Immune correlates in peripheral blood samples in a preoperative window of opportunity randomized trial of Nivolumab with or without tadalafil in resectable squamous cell carcinoma of the head and neck (SCCHN)

Larry Harshyne, PhD[1], Jessica H. Lewis[1], Adam Luginbuhl, MD[2], Joseph Curry, MD[2], Young Kim, MD PhD[3], Athanassios Argiris, MD PhD[2], Sanket Shukla, PhD[4], Ulrich Rodeck, MD, PhD[4], Jennifer M Johnson, MD PhD[1]

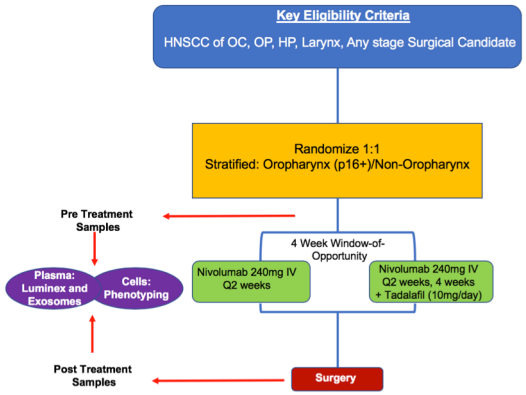
[1]Thomas Jefferson University, Dept of Medical Oncology

[2]Thomas Jefferson University, Dept of Otolaryngology Head and Neck Surgery

[3]Vanderbilt University, Vanderbilt Ingram Cancer Center, Dept of Otolaryngology Head and Neck Surgery

[4]Thomas Jefferson University, Dept of Dermatology and Cutaneous Biology

Clinical trial: NCT03238365



Introduction

- Squamous cell carcinoma of the head and neck (SCCHN) carries a poor prognosis, with low survival rates for advanced stage tumors and minimal improvement in survival prior to the advent of immune checkpoint therapy.
- HPV-associated SCCHN represents a distinct biological and clinical entity with a more favorable prognosis. To account for this we analyzed immune parameters stratified by HPV status.
- The recent CheckMate 141 clinical trial demonstrated that nivolumab, an anti-programmed cell death protein 1 monoclonal antibody, extended median overall survival (OS) in SCCHN patietns compared with standard therapies.
- Tadalafil has been reported to promote tumor immunity in HNSCC patients by reducing MDSCs and Tregs.
- This randomized window-of-opportunity trial studied peripheral blood immune markers in SCCHN patients treated with nivolumab with or without tadalafil.

Companion posters

- **Poster 1116PD -** clinical trial design and results.
- **Poster 1131 -** genetic profiling of tumors collected pre and post treatment.

Material and Methods

- **Patients:** SCCHN (n=28), Nivolumab alone (n=12), Nivo+Tadalfil (n=16); subset of patients with available data from NCT03238365
- **Sample:** Pre- and Post-treatment peripheral blood and tumor.
- **HPV Status (p16 status IHC):** HPV (+) = 19, HPV(-) =09
- **Clinical Response:** Non Responder (NR) = 13, Responder =15
- **Flow cytometry:** Samples were collected on a BD Fortessa (4 lasers, 15 colors) and analyzed using FlowJo software.
- **Luminex analysis of immune-related genes:** Millipore Milliplex kits (HCYTOMAG-60K and HCKP1-11K) was collected on a FM3D luminex machine.
- **Exosome analyses:** Plasma (20 ul) was labeled with a lipophilic dye and human monoclonal antibodies.
- **Statistical analysis:** JMP Statistical Software (version 4) Mean ± SD; p<0.05 Significance used for discriminative analysis.

Results

Figure 1. *Peripheral blood cells:* Flow cytometric analysis of peripheral blood cell subsets in pre-treatment samples. Numbers indicate the frequency of cells within a given gate. There was no difference between HPV+ and HPV- patients. **A.** Gating strategies for live cells (left) and peripheral blood mononuclear cells (right). B. CD4/CD8 T lymphocyte subset ratios as they relate to clinical response. B. Monocyte polarization states (CD163/PD-L1 expression) as they

