Integrated genomics and histology based studies of triple negative breast cancer identify ICOS as potential target for therapeutic intervention

ABSTRACT# 1678

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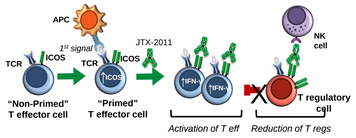
ABSTRACT

ICOS (Inducible CO-Stimulator of T cells) is a co-stimulatory molecule expressed primarily on T lymphocytes. ICOS was prioritized as a target of interest based on nonclinical and clinical data that identified ICOS as a potentially key molecule in providing optimal anti-tumor benefit following anti-CTLA-4 therapy. JTX-2011 is an ICOS agonist antibody that is designed to generate an anti-tumor immune response through stimulation of T effector (Teff) cells and preferential reduction of intratumoral T regulatory (Treg) cells. In preclinical mouse tumor models, efficacy of an ICOS agonist was greatest in tumors with the highest levels of intratumoral ICOS, suggesting a potential predictive biomarker approach for clinical development. In assessing ICOS expression across multiple tumor types at both the RNA and protein level, we have identified triple negative breast cancer (TNBC) as a potential indication for an ICOS-targeted immunotherapy approach.



JTX-2011 Dual Mechanism of Action

Integrated analysis of RNA, DNA and clinical data from the Cancer Genome Atlas (TCGA) was performed to understand the context in which ICOS is expressed. Additionally, ICOS levels were assessed by IHC in human tumor samples from an orthogonal data set. IHC and RNA analyses revealed a dynamic range of ICOS expression across indications and identified a subpopulation of breast cancer tumors enriched for high ICOS expression. Further analysis of both IHC and RNA data sets revealed that the triple negative subtype has higher enrichment of ICOS expression than other breast cancer subtypes. ICOS levels were correlated to gene signatures of immune infiltrate as well as other clinical attributes and molecular markers. There was a correlation between ICOS, ICOS signature, PD-L1 and IFNγ signatures. We then assessed TNBC samples obtained pre- and post-neoadjuvant chemotherapy treatment to further understand the impact of chemotherapy on the tumor microenvironment. This included analysis of ICOS and PD-L1 protein expression as well as assessment of tumor-infiltrating immune cell subsets. While the distribution of certain immune cell subsets differed in pre- and post-treatment samples, the expression of ICOS remained consistent. Based on these data, a TNBC cohort, enriched for the ICOS IHC biomarker, is included in the Phase 2 portion of the ICONIC study that is designed to assess the potential for a combination of JTX-2011 with a PD-1 checkpoint inhibitor in this difficult to treat patient population.

SUMMARY

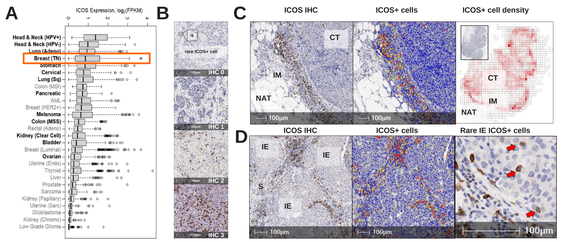
- ICOS is highly expressed across a wide range of malignancies and has a dynamic range within indications.
- Within breast cancer, the triple negative subtype has highest enrichment of ICOS expression and has been selected for Phase 2 expansions in the ICONIC trial.
- In samples from subjects on a neoadjuvant chemotherapy study ICOS expression levels do not appear to be decreased following the chemotherapy regimen.
- Understanding the interplay between ICOS/PD-L1 and other determinants of the tumor microenvironment will be critical to development of an ICOS agonist.

References:

1. Hugo W, Zaretsky JM, *et al.* Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. Cell, 2016 Mar 24; 165(1):35-44 DOI: https://doi.org/10.1016/j.cell.2016.02.065

