Use of High-dimensional and Spatial Immune Profiling to Explore Sotigalimab (CD40 Agonist) Activation of Antigen Presenting Cells and T Cells in the Tumor Microenvironment in Patients with Esophageal/Gastroesophageal Junction Cancer

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Abstract # 450

Background

Neoadjuvant chemoradiation (CRT) followed by surgical resection is standard of care for patients with locally advanced esophageal/gastroesophageal junction (E/GEJ) cancer. A pathologic complete response (pCR) at surgery is associated with improved survival outcomes. Sotigalimab (sotiga) is a potent CD40 agonist mAb capable of inducing and expanding anti-tumor immune responses.

A recently reported phase II clinical trial of sotiga combined with CRT in E/GEJ cancer patients showed increased pCR rates that compared favorably to historical data[1]. Here, deep immune profiling was performed on samples from the circulation and tumor microenvironment (TME) from a subset of patients to gain insight into the mechanism of sotiga.

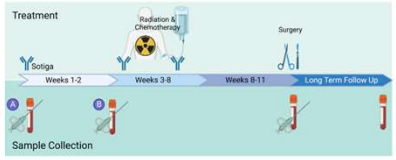
	Efficacy Population n= 29 % (n/total)	Histologic Subtype		Location
		Adenocarcinoma n= 24 % (n/total)	Squamous Cell Ca n= 5 % (n/total)	GEL Ca n= 14 % (n/total)
R0 resection	86% (25/29)	83% (20/24)	100% (5/5)	79% (11/14)
Pathologic response				
Complete response (pCR)	38% (11/29)	33% (8/24)	60% (3/5)	29% (4/14)
Major path response*	66% (19/29)	63% (15/24)	80% (4/5)	57% (8/14)
PD (before or at surgery)	7% (2/29)	8% (2/24)	0% (0/5)	14% (2/14)
Historical CRT pCR rate		19 - 23%	42 – 49%	--

*Defined as <10% viable tumor – includes pathologic CR and PR

Registered clinicaltrials.gov #NCT03165994

References: 1. Ko A, at al. A Multicenter Phase 2 Study of Sotigalimab (CD40 Agonist) in Combination with Neoadjuvant Chemoradiation for Resectable Esophageal and Gastroesophageal Junction (GEJ) Cancers. *ESMO Annual Conference*; 2022. **2.** Spitzer, M, at al. An Interactive Reference Framework for Modeling a Dynamic Immune System. *Science;* 2015. **3.** Wolf, FA, et al. SCANPY: large-scale single-cell gene expression data analysis. *Genome Biology;* 2018.

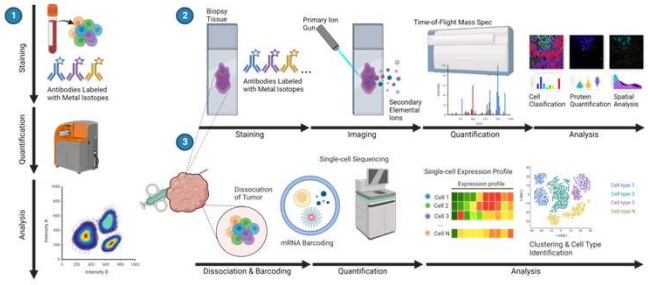
Study Protocol and Biopsy Schedule



Patient	path CR	PBMC CyTOF	Tumor scRNAseq	Tumor MIBI
01	No	Yes	Post Only	Yes
02	Yes	Yes	Pre Only	Yes
03	Yes	Yes	Pre/Post	Partial (Pre)
04	Yes	Yes	Post/ Surgery	Yes
05	Yes	Yes	Pre/Post/ Surgery	No
06	No	Yes	No	Yes

Blood samples (PBMC) were collected before and after treatment, and after surgery (N=6). Paired tumor biopsies were collected before (A) and after (B) sotiga treatment (N=4). When available, fresh tumor was collected from surgical specimens.

Analytical Methods

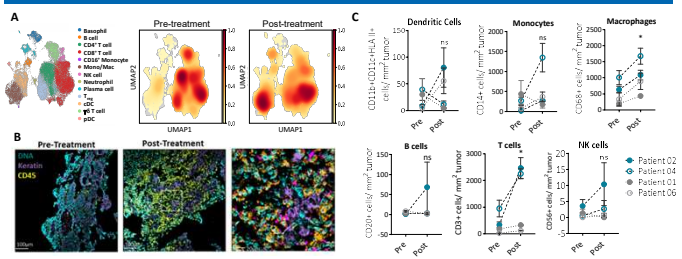


1. PBMCs were isolated from whole blood, stained and analyzed by mass cytometry (CyTOF). Scaffold was used to analyze CyTOF data.[2]

