Oral Oncology 81 (2018) 1–9

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

# Integrated genomic characterization of oral carcinomas in post-hematopoietic stem cell transplantation survivors

Glenn J. Hanna[a,*], Eric R. Kofman[a], Muhammad Ali Shazib[b], Sook-bin Woo[b,c], Brendan Reardon[a], Nathaniel S. Treister[b], Robert I. Haddad[a], Corey S. Cutler[d], Joseph H. Antin[d], Eliezer M. Van Allen[a,e], Ravindra Uppaluri[f], Robert J. Soiffer[d]

[a] Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, USA
[b] Department of Oral Medicine, Infection, and Immunity, Harvard School of Dental Medicine, Boston, USA
[c] Department of Pathology, Brigham and Women's Hospital, Boston, USA
[d] Division of Hematologic Malignancies, Dana-Farber Cancer Institute, Boston, USA
[e] Broad Institute, Massachusetts Institute of Technology, Cambridge, USA
[f] Department of Head and Neck Surgical Oncology, Dana-Farber Cancer Institute, Brigham & Women's Hospital, Boston, USA

ARTICLE INFO

*Keywords:*
Oral cancer
Bone marrow transplantation
Genomics
Immunology
Outcomes

ABSTRACT

*Objectives:* Secondary oral squamous cell carcinoma (OSCC) is a late complication in allogeneic hematopoietic stem cell transplantation (HSCT) patients, but little is known about long-term outcomes and prognostication. Additionally, molecular alterations and immunologic insights unique to this disease remain largely unexplored.
*Methods:* We present a cohort of 31 patients with post-HSCT OSCC and reported on clinicopathologic predictors of survival. Whole-exome sequencing was performed on 6 (19%) matched pairs of peripheral blood (post-conditioning, pre-HSCT) and tumor samples. The entire cohort had archival tumor available for immunoprofiling with PD-1/L1 immunohistochemistry.
*Results:* Five-year overall survival (OS) was 57% (95% CI: 46.1–69.8) with a median disease-free survival (DFS) of 13.3 months. Advanced initial staging, a buccal or oral tongue subsite, chronic oral graft-versus-host disease (GVHD) and smoking all negatively impacted survival. High tumor mutational burden (TMB) (median 11.3 vs. 5.0) and unique mutational signatures were noted between unrelated and related donor groups – with a strong correlation between infiltrating PD-1 + lymphocytes and TMB ($R = 0.98$, $p < 0.01$). Some differences were observed when comparing commonly mutated genes among our cohort and TCGA, with a predominance of TP53 events.
*Conclusion:* Survival outcomes appear similar in HSCT survivors with OSCC compared with non-HSCT OSCC populations. We identified somatic alterations in genes with therapeutic potential unique to this subpopulation of oral cancers.

## Introduction

Successes in hematopoietic stem cell transplantation (HSCT) over the last few decades to cure many patients of their underlying hematologic malignancies has resulted in a surge of patients followed long-term. To that end, it has become increasingly recognized that the risk of secondary cancers in survivors of allogeneic HSCT is high [1]. The most frequent secondary cancer is squamous cell carcinoma (SCC) of the oral cavity, representing a 16-fold higher risk among HSCT patients compared with the general population [2].

Studies have shown that chronic oral graft-versus-host disease (GVHD) is a major risk factor for the development of SCC. Additionally, patients who have received total body irradiation (TBI) as part of their conditioning regimen appear at increased risk [3]. Consensus guidelines support annual dental evaluations in these patients. Oral cancer surveillance including soft and hard palate exams (with radiographs as indicated) are recommended at least every 6 months while the patient remains on immunosuppression [4,5]. Treatment often involves surgical resection with adjuvant therapy considered for high-risk pathologic features. Our group has shown that these patients have a higher risk of recurrent and second primary oral malignancies [6] – suggesting more aggressive disease biology and a field cancerization effect,

* Corresponding author at: Instructor in Medicine, Harvard Medical School, Department of Medical Oncology, Center for Head & Neck Oncology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215, USA.
*E-mail address:* glenn_hanna@dfci.harvard.edu (G.J. Hanna).

https://doi.org/10.1016/j.oraloncology.2018.04.007
Received 5 February 2018; Received in revised form 15 March 2018; Accepted 7 April 2018

respectively. Despite these early observations, the underlying mechanisms of oral carcinogenesis among HSCT patients have not been elucidated. Chronic GVHD reflects a combined autoimmune and alloimmune inflammatory process, and it is hypothesized that T cell-mediated mucosal injury may predispose to genomic instability and eventual malignant transformation [7]. Here we present our institutional experience with a cohort of patients with oral cancer after HSCT matched with whole-exome sequencing data and immunologic parameters in a subset of patients to further elucidate mechanisms of oral carcinogenesis in this population.

## Methods

### *Study participants*

Thirty-one men and women with a diagnosis of biopsy-proven oral cavity squamous cell carcinoma (OSCC) following allogeneic HSCT for hematologic malignancy or an associated disorder, who had received treatment at our institution between 2004 and 2016, were identified retrospectively following institutional review board approval. Patient demographics, clinicopathologic features and treatment outcomes were recorded. All patients were in remission for their respective hematologic disorder with a median disease-free survival (DFS) from day 0 of HSCT of 7.3 years (range, 2.7–26.8). HPV status was reported if evaluated (n = 2), although testing is not standard at our institution for OSCC tumors. Of the 31 patients, 22 (71%) had adequate archival tumor material available for whole-exome sequencing (WES), but only 13 (42%) had matched peripheral blood samples obtained following conditioning but prior to HSCT. Matched peripheral blood samples were obtained in allogeneic HSCT patients following myeloablative conditioning but before stem cell infusion to minimize detection of circulating malignant clones. Of these 13 paired samples, a remaining 6 (19%) had adequate DNA and met strict quality metrics for sequencing. All 31 patients had formalin-fixed, paraffin-embedded (FFPE) archival tumor samples facilitating immunohistochemistry (IHC) to determine PD-1/L1 status.