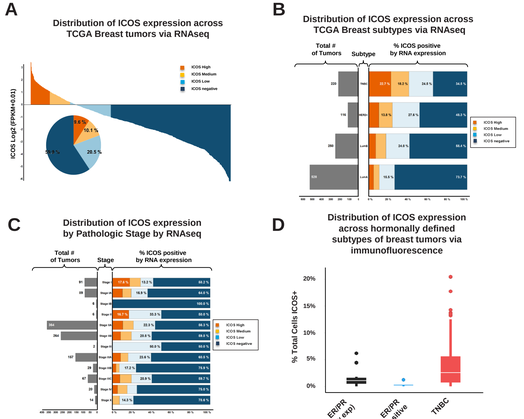
RESULTS

Figure 1: Analysis of ICOS mRNA and protein expression in human tumors



(A) FPKM values for the ICOS gene across tumor types in TCGA. **(B)** Examples of ICOS IHC scoring based on the percentage of ICOS positive immune cell infiltrate within the tumor. **(C)** a number of evaluable specimens (in 12/35 TNBC resections) showed increased ICOS+ cells at the invasive margin (IM); CT = center of tumor; NAT = normal adjacent to tumor. **(D)** ICOS+ cells preferentially resides in the stromal- (S) compared to the intra-epithelial- region (IE) of the tumor in 42/45 TNBC.

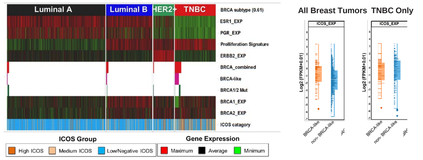
Figure 2: Exploration of ICOS expression distribution across breast cancer tumors



(A) Distribution of ICOS expression across breast cancer tumors from TCGA RNAseq datasets. RNAseq samples were binned into high, medium, low, and absent ICOS using thresholds based on proportions observed in across multiple indications using IHC. **(B)** Percentages of tumors within each subtype that are ICOS positive by subtype based on RNAseq. **(C)** Percentages of tumors that are ICOS positive by tumor stage based on RNAseq. **(D)** ICOS protein levels in breast cancer subtypes based on immunofluorescence.

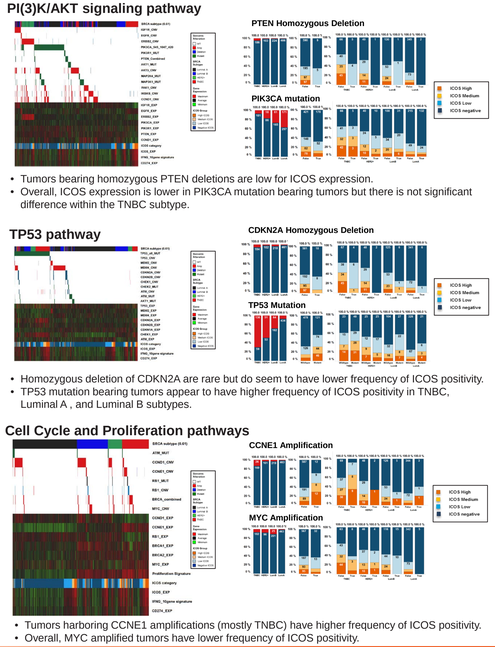
- ICOS exhibits a dynamic range of expression within breast cancer with only a subset of tumors ranked as ICOS high (~20% by RNA).
- The TNBC subtype exhibits the highest percentage of tumors considered ICOS high both at the RNA and protein level.

Figure 3: BRCA-like tumors vs ICOS expression



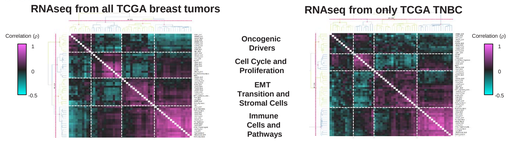
- While overall BRCA-like tumors exhibit higher ICOS expression, it is not differential within the triple negative subtype.

Figure 4: Association of ICOS with key genomic alterations within breast cancer



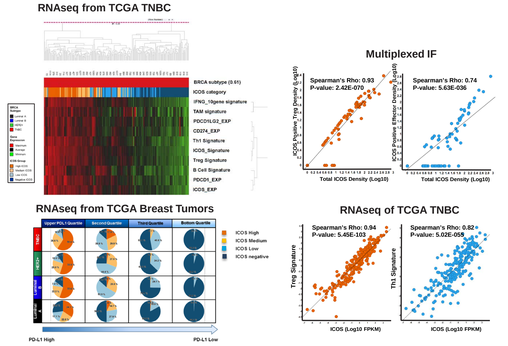
- Tumors bearing homozygous PTEN deletions are low for ICOS expression.
- Overall, ICOS expression is lower in PIK3CA mutation bearing tumors but there is not significant difference within the TNBC subtype.
- Homozygous deletion of CDKN2A are rare but do seem to have lower frequency of ICOS positivity.
- TP53 mutation bearing tumors appear to have higher frequency of ICOS positivity in TNBC, Luminal A , and Luminal B subtypes.
- Tumors harboring CCNE1 amplifications (mostly TNBC) have higher frequency of ICOS positivity.
- Overall, MYC amplified tumors have lower frequency of ICOS positivity.

