**RESEARCH TOPIC MECM_26**

**Translational correlates of 3 clinical trials aimed to predict and intercept the malignant transformation of oral potentially malignant disorders and to treat oral cavity cancers**

**Curriculum**

MECM Standard

**Research Area**

Onco

**Laboratory name**

Translational Genomic Unit, Humanitas University

**Research Supervisor**

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**Abstract**

Background and rationale

An estimated 350,000 new cases of oral squamous cell carcinoma (OSCC) are diagnosed globally each year, with a 5-year overall survival rate of roughly 50%; the diagnosis is frequently made at a late stage. Lesions in the oral cavity that pose a risk of developing into cancer are known as oral potentially malignant disorders (OPMD); they are quite frequent (global prevalence of 4.5%) and they carry a risk for malignant transformation of 15.3% in dysplastic cases (4.8% to 51.3%). Incisional biopsy combined with histological analysis is the conventional procedure for evaluating the mucosa; nevertheless, it is invasive, and it should be repeated frequently to rule out the risk of cancerization. Oral brushing represents the ideal tool to continuously monitor patients, as being easily implementable in different clinical settings, non-invasive and potentially as informative as histological assessments.

Once diagnosed with a risk of cancer transformation, the issue of cancer interception becomes crucial, by intervening on molecular/immunological factors that could lead to cancerization. One of this strategy, called “immuno-interception” aims at eliminating neoplastic lesions at their earliest stages by mobilizing a specific immune response. Preliminary results showed that patients with oral proliferative leucoplakia who were treated with anti-PD1 nivolumab experienced a regression rate of 37%. However, even in presence of regression, malignant transformation may happen, and the systemic immunotherapy is not without toxicities that are less acceptable in patients with pre-malignant conditions.

Cluster of differentiation 40 (CD40) is a co-stimulatory receptor of the tumor necrosis factor (TNF) receptor superfamily. CD40 is expressed on numerous cell types, including antigen presenting cells such as dendritic cells (DCs) and macrophages. Reduced expression of CD40 pathway components has been associated with a higher risk of OPMD malignant transformation6; conversely, activating the CD40 pathway inhibited malignant transformation of OPMD in a carcinogen-induced animal model of oral squamous cell carcinoma. Agonistic monoclonal antibodies to CD40 can: stimulate DCs to activate cytotoxic CD8+ T-cells in the absence of CD4+ T-cell help; reprogram macrophages to kill tumor cells in a T-cell independent

fashion and activate cytotoxic NK cells and neutrophils. Mitazalimab is an agonistic human monoclonal (IgG1) antibody targeting CD40; its intralesional administration may allow a safer and more active stimulation of immune system to modulate the immune equilibrium towards elimination of OPMD.

Instead, when facing a locally advanced OSCC, the prognosis is less favourable, and the risk of locoregional/distant relapse is high even with surgery and postoperative (chemo)radiation approach. Recently, the use of immune checkpoint inhibitor pembrolizumab in neoadjuvant/adjuvant setting has demonstrated a benefit in improving event-free survival (Keynote 689 trial). However, the treatment was offered to all the patients with no selection factors, and the response rate in neoadjuvant setting is limited to about 1 out of 5 patients. In this regard, combining chemotherapy and immunotherapy in a selected population of patients with OSCC is a reasonable choice to enhance treatment efficacy in those more likely to respond while avoiding unnecessary toxicities in patients resistant to the drug combination.

The current project for the PhD candidate will be focused on the translational activities linked to ongoing clinical trials and it is divided into 3 Working Packages.

Working package 1: Accuracy of oral brushing in respect to histology for molecular characterization of OPMD.

After the approval of the Ethical Committees, a consecutive series of OPMD patients will be collected. Fifty patients with any type of OPMD will undergo oral brushing. DNA purified from cytobrush will be used to infer the presence of DNA aberrancies by scanning the entire genome for the presence of marked chromosomal imbalances, such as loss of heterozygosity (LOH), gain or loss of genomic material or single nucleotides variants. To this aim, 20-50 ng of genomic DNA will be used to generate genomic libraries using a in house previously developed hybrid capture solution. Probes have been designed to simultaneously capture both structural regions of the genomes (“backbone panel”, 14 Mb in size) and single nucleotides variation in the full-length coding sequence of 375 genes (“comprehensive panel”, 1.6 Mb in size). Libraries are then pooled and sequenced on a median coverage of 200x on a benchtop sequencer (NextSeq2000, Illumina). Downstream bioinformatic analysis has been previously developed in the laboratory of Dr. Marchini to scan the entire tumor genome for the presence of large genomic rearrangements, such as LOH, telomeric allelic imbalance (TAI) or Large-Scale Transition (LST) or single point mutations in high grade serous ovarian cancer cases. Therefore, we expect that this solution is suitable to identify LOH at genome scale also in DNA from oral brush derived from OPMD. Conventional cytology will be also performed, and the description of the results will be collected. After oral brushing, a diagnostic biopsy (incisional) or a diagnostic-therapeutic (excisional) exeresis will be accomplished. The same molecular analysis as above will be performed on the histological sample, as well as the pathological assessment.

