Preclinical Evaluation of JTX-8064, an Anti-LILRB2 Antagonist Antibody, for Reprogramming Tumor-Associated Macrophages

ABSTRACT# 5007

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ABSTRACT

Introduction: Jounce has generated cell type-specific gene signatures as a means of probing The Cancer Genome Atlas and other large datasets to identify targets that may be important immune checkpoints. Using a tumor-associated macrophage (TAM) gene signature, we have found a strong correlation and coherence between TAMs and LILRB2 (leukocyte immunoglobulin like receptor B2; ILT4) across multiple tumors types. LILRB2 is a myeloid cell surface receptor containing four extracellular immunoglobulin domains, a transmembrane domain, and three cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs). Ligation of LILRB2 on myeloid cells, via its endogenous ligands (classical MHC I molecules [e.g. HLA-A, HLA-B] and non-classical MHC I molecules [e.g. HLA-G]), provides a negative signal that inhibits stimulation of an immune response. HLA-G is recognized as an important immunosuppressive molecule playing a role in maternal-fetal tolerance and being overexpressed in cancer – often associated with advanced disease stage and poor prognosis. As tumor-associated macrophages are known to suppress the anti-cancer immune response, these findings provide rationale for targeting LILRB2.

Methods and Results: We have generated a panel of monoclonal antibodies that bind specifically to LILRB2, but not other LILR family members, and can block binding of LILRB2 to MHC I molecules (i.e. HLA-A and HLA-G). In vitro differentiated human monocyte-derived macrophages (HMDMs) cultured for 24h in the presence of anti-LILRB2 antibodies and lipopolysaccharide (LPS) show polarization toward a more inflammatory phenotype - secreting higher levels of TNF-α and IL-6 with decreased amounts of IL-10 and CCL2 as compared to an isotype control antibody. NanoString mRNA analysis revealed that, in the absence of LPS or any additional stimuli, MDMs cultured with anti-LILRB2 antibodies showed gene changes consistent with inflammatory or M1-like polarization of macrophages. Anti-LILRB2 antibodies were also evaluated in human tumor histoculture and induced pharmacodynamic responses consistent with macrophage and T cell activation in a variety of tumor types. While mice do not express LILRB2 specifically, they do express a LILRB-like molecule known as Pirb. Mice that are deficient in Pirb display resistance to mouse colon 38 (MC-38) tumor growth suggesting this pathway functions as immune checkpoint in cancer.

Conclusions: Based on these preclinical data, JTX-8064, a high affinity LILRB2-specific humanized antagonist monoclonal antibody, is being developed as an immunotherapeutic to reprogram suppressive macrophages within the tumor microenvironment.

RESULTS

LILRB Proteins: Inhibitory Receptors Expressed on Immune Cells

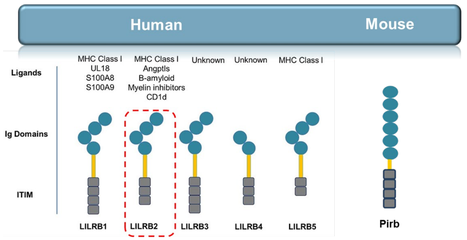

Figure 1. The LILRB family of proteins consists of 5 distinct members that vary in the number of Ig-like extracellular and immunoreceptor tyrosine-based inhibitory (ITIM) cytoplasmic domains. Several members bind MHC class I molecules (e.g. HLA-A and HLA-G), but with differential affinity particularly to β2m-free forms of HLA. Humans also contain 6 activating LILRA receptors with highly similar Ig-like domains that can bind MHC class I molecules. Mice contain only one inhibitory receptor (Pirb) and one activating receptor (Pira).

