

CD47/SIRP $\alpha$  interaction and induces phagocytosis of tumor cells, but it also has a direct killing mechanism (via PCDIII) and induction of immunogenic cell death, leveraged by preferential binding to tumor versus normal cell CD47.

**Methods** CD47 and  $\beta$ 1 integrin expression and localization were evaluated using a combination of flow cytometry, western blotting, confocal microscopy and immunohistochemistry.

**Results** Previously, we described that the preferential binding of AO-176 to tumor versus normal cells was due to its interaction with CD47 molecules that were pre-complexed to  $\beta$ 1 integrin. This finding was particularly important and suggestive of why AO-176 does not bind red blood cells since they do not express  $\beta$ 1 integrin. We have extended these findings to show that  $\beta$ 1 integrin as well as CD47 are also expressed at lower levels in normal versus tumor cells, and that solid and hematologic tumor cells overexpress both CD47 and  $\beta$ 1 integrin which correlate with poor prognosis in cancer. In addition, we show that AO-176 is able to bind and occupy CD47/ $\beta$ 1 integrin complexes to a greater extent at acidic versus physiological pH such as would be found in tumor microenvironments, an observation that also contributes to the enhanced targeting of AO-176 to tumor cells. Taken together, these findings add further insight into the preferential binding of AO-176 to tumor versus normal cells.

**Conclusions** The context dependent binding of AO-176 to CD47, when complexed to  $\beta$ 1 integrin, is unique among CD47 axis targeting agents and together with its direct killing mechanism of action offers a potentially better safety profile and opportunity for a therapeutic advantage. AO-176 is currently being evaluated in Phase 1 clinical trials for the treatment of patients with select solid tumors (NCT03834948) and multiple myeloma (NCT04445701).

**Trial Registration** NCT03834948, NCT04445701.

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#### ANTI-TUMOR ACTIVITY OF IOSH2 BY BLOCKING LILRB2 RECEPTOR SIGNALLING

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**Background** The human leukocyte immunoglobulin-like receptor family B (LILRB) acts as check point blockade of the innate immune system by inhibiting leukocyte activation through SHP phosphatase recruitment. Some of the physiological ligands include classical HLA class I molecules, including beta-2-microglobulin (B2M) free open conformers (OC). Natural HLA-OC expression is known from autoimmune disease leading to immune activation by pleiotropic effects since they bind to LILRB and KIR family members reducing Treg and MDSC numbers and increased effector T-cell and NK-cell activation, respectively. We have generated an IgG4-HLA-57 open conformer (OC) molecule (iosH2) with high affinity for LILRB molecules and demonstrate its anti-cancer activity in vitro and in vivo.

**Methods** iosH2 was produced by transient gene expression in CHO cells and purified by standard chromatography. Affinity of iosH2 binding was quantified by ELISA and SPR analysis. HLA-G mediated signaling and competition was assessed using functional cell lines. Effect of iosH2 on activation of SHP1/2 was assessed using Western Blot. Functional assays including in vitro polarization and phagocytosis potential of primary macrophages was assessed by flow cytometry in the presence of

iosH2 or isotype control. Effect of iosH2 on T cell activation was evaluated in co-cultures of cancer and T cells. Mouse models were used to assess in vivo activity.

**Results** iosH2 binds to LILRB2 with high affinity and blocks the activation of HLA-G. In addition, iosH2 blocks receptor-mediated activation of SHP1/2. iosH2 promotes a shift from M2 to M1 macrophages with enhanced tumor cell phagocytosis in vitro. iosH2 enhances activation and killing potential of T cells in cancer cells and T cells co-culture assay. iosH2 exerts therapeutic efficacy in mouse transgenic (melanoma) and different syngeneic tumor models (e.g. pancreatic, colon and breast cancer) as monotherapy. Moreover, it acts synergistically in vivo with PD1 blocking antibodies achieving long-term tumor control. Ex vivo tumor sample analysis demonstrates a significant reduction of MDSC and Tregs and a shift towards an activated inflammatory M1 macrophage phenotype. Loss of MDSC functionality was paralleled by enhanced CD8 + T cell expansion and activity.

**Conclusions** iosH2 binds to LILRB2 with high affinity, restores immune cell function in vitro and demonstrates anti-tumor activity in different in vivo mouse models. In addition, it acts synergistically in vivo with PD1. iosH2 is a first-in-class OC therapeutic with robust anti-tumor activity by promoting key components of the innate immune system. Clinical development is under way and phase I trial in preparation.

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#### EVALUATING BIOMARKERS OF JTX-8064 (ANTI-LILRB2/ILT4 MONOCLONAL ANTIBODY) IN AN EX VIVO HUMAN TUMOR HISTOCULTURE SYSTEM TO INFORM CLINICAL DEVELOPMENT

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**Background** Leukocyte immunoglobulin-like receptor B2 (LILRB2; ILT4) is an immunoinhibitory protein expressed on the surface of myeloid cells that has been increasingly recognized as a therapeutic target of interest in immuno-oncology (IO). Upon binding its ligands, MHC I molecules (e.g. HLA-G/HLA-A), LILRB2 inhibits myeloid cell activation and promotes an M2-like (anti-inflammatory) state. LILRB2 was the first target prioritized from a macrophage discovery effort leading to the development of JTX-8064, a humanized monoclonal antibody that specifically binds to and antagonizes LILRB2. JTX-8064 has been shown to induce an M1-like (pro-inflammatory; anti-tumor) functional state in macrophages. Rodents do not express LILRB proteins limiting their usefulness as a model for preclinical study of JTX-8064. To overcome this limitation, we conducted an ex vivo human tumor histoculture study to assess the pharmacodynamic effects of LILRB2 antagonism. Protein and/or gene expression analysis of matched tumor samples enabled the discovery of predictive biomarkers associated with the induction of specific pharmacodynamic signatures in ex vivo-cultured human tumors in response to JTX-8064. Finally, tumor types were identified that had a high prevalence of these predictive biomarkers suggesting they may be priority indications for JTX-8064 therapy.

**Methods** More than 100 fresh treatment-naïve human tumor samples obtained post-surgery from kidney, lung, and head and neck cancer were treated with JTX-8064 or isotype