A Study to Evaluate Immunological Response to PD-1 Inhibition in Squamous Cell Carcinoma of the Head and Neck (SCCHN) using novel PET Imaging with [18F]F-AraG

A. Dimitrios Colevas, Nikita Bedi, Serena Chang, Uriel Yojanan Moreno Nieves, Susmita Chatterjee, Guido Alejandro Davidzon, Shyam Srinivas, Quynh-Thu Le, Aruna Gambhir, John B. Sunwoo

Stanford Cancer Institute, Stanford, CA; Stanford Clinical Trials Office, Stanford, CA; Institute for Immunity, Transplantation and Infection, Stanford School of Medicine, Stanford, CA; Stanford Cancer Institute, Palo Alto, CA; OHNS/Research Division, Stanford, CA; Stanford University Medical Center, Stanford, CA; Cellsight Technologies, San Francisco, CA; Stanford University, Stanford, CA.

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ABSTRACT

Background: Immune checkpoint blockade has demonstrated remarkable responses in a subset of patients with head and neck squamous cell carcinoma (HNSCC). However, the response rate is only ~20% or less for HNSCC. Currently, there are no good biomarkers to predict and assess responses after patients have initiated therapy. We evaluated the ability of a PET metabolic tracer ([^{18}F]F-AraG), that preferentially accumulates in activated CD8$^+$ T cells, to assess response to anti-PD-1 ab. We hypothesize that uptake of this agent within the tumor will correlate with the accumulation and activation of T cells within the tumor. **Methods:** Locally advanced HNSCC patients undergoing surgical resection received an infusion of anti-PD-1 ab in a window-of-opportunity study. A novel radiofluorinated AraG imaging agent, [^{18}F]F-AraG (Cellsight), was used to image patients by PET/CT before and 2-3 weeks after their infusion. The tumor volume of interest (VOI) for the pre- and post-infusion [^{18}F]F-AraG PET/CT scans was defined using pre-treatment conventional FDG-PET/CT scans. To assess for correlation of the [^{18}F]F-AraG with immune response, pre- and post-infusion samples of the patients' tumors were obtained and dissociated into cell suspensions. Tumor-infiltrating T cells were evaluated by flow cytometry to determine T cell infiltration and activation. **Results:** In a patient with oral cavity HNSCC, there was an approximately 50% increase in total [^{18}F]F-AraG SUV in the VOI representing the tumor volume, following anti-PD-1 ab. Concurrently we observed a substantial increase in the proportion of CD8+ T cells. The CD8+ T cells exhibited an activated state based on surface marker expression. **Conclusions:** [^{18}F]F-AraG accumulation in the tumor tissue correlates with an increase in T cell infiltration and activation. Further study of this novel PET tracer is underway in HNSCC to assess its utility for predicting clinical response.

BACKGROUND

While immune checkpoint blockade has demonstrated remarkable responses in a subset of cancer patients, the need for noninvasive means of predicting response to tailor therapy is greatly needed. This project assesses the ability of an imaging agent, [18F] F-AraG (Cellsight Technologies), to determine response to anti-PD-1 antibody therapy in patients with head and neck squamous cell carcinoma.

Anti-PD-1 antibody (PD-1 ab) induction of tumor-specific T cell activation and expansion results in T cell receptor (TCR) repertoire changes. This study will (1) evaluate correlations between clinical response, [18F]F-AraG imaging changes, and tumor-infiltrating CD8+ T cell activation.

We hypothesize that characterization of these changes in the immune system and changes in [18F] F-AraG uptake within the tumor may allow us to better predict:

1. Which patients will go on to benefit from PD-1 therapy.
2. Which combinations of immunotherapy will be most beneficial for a particular patient.

OBJECTIVES

1. To collect adequate pre and post immunotherapy blood and tissue samples to perform the above analysis
2. To assess whether an ^{18}F-labeled metabolite analogue that accumulates in sites of inflammation can be used for noninvasive imaging and assessment of T cell activation and expansion in the tumor microenvironment.

REFERENCES

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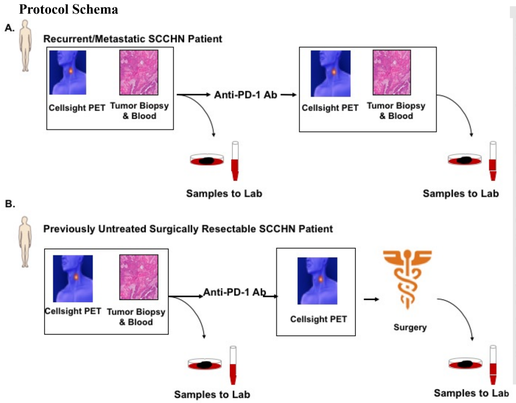
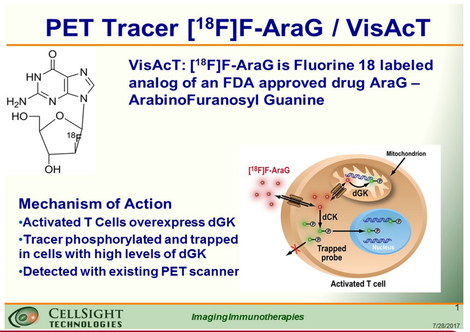
METHODS



Study Schema. Two cohorts are being studied: (A) Recurrent/metastatic (R/M)SCCHN patients will have a PET scan with the Cellsight [18F]F-AraG tracer, and then a baseline biopsy of the tumor and blood sample will be collected before the first infusion of anti-PD-1 antibody. Following the initiation of therapy, a second Cellsight PET scan will be performed, and another biopsy of the tumor and peripheral blood will be collected for analysis. Tumor-infiltrating T cells will be analyzed by flow cytometry. (B) Previously untreated surgically resectable SCCHN patients will be studied in a similar fashion with the anti-PD-1 antibody given preoperatively 2-3 weeks before surgery. A post-infusion Cellsight PET will be obtained just before surgery. Tumor samples and peripheral blood will be collected at the time of surgery and analyzed as above.