Knockout Mice Highlight Importance of Pirb (LILRB) as a Potential Immune Checkpoint in Cancer

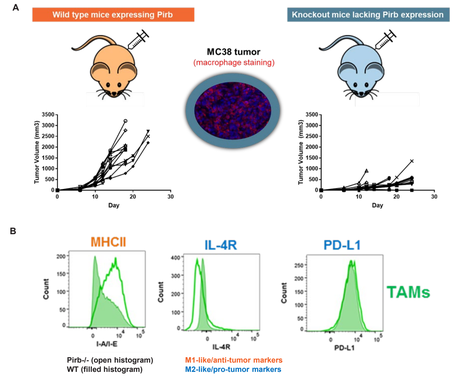

Figure 2. Pirb knockout mice show significantly delayed mouse colon-38 (MC-38) tumor growth. **A)** MC-38 tumor cells were injected subcutaneously into the flanks of wild type (WT) C57BL/6 or Pirb knockout C57BL/6 mice (Dr. Toshi Takai, Tohuku University).In comparison to WT mice, Pirb knockout mice experienced significantly delayed tumor growth suggesting a key role for Pirb in inhibiting the anti-tumor response. **B)** Tumor-associated macrophages (TAMs) from WT or Pirb knockout mice were stained for the presence of surface markers commonly associated with M1-like or M2-like macrophages. Pirb knockout mice show significantly more MHC class II and decreased IL-4R expression than WT mice - consistent with a more M1-like phenotype.

Proposed Model: Dual Mechanism of Action of JTX-8064 on Myeloid Cells

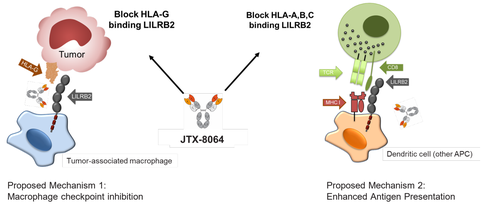

Figure 3. JTX-8064 promotes anti-tumor immunity by 1) blocking a potential macrophage 'checkpoint' thereby shifting macrophages to a more pro-inflammatory or M1-like state and 2) enhancing the function of antigen presenting cells resulting in T cells with greater effector functions.

JTX-8064 Binds With High Affinity to LILRB2 in Region Known to Interact with HLA-G

mAb	ka(1/M^{-1}s^{-1})	kd(1/s)	K_D(M)
JTX-8064	7.05E+05	1.76E-03	2.49E-09

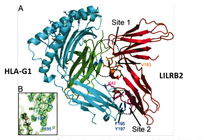

Figure 4. JTX-8064 binds to LILRB2 with low nanomolar affinity in a site previously described to be important for HLA-G binding. The affinity of HLA-G (and other class I HLAs) is reported to be in the micromolar range and JTX-8064 exhibits the ability to out-compete HLA for binding LILRB2 using on-cell binding assays (Figure 5).

JTX-8064 Potently Blocks Binding of HLA-G and HLA-A2 to LILRB2

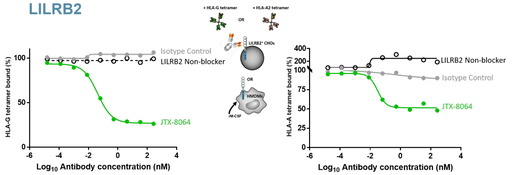

Figure 5. JTX-8064 potently blocks HLA-G and HLA-A2 tetramers from binding to human monocyte-derived macrophages (HMDMs; data above) and CHO cells overexpressing LILRB2 (data not shown). JTX-8064 or control antibodies were added in dose titration to HMDMs along with HLA-G or HLA-A2 tetramers labeled with an APC conjugate. Maximal tetramer binding is the FACS-determined MFI of HMDMs plus tetramer in the absence of antibodies. Note: HMDMs also express other receptors for HLA-G and HLA-A2 thus total tetramer blocked is less than 100%.

JTX-8064 Binds Specifically to LILRB2 Not Other LILRB/A Family Members

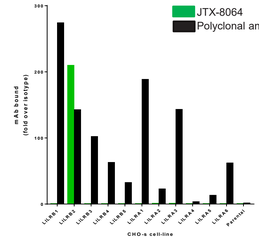

Figure 6. JTX-8064 (10 μg/mL) binds specifically to LILRB2 overexpressing CHO-S cells and not parental CHO-S cells or CHO-S cells overexpressing other LILRB or LILRA family members as measured by FACS. Cell surface expression of LILRB/LILRA was confirmed by staining all cell lines with polyclonal anti-LILRB antibody (AF2078). LILRB2-specific binding was also observed at 100 μg/mL of JTX-8064, the highest concentration tested (data not shown).

