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

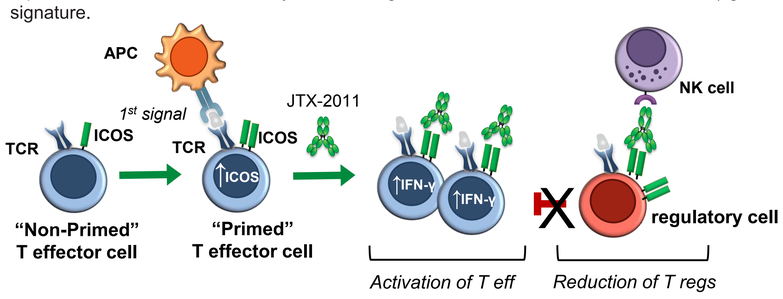
ABSTRACT# P39

Heather A. Hirsch, Jason Reeves, Tong Zi, Amit Deshpande, Guang Yang, Alexander Needham, Jenny Shu, Christopher Harvey, Sriram Sathyanaryanan, Jennifer Michaelson, Emma Lees, Elizabeth Trehu, Deborah A. Law

Jounce Therapeutics Inc., Cambridge, MA

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 IFNγ gene signature.



Methods

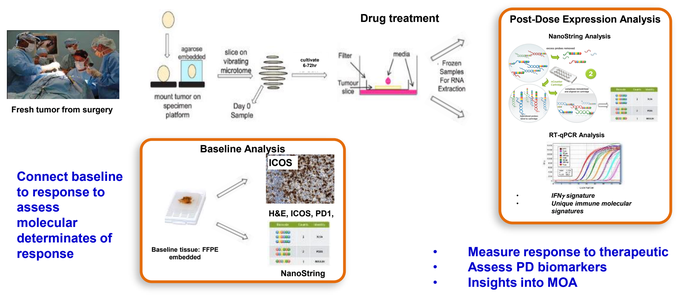
Tumor processing: Fresh human tumor samples were obtained post-surgery. A section of each tumor was cut and fixed for IHC. 300 μM slices of remaining tumor were placed in a 6-well plate. Treatments were added into the medium and plates were incubated at 37° C. Tumor slices were stored in RNAlater after 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 Quibit, and QC'd using AATI'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.

A



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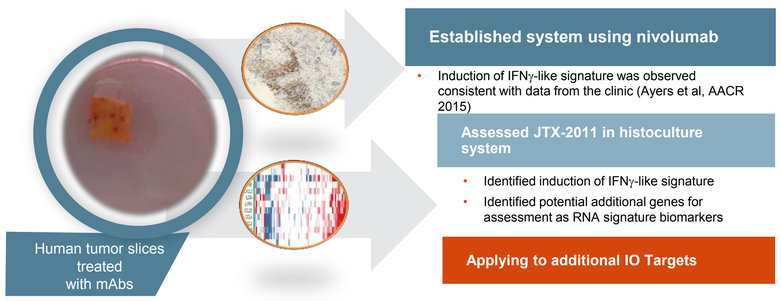
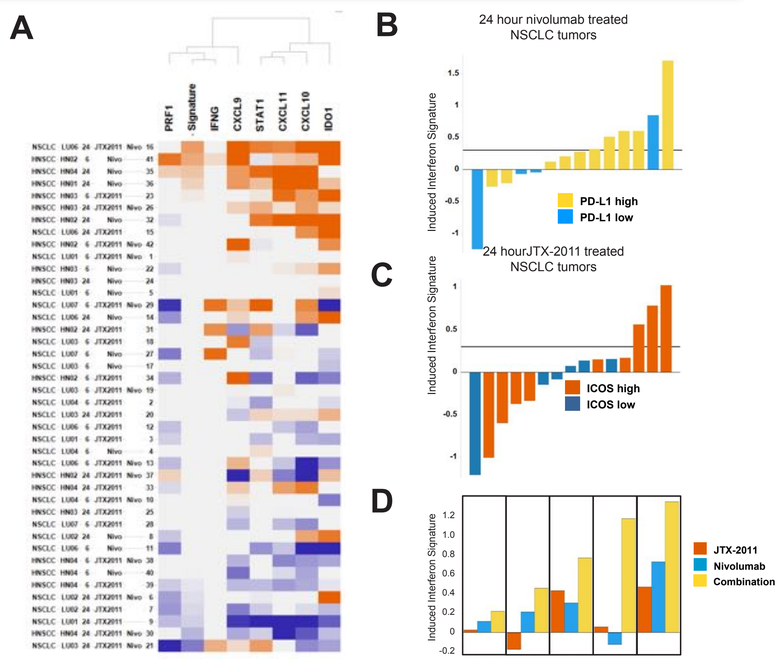


(A) Schematic of the process by which histoculture is performed. (B) Human histoculture is used as a functional assay to test both indication selection biomarkers as well discovery of potential PD biomarkers

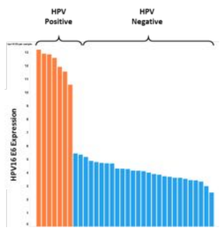
Establishment of histoculture system and association of IFNγ signature induction with treatment