Working Package 2: Translational analysis on the samples collected in “Aphrodite” trial with intralesional mitazalimab to reduce the risk of cancerization of OPMD.

Main aim is to identify immune dynamics following intratumoral injection in patients with OPMD. To reach this objective, we will:

a) Collect peripheral blood samples at baseline, at d7 and d14 after each of the 4 cycles and at surgery/biopsy (end of treatment). We will initially perform 50-parameter flow cytometry to evaluate changes in the maturation, activation, proliferation and effector functions of several peripheral blood mononuclear cell subsets. We expect dendritic cell maturation and T

cell activation as assessed by specific markers (e.g., Ki-67, HLA-DR, CD38, CD25, etc.). In this way, we will be able to focus on specific time points for more in depth molecular analyses as follows;
b) Perform single cell RNA-sequencing on CD45+ cells from a subset of patients (n=10) at baseline and at the peak of the immune response (as informed by flow cytometry) to identify the molecular features of immune cells responding to mitazalimab (changes in immune-related pathways, transcription factor networks, etc.);
c) Sequence T cell receptor (TCR) at the level of single cells simultaneously to define the clonality of the T cell response. Proliferating/activated cells expected to be seen after immunotherapy are hypothesized to be primed by tumoral antigens, therefore we will be able to identify candidate anti-tumor TCRs expressed by these cells;
d) Analyse longitudinally plasma proteomics (at selected time points) and untargeted metabolomics (all time points) to identify inflammatory mediators and metabolites, respectively. Advanced bioinformatics will be used to integrate the different multiomic datasets and thus identify the molecular features associated with dendritic cell reprogramming and tumor-reactive T cell expansion.
Working Package 3: Translational analysis on the samples collected in “Persephone” trial with chemo-immunotherapy in selected CPS-positive oral cavity cancer patients.
Main aim is to identify translational correlates of response/resistance to neoadjuvant treatment with platinum, taxane and tislelizumab in oral cavity cancer patients. To reach this objective we will:
a) Perform Immunological analysis at baseline as predictor of pCR/MPR or no response. To achieve this objective, we will perform spatial transcriptomics analysis to map the immune landscape at the tumor site, identifying specific immune cell populations, their spatial organization, and gene expression profiles. This analysis will allow us to correlate baseline immune characteristics with pCR (analysis of 3 baseline samples who achieved pCR/MPR) and with no response (analysis of 3 baseline samples who did not achieve pCR/MPR).
b) Longitudinal analysis of circulating tumour DNA (ctDNA) as blood biomarker to define the response obtained with chemo-immunotherapy in oral cavity cancer. To define a more accurate tool than radiology in identifying response to chemo-immunotherapy, we will collect blood samples at baseline and after the 2nd and 3rd cycle of chemo-immunotherapy and we will correlate the changes in the ctDNA with the pathological response obtained after surgery. ctDNA will be evaluated using a tumour uninformed approach. The levels of ctDNA will be followed in a longitudinal manner throughout treatment in plasma. This will allow exploration of whether non-invasive analyses can be used in place of tissue biopsies to predict for response to treatment. In the second step of analysis, the same tube will be analysed through a panel of genes customized on their potential role on mechanism of resistance or as actionable genes.

**Main technical approaches**

Experience in genomic and epigenomic analyses, including DNA extraction from tumor tissue and liquid biopsy samples (plasma/saliva).



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Expertise in circulating tumor DNA (ctDNA) analysis, including sample processing, quantification, and quality assessment.
Preparation of next-generation sequencing (NGS) libraries, including target enrichment and quality control workflows.
Knowledge of molecular biology techniques, such as PCR/qPCR, and nucleic acid quantification.
Experience in head and neck cancer research, with understanding of tumor biology, biomarkers, and oncology approaches.
Skills in experimental planning, data interpretation, and scientific reporting in an interdisciplinary research environment.

**Scientific references**


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**Type of contract**

Scholarship of € 24.500 gross per year awarded by Istituto Clinico Humanitas. This sum is subject to IRPEF income tax and exempt from social security contributions.

Borsa di studio pari a € 24.500 annui lordi erogata da Istituto Clinico Humanitas. Importo soggetto a tassazione IRPEF ed esente da contribuzione previdenziale.



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