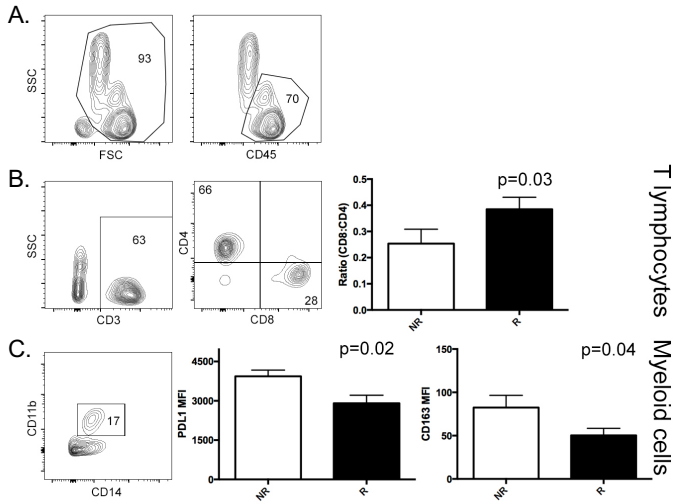


Figure 2. *Plasma:* Principal component analysis of soluble immune parameters in post-treatment plasma samples collected from SCCHN patients treated with nivolumab +/- tadalafil. **A.** PCA representation of soluble circulating factors associated with Type I/II immunity and immune checkpoints. Patients were stratified according to clinical response (red=non-responder, NR and green = responder, R). **B.** Pre and post-treatment levels (pg/ml) of type I immune cytokines stratified by HPV status and clinical response. Statistical significance was assessed using ANOVA followed by Dunnett's post test (* = p<0.05 and **= p<0.005).

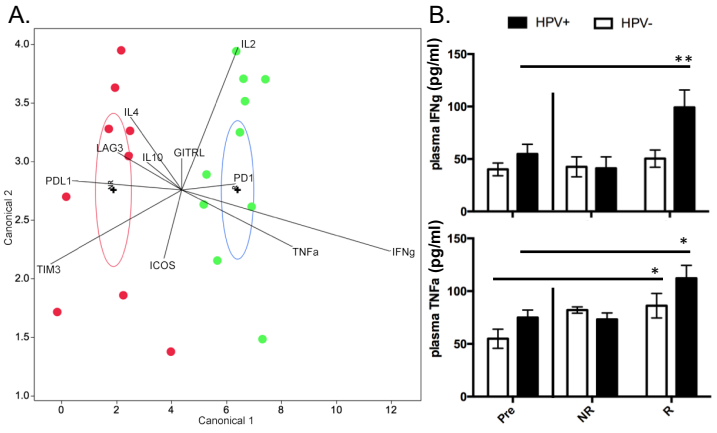
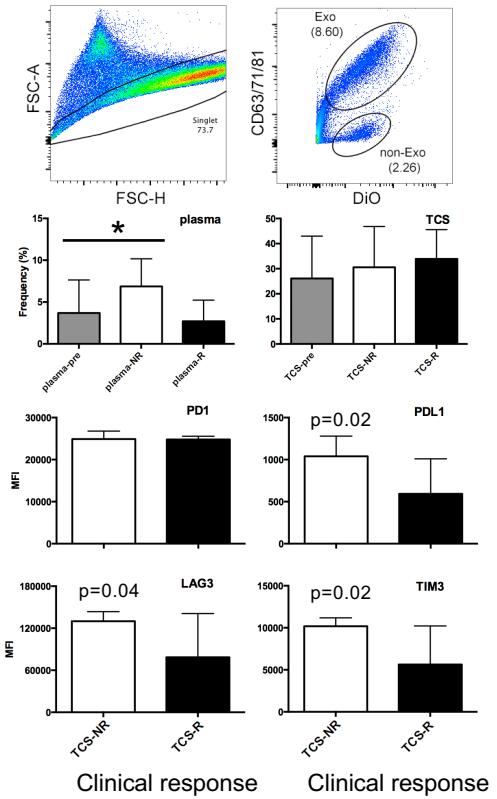


Figure 3. *Exosomes:* Flow cytometric analysis of exosomes in pre- and post- treatment plasma and supernatants from short-term ex vivo tumor cell cultures. **A.** Gating strategies for single events (left) and exosomes (right). **B.** The frequency of circulating exosomes in pre-treatment plasma (left) and overnight biopsy cultures (right) as they relate to clinical response. **C.** Bar charts show expression of immune checkpoint receptors on exosomes present in post-treatment tumor culture supernatant as they relate to clinical response.



Conclusion

- Higher levels of circulating factors and cells associated with type I immunity and/or lower levels of type II immunity are associated with clinical response.
- PCA analysis of this limited dataset suggests that clinical responses are associated with type I immune markers and lack of response is associated with a type II immune response and higher expression of alternative immune checkpoints.
- Treatment induced changes in frequency of circulating exosomes and phenotypic changes in exosomes secreted by tumor cells in overnight cultures which were associated with clinical response.

Acknowledgements

- Samples from NCT03238365 (PI:Luginbuhl)
- Corresponding author: Larry.Harshyne@jefferson.edu
- The authors declare no conflicts of interest.