Development of AO-176, a next generation humanized anti-CD47 antibody with novel anti-cancer properties and negligible binding to red blood cells

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Abstract

Blocking agents to inhibitory immune checkpoints has shown significant advances in cancer treatment and has focused many on enhancing adaptive immune responses. CD47, a cell surface glycoprotein, is an innate immune checkpoint receptor broadly expressed in normal tissues. Binding of CD47 to signal regulatory protein alpha (SRPα) on macrophages and dendritic cells delivers a ‘don’t eat me’ signal that inhibits phagocytosis. Several hematologic and solid tumors escape innate immune surveillance by overexpression of surface CD47 preventing engagement and the interaction of SRPα/CD47. The ability of AO-176 to induce direct tumor cytotoxic cell death in hematologic and solid human tumor cell lines by a cell autonomous mechanism (not ADCC), while sparing primary T cells and other normal tissues (endothelial, skeletal and epithelial). Secondly, AO-176 has preferential binding to tumor cells compared to normal cells, including ex vivo red blood cells (RBC’s) (cynomolgus monkey and human). T cells, and other normal human cells. The negligible binding to RBC’s is expected not only to lower the antigen sink and not sequester AO-176 away from tumor cells, but also to minimize on-target clinical adverse effect observed following treatment with other RBC-binding CD47 antibodies. When tested in cynomolgus monkeys, no adverse effects were observed with respect to RBCs and correlated well with in vitro results. A third novel property of AO-176 is its enhanced binding to tumor cells at acidic pH. AO-176 binds to human tumor cell lines with a mean or range of 20-fold higher at an acidic pH of 6.5 compared to a physiological pH. Because the microenvironment of solid tumors has an acidic pH of 6.4-7.2, this enhanced binding of AO-176 at low pH has the added advantage of tumor targeting. Lastly, we have demonstrated a dose-dependent anti-tumor activity in tumor xenograft models. Taken together, the unique functional characteristics of AO-176, including induction of tumor-specific cell-autonomous killing while sparing T cells and other normal tissues, enhanced binding to tumor cells at acidic pH, tumor-specific reduced binding to RBCs and potent in vivo efficacy should allow for enhanced anti-tumor efficacy and reduction in off-target toxicity (anemia). We believe that AO-176 development will allow for the generation of a therapeutically more superior anti-CD47 blocking antibody.

AO-176: A Next-Generation Humanized anti-CD47 mAb

- Humanized IgG2
  - Blocks CD47/SIRPα interaction to induce phagocytosis of tumor cells
- Selectively and potently binds to human CD47 on tumor cell lines
- Reduced binding to normal cells and negligible binding to human RBC without hemagglutination
- Greater binding affinity at acidic pH (pH is in pH 6.5-6.5; potential tumor targeting
- Direct killing of tumor cells (non-ADCC) programmed cell death type II and previously shown to be anti-tumor cell death characterized by SIRPα induction
- Antitumor efficacy in xenograft models
- Induced recruitment and dendritic cell infiltration to Raji and MDA-MB-231 tumors in vivo
- Very well tolerated in IND enabling toxicology studies

AO-176 Preferentially Blocks CD47 on Tumor Cells

- CD47 blocking by AO-176
- Cytokine release
- ADCC
- Complement-mediated lysis
- Apoptosis
- NK cell killing

AO-176 Does Not Kill Normal Cells

- CD3
- HAEC
- SMAC

AO-176 Kiling & Phagocytosis of Human Tumor Cells

AO-176 Mediates Cell-Autonomous Early and Late Apoptotic Killing of Tumor Cells

AO-176 Inhibits Growth of TNBC and B Cell Lymphoma Xenografts and Increases Macrophage and DC Tumor Infiltrates

AO-176 inhibits growth of Raji B cell lymphoma by promoting macrophage/DC recruitment and cytokine/chemokine induction in the tumor microenvironment

AO-176's unique killing profile coupled with phagocytosis induction and preferential binding to tumor versus normal cells suggest that AO-176 will have an improved therapeutic index compared to current clinical candidates and suggest further clinical investigation.

Conclusions

- AO-176 demonstrates preferential binding to tumor versus normal cells especially RBCs.
- AO-176 shows enhanced binding and function at acidic pH (such as in tumor microenvironment), a potential tumor targeting mechanism.
- In addition to promoting phagocytosis, AO-176 induces FcγR signaling as well as immunocytic cell death (CD47 is an ADCC and a phagocytic targeting mechanism) and some normal tumor cells but sparing normal cells (i.e. T cells)
- AO-176 showed significant tumor growth inhibition in B cell lymphoma and TNBC xenograft models.
- The observed tumor growth inhibition was accompanied with recruitment of microphages and dendritic cells (DCs) to the TME for both Raji B cell lymphoma and MDA-MB-231 TNBC models.
- Macrophages and DC infiltrates correlated with an increase in murine cytokines and chemokines in B cell lymphoma xenografts.

Summary Table of Phagocytosis and Killing of Solid Tumors

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