### *Whole-exome sequencing (WES) and genomic analysis*

All sequenced patients consented to our institutional Cancer Research Study [8]. Of the 6 tumor samples adequate for sequencing, all were obtained from the primary oral cancer site. For FFPE tumors, hematoxylin-eosin–stained slides were prepared and reviewed by an oral maxillofacial pathologist [S.W.] to identify areas of > 20% tumor. WES was performed on DNA extracted from FFPE archival tumor and matched blood samples. Briefly, DNA was sequenced following Illumina ICE hybrid-capture to a minimum mean target coverage of 60X across all samples except for the tumor sample for case 11, which had a mean target coverage of 27X. Mutation Annotation Format (MAF) files containing all sequenced variants against matched normal tissue are included as supplemental material.

Variant calling for single nucleotide variants (SNVs) and indels (insertions and deletions) was performed on the Broad Institute's Firehose pipeline running Mutect 1.0 [9] and Strelka 1.0 [10], respectively. OxoG and FFPE filters were used to remove false positives arising from sequencing and handling artifacts, a panel of normal samples was referenced to filter out likely germline mutations, and deTiN was applied to filter out instances of tumor contamination of normal samples. The Oncotator tool [11] was used to annotate SNV and indel calls with gene information. Tumor mutational burden (TMB) was calculated by determining the number of non-synonymous somatic mutations that occur per megabase of exonic sequence data across all genes. Mutational signature analysis was performed with deconstructSigs [12], using COSMIC signatures [13].

ReCapSeg [14], a circular binary segmentation algorithm was used to estimate total copy number across the exome. This was then used in conjunction with approximately 10,000 known heterozygous sites to infer allele-specific copy number using AllelicCapseg. Absolute copy number, purity and ploidy were then determined using ABSOLUTE [15]. Supplemental Fig. 1 diagrams the WES workflow and method parameters employed.

### *Immunologic characterization*

Oral cancer tumor biopsies from 31 patients consisted of the primary tumor or excised regional lymph nodes. Tissue slides were prepared from FFPE tissue blocks [16]. Independent pathologic review of hematoxylin-eosin stained slides confirmed the presence of tumor in each case. IHC studies were performed with previously validated, in-house analytic PD-L1 (clone 9A11, mouse antibody) and PD-1 (clone EH33, mouse antibody) antibodies. An expert oral and maxillofacial pathologist blinded to clinical and survival data [S.W.] analyzed the presence and distribution of both membranous and cytoplasmic tumoral PD-L1 immunoreactivity. PD-L1 expression intensity was quantified using the *H*-score: a combination of both the intensity (scored: 0, 1+ weak, 2+ moderate, and 3+ strong) and the percentage of positive-stained tumor cells, as previously described [17]. The presence of tumor-infiltrating lymphocytes (TILs) was also quantified (scored: 0, 1+ weak, 2+ moderate, and 3+ strong). PD-1 immunoreactivity was characterized among TILs with the absolute number of PD-1 positive TILs counted under 40x middle power field [18]. PD-L1 expression was also evaluated among TILs, but was negligible. For each slide, five representative areas were counted and the average absolute number was recorded.

### *Statistical analysis*

Overall survival (OS) was determined from the date of oral cancer diagnosis to death from any cause, otherwise this was censored at date of last known follow-up. DFS was determined from the date of completion of oral cancer therapy to first progression or recurrence of oral cancer or death from any cause, whichever occurred first. Time to recurrence (TTR) was determined from the date of completion of initial oral cancer treatment to recurrence – and Kaplan-Meier statistics were applied with log-rank testing used to compare survival among subgroups. Multiple regression utilized Cox proportional hazards modeling (only if $n \geq 6$ patients were available in each subgroup) and proportional hazards assumption testing was verified. Binary multiple logistic regression analysis was performed to assess predictors of recurrence. Bootstrap methods were used to improve the reliability of confidence intervals (CIs). Spearman rho (ρ) was used to measure the strength of association between clinicopathologic variables, genomic data and immune metrics. All p-values reported are two-sided and a p-value $< 0.05$ (*) was considered statistically significant. Data were analyzed using Stata 14.2 software package (StataCorp LP, College Station, TX, USA).

## Results

### *Clinical characterization of the cohort*

Table 1 summarizes demographic and clinicopathologic features of 31 patients with a diagnosis of OSCC following allogeneic HSCT. The cohort was comprised mostly of men (23, 74%) and never or light smokers (≤10 pack-years) (23, 74%). Twenty-eight (90%) presented with T1-2 primary disease and 80% were clinically node-negative – resulting in 24 (77%) patients with early stage disease at initial presentation. All were treated with surgery with or without the addition of adjuvant radiation and/or chemotherapy. Median age at OSCC diagnosis was 51.5 (15–69), and 42 (8–62) for the prior hematologic disorder. Most received myeloablative conditioning (23, 74%) and had matched-unrelated donors (MUD) (22, 71%). Chronic GVHD was