CD47/SIRPα 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 β1 integrin expression and localization were evaluated using a combination of flow cytometry, western blotting, confocal microscopy and immunohistochrometry.

**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 β1 integrin. This finding was particularly important and suggestive of why AO-176 does not bind red blood cells since they do not express β1 integrin. We have extended these findings to show that β1 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 β1 integrin which correlate with poor prognosis in cancer. In addition, we show that AO-176 is able to bind and occupy CD47/β1 integrin complexes to a greater extent at acidic versus physiologic 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 β1 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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**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 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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control antibody for 24 hrs in the histoculture system. RNA was isolated from tumors prior to any treatment as well as from JTX-8064 and isotype control treated samples. Gene expression was analyzed using the NanoString nCounter® and qPCR assays. Additional IHC analyses were performed on baseline untreated tumor samples.

Results JTX-8064 was shown to induce pharmacodynamic responses to treatment significantly above control indicative of macrophage polarization, IFNg-signaling, and T cell inflammation. To identify predictive biomarkers of pharmacodynamic response to JTX-8064, matched untreated samples were characterized by gene expression analysis and by IHC (CD8, CD163, and HLA-G proteins). Numerous LILRB2 pathway-related molecules (e.g. HLA-A, HLA-B, CD163, LILRB2) and gene signatures were found to be statistically significantly higher in the untreated kidney, head and neck, and lung cancer samples of matched pharmacodynamic responders compared to non-responders. Further bioinformatics analysis revealed additional cancer subtypes where these biomarkers are enriched.

Conclusions These data will inform indication selection and combination strategies for JTX-8064 to maximize potential therapeutic benefit for patients with solid tumor malignancies.

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218 A PRECLINICAL STUDY OF IMC-002, A FULLY HUMAN THERAPEUTIC ANTIBODY SAFELY TARGETING CD47 IN CANCER
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Background Immunotherapy with immune checkpoint inhibitors such as PD-(L)1 and CTLA-4 blocker has become an important part of cancer treatment. For the cancers resistant to these drugs, however, many other therapeutic targets are being tested to modulate the tumor microenvironment (TME) toward anti-cancer immunity. Due to the functional flexibility, macrophages play an essential role in orchestrating tissue immunity including TME. CD47 is one of the key targets that modulate macrophages, which is often overexpressed on cancer cells.1 When it binds to its receptor, SIRPα, it gives a ‘don’t-eat-me’ signal and inhibits phagocytosis of cancer cells by macrophages.2 IMC-002 is a fully human IgG4 monoclonal antibody targeting human CD47, which has been engineered to possess optimal efficacy and safety profile. IMC-002 does not induce hemagglutination and contains a hinge stabilizing S228P mutation to prevent Fab arm exchange.

Methods A series of in vitro functional assays including ligand binding, cell surface binding and phagocytosis assays were performed. Putative epitopes for IMC-002 were identified using synthetic peptide libraries. In vivo efficacy of IMC-002 was tested in human breast cancer models. Pharmacokinetic parameters and toxicity profiles were assessed in mice and cynomolgus monkeys.

Results IMC-002 strongly bound to CD47 ligand and to various types of CD47-expressing cancer cells including solid and hematological cancers. IMC-002 also bound to human CD4 T cells and, to a lesser degree, to CD8 T cells, but not to NK or B cells. Interestingly, IMC-002 showed no binding to RBCs which highly express CD47 and thus, did not induce RBC agglutination in vitro. IMC-002 induced phagocytosis of cancer cells by human blood CD14+ monocyte-derived macrophages and strongly suppressed tumor growth in a dose-dependent manner in xenograft animal models. Treating IMC-002 with tumor antigen targeting IgG1 type therapeutics increased phagocytosis compared to single treatment. Epitope mapping analysis revealed that compared to RBC-binding anti-CD47 antibody and a natural ligand, SIRPαFc, IMC-002 bound to distinct parts of CD47 antigen, which may be responsible for the cell-selective binding of IMC-002. Consistent with the in vitro data, IMC-002 was well tolerated in cynomolgus monkeys with no adverse effects including hematologic toxicity at doses up to 100 mg/kg. IMC-002 showed a typical pharmacokinetic profile of therapeutic antibody with a half-life of 5–10 days. Given its differential binding profile toward tumor cells vs normal cells such as RBC, preclinical data was thoroughly analyzed to simulate human PK and to come up with the optimal first-in-human dose.

Conclusions Preclinical efficacy and safety profiles of IMC-002 provide a strong rationale for assessing therapeutic potential in clinical studies. Particularly, IMC-002 is expected to be beneficial for hematologic cancer patients because it has been engineered to minimize hematologic toxicities such as anemia which is a class effect of the CD47-targeting antibodies. The first-in-human (FIH) study of IMC-002 is ongoing in the US sites. The purpose of the study is to assess the safety and tolerability of IMC-002 and determine the recommended Phase 2 dose (RP2D) of IMC-002 in subjects with metastatic or locally advanced solid tumors and relapsed or refractory lymphomas.

Ethics Approval All experimental procedures were performed according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the contract research organizations.

REFERENCES

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219 LONG-TERM CLINICAL OUTCOMES ASSOCIATED WITH SEQUENTIAL TREATMENT OF BRAF M UTANT ADVANCED MELANOMA PATIENTS
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Background Patients with BRAF mutant advanced melanoma can be treated sequentially with immunotherapies (IO) and BRAF+MEK inhibitors. We evaluated the clinical outcomes associated with various treatment sequences for BRAF mutant advanced melanoma based on the 5-year follow-up data from clinical trials.

Methods In the absence of head-to-head trial data, a matching-adjusted indirect comparison (MAIC) was conducted for IO vs. BRAF+MEK inhibitors, using the longest follow-up available in the published literature. Multivariate risk models.