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 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 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 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 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
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, SIRPα-Fc, 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 hemato logical 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 hematological 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