microenvironment and anticancer treatments.

Taken together these results confirm the feasibility of characterising the oral microbiome in HNSCC patients receiving immune checkpoint inhibitor therapy using minimally invasive saliva and oral wash sampling. Given the established links between oral dysbiosis and HNSCC risk, and precedent from the gut microbiome-immunotherapy literature in other cancers, the oral microbiome represents a promising and accessible avenue for future biomarker discovery in HNSCC.

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