PET Tracer Mechanism. Molecular imaging of immune cell activation by PET is a potentially powerful noninvasive strategy to monitor immune activation after treatment with immunotherapy. Increased activity of nucleoside salvage pathways has been associated with the proliferation of adaptive and innate immune cells. In preliminary studies, [^{18}F]F-AraG preferentially accumulates in murine and human activated T cells compared to naive T cells.



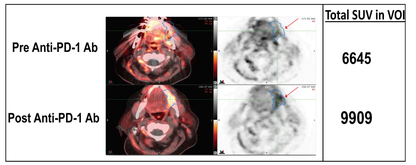
RESULTS

	Surgical Cohort	R/M Cohort	Total
# patients enrolled	6	3	9
# patients with paired blood	6	3	9
# patients with paired biopsy	6	3	9
# patients with paired imaging [18F]F-AraG PET	2	1	3
# patients will all 3 paired	2	1	3

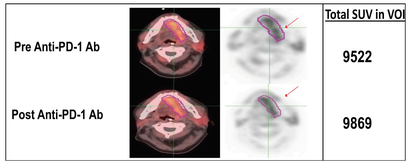
In the Surgical Cohort- 4/6 patients were enrolled in the imaging portion of study. In the R/M Cohort- 1/3 patients were enrolled in the imaging portion of the study. We were unable to get matched sets for [18F]F-AraG PET imaging for 2 patients, due to synthesis failure. For the 3 patients that we were able to get matched sets (Patient 1, 11 and 13), Patient 1's imaging did not show significant signal, and thus their information is not included.

SURGICAL COHORT

Patient 11



Patient 13

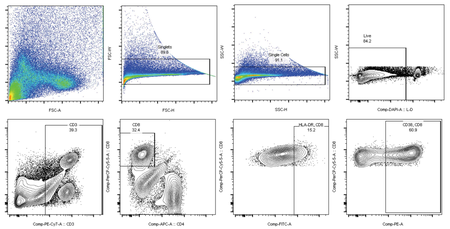


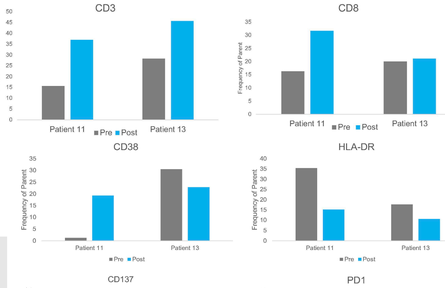
Imaging: The conventional FDG PET scan was used to determine the volume of interest (VOI) of the tumor in the patients, which was superimposed on the F-AraG PET scans and the total standardized uptake value (SUV) was quantified in the VOI. The SUV represents the amount of F-AraG tracer in the VOI.

Assessment of Activation Markers on CD8+ T Cells within Tumor Biopsy Samples Taken Before and After Anti-PD-1 Antibody Therapy. Pre- and post-therapy biopsy samples were dissociated by mechanical digestion. Cells were stained and analyzed using flow cytometry. We gated out dead cells, doublets and debris and continued hierarchical gating to isolate singlet, live, CD3+, CD8+ T cells. Activation markers, CD38, HLA-DR, and CD137, an inducible CD8+ costimulatory receptor, along with checkpoint blockade, PD1, markers were analyzed using the CD8+ parental subset. The bar graphs represent the number of CD38, HLA-DR, CD137 or PD1 positive cells from the CD8+ parental gate, shown as frequency(%) of parent.

Preliminary Results from Imaging: Patient 11 had a significant increase in the total SUV and also an increase in the CD8+ T cells. Conversely, Patient 13 did not have an increase in the total SUV, and there was not an increase in the CD8+ T cells. Importantly, the change of the total SUV and the change in CD8+ T cells is more important than the total baseline values because this change is potentially the result of the PD-1 ab. In addition, the activation marker CD38 goes up on the CD8+ T cells only in Patient 11."

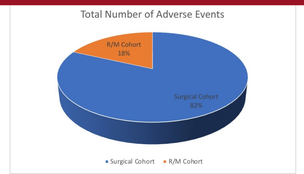
Gating Hierarchy





SAFETY

Variable	Surgical Cohort	R/M Cohort	Related to PD-1 MoAb
CTCAE Grade 1 and 2	7	2	
Fatigue	2	0	Possible
Parasthesia	1	0	Not Related
Fever	1	0	Possible
Tumor Pain	3	2	Not Related
CTACE Grade 3 and 4	2	0	
Fever	1	0	Possible
Hypotension	1	0	Possible



Patients in the surgical cohort are administered a single dose of PD-1 ab in a neo-adjuvant setting. In the six patients we have enrolled in the surgical cohort, we have seen 2 counts of possibly related grade 3 events.

CONCLUSIONS

We have consented 19 patients on the study, with nine patients enrolled on the study blood and biopsy study, and five patients enrolled in the imaging study. All nine patients completed blood and biopsy collection of the study, and three patients completed all three procedures i.e. blood, biopsy and Imaging. In the patients that have had all three procedures done i.e. blood, biopsy and imaging, we have observed a correlation in the change in total SUV and change in the CD8+T cells post PD-1 ab. This is an ongoing study, and we are continuing to enroll more patients. Based on the patients we have enrolled thus far, we conclude that imaging and tissue collection in this study design is feasible.

For further questions, please contact A. Dimitrios Colevas at colevas@stanford.edu.