2. Tumor samples from biopsies were analyzed by IonPath using Multiplexed Ion Beam Imaging Technology (MIBI).

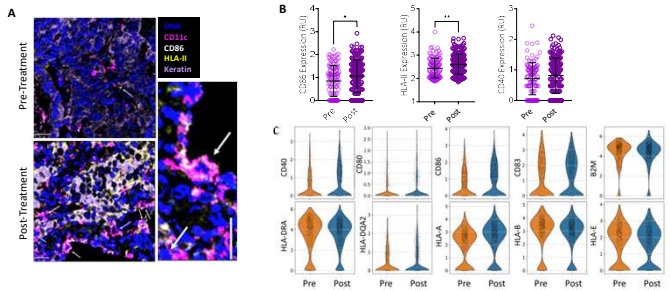
3. Tumor samples were also dissociated and single cell RNA sequencing (scRNAseq) and T cell receptor (TCR) sequencing was performed using the 10X Genomics platform. Data was analyzed using SCANPY. [3]

Sotiga Induces Immune Cell Infiltration in the TME



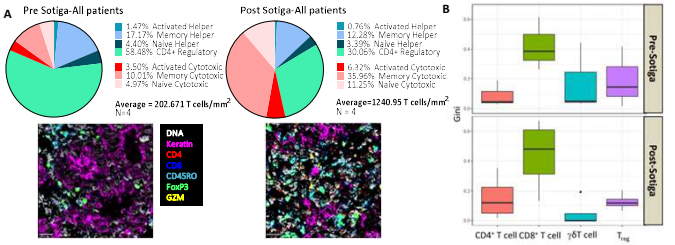
A. Uniform Manifold Approximation and Projection (UMAP) plot demonstrating all immune cells from tumor biopsies with cell type over-laid (left) or by density heatmap showing relative abundance of cell type by pre/post-treatment, demonstrating altered immune cell composition with sotiga. **B.** Staining of tumor tissue pre and post-treatment by MIBI showing increased immune infiltrate post-sotiga. **C**. Quantification of cell type using MIBI; patients that achieved pCR ●○ or did not ●○.

Infiltrating Myeloid Cells Display Inflammatory Markers



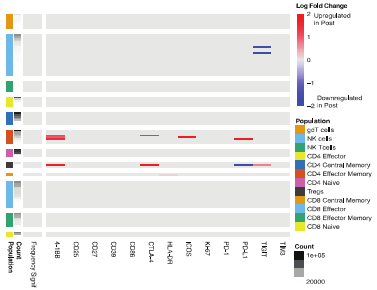
A. Images from MIBI showing increased CD11c$^+$CD86$^+$HLA-II$^+$ myeloid cells post-sotiga. **B.** CD86 and HLA-II were significantly increased in expression on dendritic cells post-sotiga. **C.** Expression of activation markers on dendritic cells pre vs post-sotiga by scRNAseq. All transcripts shown were significantly increased post-sotiga.

Sotiga Increased Infiltration of CD8$^+$ T Cells and Decreased CD4$^+$ Regulatory T cells in TME



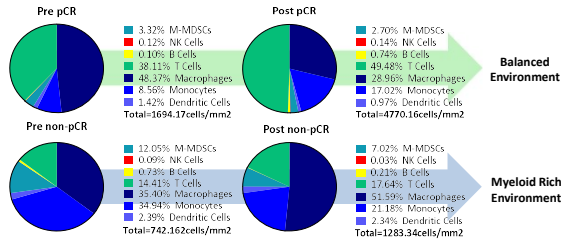
A. T cell composition pre and post-sotiga by MIBI. **B**. Gini co-efficient for T cell sub-types pre and post-sotiga, demonstrating a a trend towards higher clonality of intra-tumoral CD8$^+$ T cells and lower clonality of Tregs post-sotiga.

Sotiga Activates Circulating NK and T cells



Heatmap demonstrating the frequency of immune clusters and expression of activation and inhibitory markers on T and NK cells in circulation of patients with E/GEJ cancer pre and post-sotiga, as analyzed using CyTOF. There were no differences in the frequency of clusters of T and NK cells, but there was increased expression of 4-1BB, CTLA-4, ICOS on T cells and up- or down-regulation of PD-L1 and TIGIT on different NK and T cell clusters.

Higher T cell Density in TME at Baseline is Associated with Tumor Response



Immune composition pre and post-sotiga in tumors that had a pCR following sotiga + CRT compared to tumors that did not have a pCR.

Conclusions

Sotiga induced dramatic changes in the tumor microenvironment and converted "cold" tumor to "hot" by increasing the frequency of activated T cells and antigen presenting cells (APCs) and decreasing frequency of T_{regs}. In circulation, treatment with sotiga activated NK and T cells. A distinct signature of T cell infiltration in baseline tumor biopsies was observed in patients who achieved a pCR versus those who did not, potentially identifying patients that may benefit from this novel treatment strategy. This is the first demonstration that single-agent sotiga can induce significant inflammatory responses in the TME. The conversion of "cold" tumors to "hot", a key MOA of sotiga, is the foundation of immunotherapy.