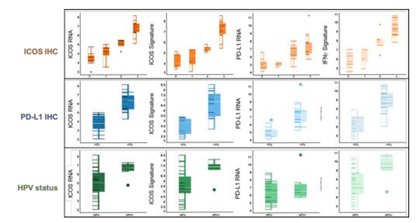
(A) Post-dose profiling of NSCLC and HNSCC to establish IFNγ induced signature. Threshold across treatments was set based on PHA treated positive control samples. (B) *Ex vivo* treatment of NSCLC tumors with nivolumab. (C) *Ex vivo* treatment of NSCLC with JTX-2011. Samples compared to response of PHA at 24hrs (D) In some tumors, treatment with both JTX-2011 and nivolumab results in increased IFNγ 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

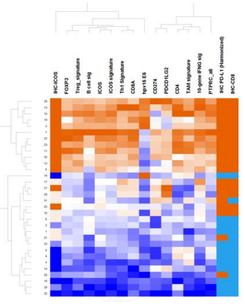
A Determination of HPV status



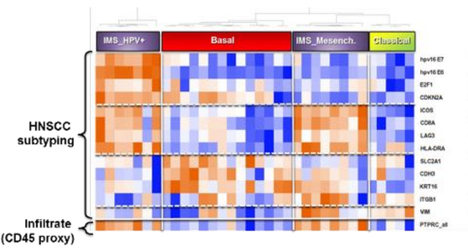
B Concordance of RNA and IHC



C Integrative analysis of RNA expression, RNA signatures and IHC



D Molecular subtyping



QC metrics and molecular characterization of baseline tumors:
(A) HPV status was determined by NanoString analysis of HPV16 E6 mRNA in baseline tumors samples. (B) Box plots showing concordance of IHC and relevant RNA expression at baseline as well as the expression of these genes in HPV+ tumors. (C) Integrative analysis of IHC and mRNA data using complete linkage clustering with correlation distance. (D) Molecular subtypes (Keck et al, 2015) were determined via two dimensional clustering using representative genes from each subtype as well as HPV E6 and E7 mRNAs.

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

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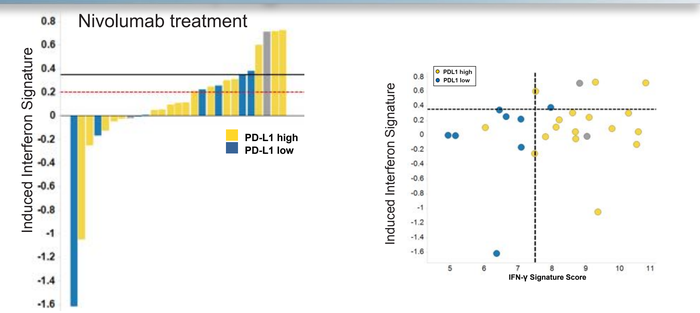

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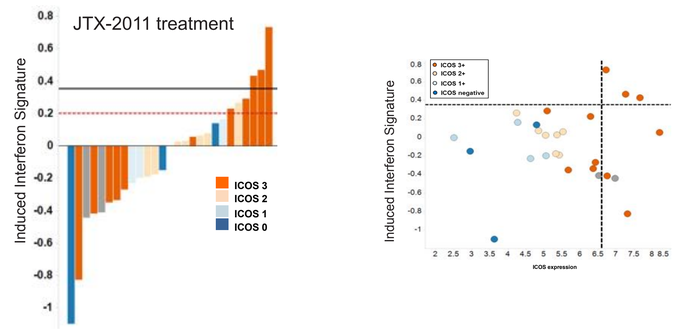

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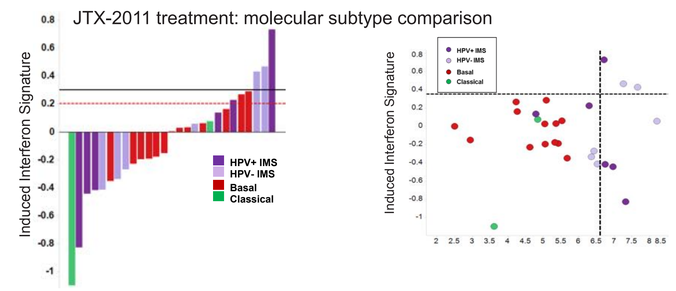
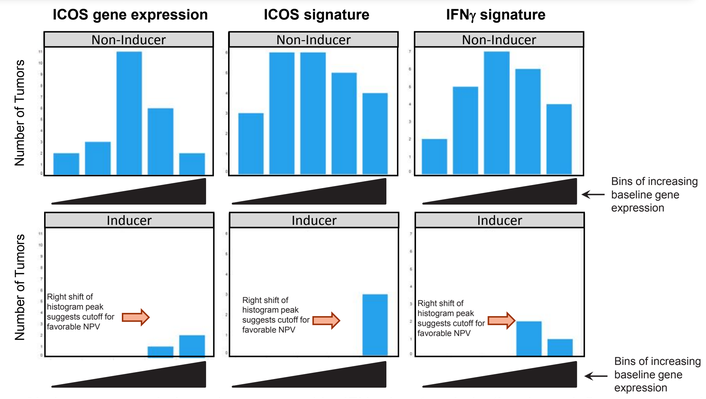


(A) *Ex vivo* histoculture analysis of anti-PD1 treatment of HNSCC tumors. Induction of IFNγ signature is used as a proxy for tumor response to anti-PD1 therapy. The black line shows strong IFNγ induction and dashed red lines shows moderate IFNγ induction (B) *Ex vivo* histoculture analysis of JTX-2011 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-inducer (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 signature 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 and 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 RNA 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.

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