JTX-8064 Enhances Pro-Inflammatory (M1-like) Activation of Macrophages

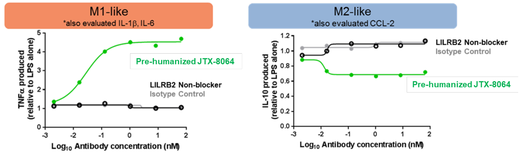

Figure 7. JTX-8064 promotes induction of pro-inflammatory (M1-like) cytokines while reducing M2-like cytokines. HMDMs were stimulated with LPS and JTX-8064 for 24 hr at which time supernatants were assayed for TNF-alpha or IL-10 using cytokine bead arrays. Isotype control and mAbs that bound specifically to LILRB2 but did not block the LILRB2/HLA-A(G) interaction had no effect on pro-inflammatory cytokine induction or IL-10.

Transcriptomic Evidence of a Unique M1-like Shift in anti-LILRB2 Treated Macrophages

Figure 8. Transcriptomic analysis suggests blocking the LILRB2/HLA interaction shifts macrophages to an M1-like state. HMDMs were cultured with 3 LILRB2-specific antibodies, two macrophage targeting antibodies (CSF1R, TIM-3), or isotype control in the absence of any additional stimulation. After 24 hr RNA was harvested for NanoString analysis. LILRB2 mAbs #1-3 (including pre-humanized JTX-8064 - Blocker #2) potently block the LILRB2/HLA interaction and induce the upregulation of several M1-associated genes while downregulating M2-associated genes in comparison to isotype control. The anti-LILRB2 antibodies induce a distinct transcriptional profile compared to macrophage targeted mAbs CSF1R and TIM-3.

JTX-8064 Induces Both Inflammatory Myeloid and IFN-γ Gene Signatures in Human Tumor Histoculture

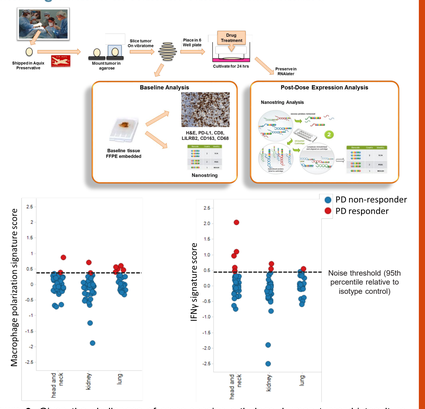

Figure 9. Given the challenges of cross-species orthologs, human tumor histoculture was utilized to analyze the effect of JTX-8064 on the tumor microenvironment of 3 different cancers. A) Schematic overview of the tumor histoculture technique B) Single agent JTX-8064 induces both a macrophage inflammatory gene signature (left) and an IFN-γ gene signature (right) in a subset of tumor samples (red pharmacodynamic (PD) responders; blue PD non-responders). The IFN-γ signature is comprised of genes generally associated with response to T cell checkpoint therapy, suggesting innate and adaptive immune cell modulation. We are assessing the ability of JTX-8064 to combine and/or synergize with checkpoints and other therapies. Additional work is ongoing to expand this analysis and understand potential predictive biomarkers associated with PD response in histoculture.

SUMMARY

- LILRB2 is part of the LILRB family of inhibitory proteins and suppresses the activation of myeloid cells
- Pirb knockout mice show substantially delayed growth of MC-38 tumors highlighting the importance of this pathway as a potential checkpoint in anti-tumor immunity
- JTX-8064 is a LILRB2-specific mAb that potently blocks binding of classical and non-classical HLA class I molecules to LILRB2
- JTX-8064 induces pro-inflammatory cytokine secretion and a unique transcriptional profile suggestive of an M1-like shift in human macrophages
- JTX-8064 induces both myeloid inflammatory and IFN-γ signatures in a subset of human tumor slices treated suggesting beneficial innate and adaptive immune cell modulation of the tumor microenvironment.
- Based on these pre-clinical data JTX-8064 is being developed as a novel immunotherapy to reprogram suppressive macrophages and enhance anti-tumor immunity

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