Figure 5: Expression of ICOS within the context of the immune microenvironment



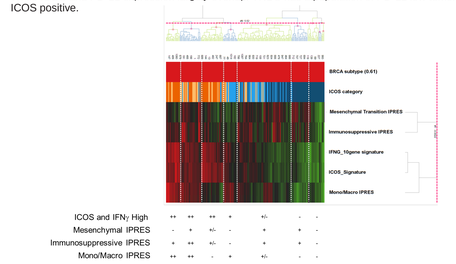
Covariance of gene expression within breast tumors

- ICOS gene expression correlates with other markers of immune infiltrate.
- Oncogenic drivers do not tend to cluster with ICOS expression and other markers of immune infiltrate.



Comparison of ICOS to PD-1 axis and signatures of immune cells

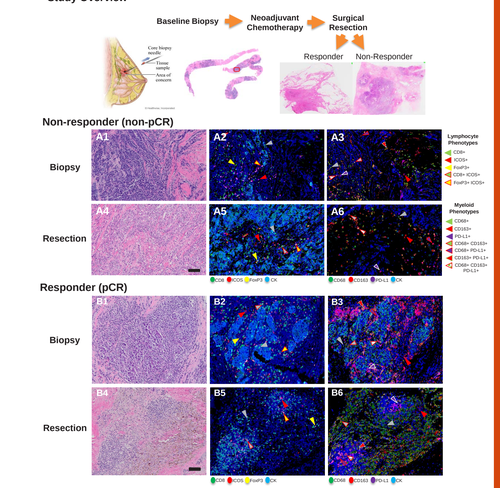
- ICOS is highly correlated to cell type specific signatures of immune infiltrate as well as PD-1 biology.
- ICOS is more correlated with Treg cells than with Teff cells.
- While ICOS and PD-L1 expression largely overlap, TNBC has a population of PD-L1 low tumors that are ICOS positive.

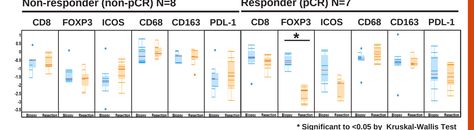


ICOS RNA expression compared to innate PD-1 resistance expression signatures (IPRES)[1]

- There are distinct subpopulations of ICOS high tumors that also express signatures of resistance.
- There are also distinct subpopulations that do not express signatures of resistance.

Figure 6: Contextual analysis of neoadjuvant treated TNBC





Analysis of immunological phenotypes in neoadjuvant non-responsive and responsive TNBC

- Morphological overview and immunofluorescent staining (IF) of pre-treatment biopsies and post treatment resections (magnification bars = 100um), in neoadjuvant responders (A) and non-responders (B).
- IF was performed in serial sections for CD8, ICOS, FoxP3, and CK (Lymphocyte panel) or CD68, CD163, PD-L1, and CK (myeloid panel), allowing for identification of phenotypes from lymphocytes and myeloid cells:
  - Lymphocyte: CD8+; ICOS +; FoxP3+; CD8+ ICOS+; and FoxP3+ ICOS+
  - Myeloid: CD68+; CD163+; PD-L1+; CD68+ CD163+; CD68+ CD163+ PD-L1+; CD163+ PD-L1+; and CD68+ PD-L1+
- Both manual and digital analyses examined the relative abundance of CD8, ICOS, FoxP3, CD68, CD163, and PD-L1 (digital analysis plots shown).
- In the neoadjuvant non-response group, across all cell types, there was no change in the amount of positive signal between pre-treatment biopsy and post-treatment resection.
- In the neoadjuvant response, there is a significant decrease in the overall FoxP3 signal between the biopsy and the resection (B7, H = 8.33$_{1,12}$, p = 0.004). All other markers remained unchanged between pre-treatment biopsy and post-treatment resection in the neoadjuvant response condition.

Presented at the American Association of Cancer Research (AACR) Annual Meeting ● April 14-18, 2018 ● Chicago, Illinois