Use of ex vivo Histoculture to identify potential predictive biomarkers for the ICOS agonist antibody, JTX-2011

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**Background**

ICOS (Inducible T cell CO-Stimulator) is a co-stimulatory molecule expressed primarily on T lymphocytes. Clinical correlations and preclinical data suggest that ICOS plays an important role in the immune response to cancer. Therefore, we generated JTX-2011, an ICOS agonist antibody currently in clinical development in advanced solid tumors in the ICONIC trial. In preclinical studies, single agent efficacy correlates with the percentage of ICOS-expressing T cells within the tumor. Thus, ICOS expression is being used as a biomarker to enrich for patients in Phase 2 of the ICONIC trial. Building on our biomarker-driven strategy, we have explored additional potential predictive biomarkers using ex vivo tumor histoculture which allows for in functional analysis of therapies using patient intact tumor tissue. Herein, we report on the results of such analysis, including assessment of the induction of an IFN-γ gene signature.

**Methods**

Tumor processing: Fresh human tumor samples were obtained post-surgery. A section of each tumor was cut and fixed for IHC. 300 µM slices of remaining tumor were placed in a 6-well plate. Treatments were added into the medium and solid tumors were incubated at 37° C. Tumor slices were stored in RNALater after incubation.

RNA extraction and QC: Tumor slices were lysed using Qiagen’s TissueLyser processor and FFPE samples were deparaffinized. RNA was extracted from FFPE and fresh tumor samples, quantified using Qubit, and QCl’d using Agilent’s Fragment Analyzer.

Gene expression: Gene expression for interferon-γ signature and other genes of interest were performed using Taqman qPCR probes or NanoString nCounter using the Human Immunology V2 panel.

IHC: ICOS (Spring SP98) and PD-L1 (CST E1L3N) levels were assessed by IHC. Samples were considered to be PD-L1 high if they were 5% positive for PD-L1. Samples are considered ICOS high with an ICOS IHC score of 2 or 3.

**Establishment of histoculture system and association of IFNγ signature induction with treatment**

(A) Post-dose profiling of NSCLC and HNSCC to establish IFN-γ induced signature. Threshold across treatments was set based on PHA treated positive control samples. (B) ex vivo treatment of NSCLC tumors with nivolumab. (C) ex vivo treatment of NSCLC with JTX-2011. Samples compared to response of PHA at 24hrs (D) in some tumors, treatment with both JTX-2011 and nivolumab results in increased IFN-γ signature induction that is greater than single agent alone. The bar chart above compares single agent treatment to combination treatment.

**Expanded assessment of histoculture platform and characterization of baseline predictive markers**

(A) Schematic of the process by which histoculture is performed. (B) Human histoculture is used as a functional assay to test both indication selection biomarkers as well discovery of potential PD biomarkers

**Correlating potential predictive biomarkers with induction of IFNγ signature in HNSCC tumors**

(A) ex vivo histoculture analysis of anti-PD1 treatment of HNSCC tumors. Induction of IFNγ signature is used as a proxy for tumor response to anti-PD1 therapy. The black line shows strong IFNγ induction and dashed red lines shows moderate IFNγ induction. (B) ex vivo histoculture analysis of JTX-2011 treatment of HNSCC tumors suggests ICOS high tumors are more likely to respond. (C) ex vivo histoculture analysis of JTX-2011 treatment of HNSCC tumors suggests greater response in IMS molecular subtype.

**RNA signatures as potential predictive biomarkers**

Histogram of inducers vs non-inducers (as measured by IFNγ signature inductions) are defined as shown in the waterfall plots above using the cutoff for the strong inducers. Tumors are placed into 5 equal groups based on range of signature expression at baseline. In tumors where JTX-2011 induced IFNγ signature, baseline scores are higher as illustrated by right shift of the Histogram suggesting ICOS expression, ICOS signature and 10-gene IFNγ signatures are potential predictive biomarkers.

**Summary**

- We have confirmed that treatment of NSCLC and HNSCC histoculture samples with nivolumab leads to induction of an IFNγ gene signature in a subset of the treated samples.
- We have established that JTX-2011 induces IFNγ signature both as a monotherapy in combination with nivolumab.
- Additionally, induction correlated with baseline ICOS expression for JTX-2011-treated samples, and with baseline PD-L1 expression for nivolumab-treated samples.
- Baseline expression of ICOS mRNA, ICOS mRNA gene signature, and 10-gene IFNγ signature correlated with IFNγ induction in JTX-2011-treated samples providing novel predictive biomarkers to assess in the ICONIC clinical trial.
- In conclusion, ex vivo Histoculture is a robust tool that can be used to assess candidate predictive biomarkers, identify novel potential biomarkers, and interrogate post-dose responses to novel therapeutics.