AO-176, a highly differentiated humanized anti-CD47 antibody, exhibits single-agent and combination anti-tumor efficacy with chemotherapy and targeted antibodies in pre-clinical models.

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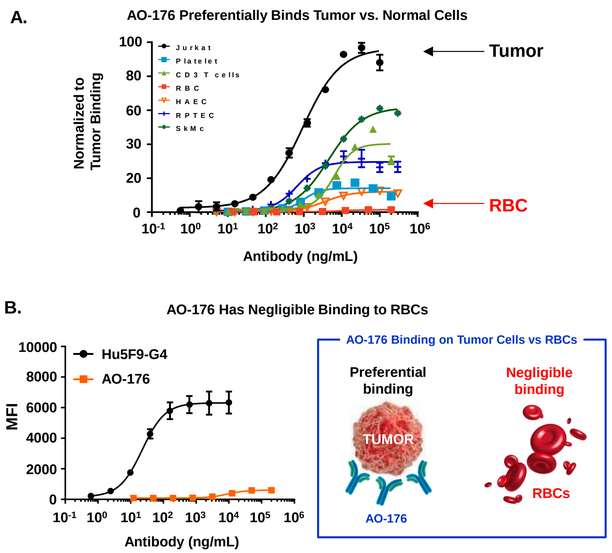
Abstract #403

Abstract

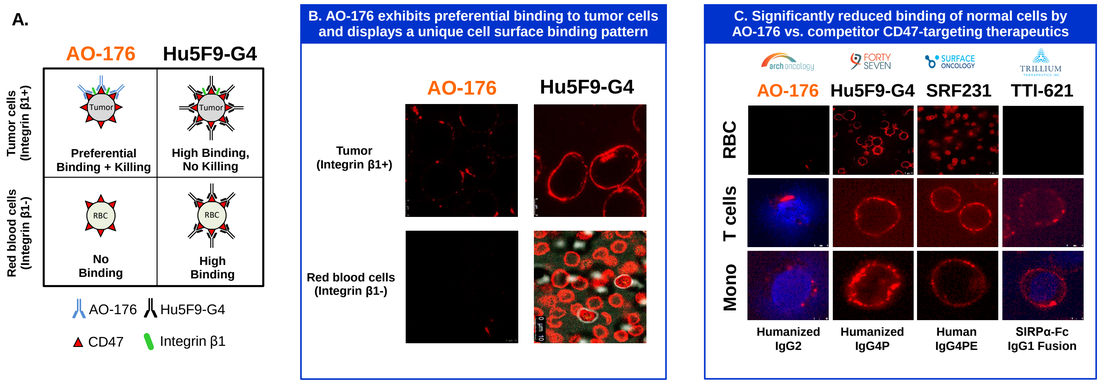
Purpose of study: 1) To define the mechanism by which AO-176, a highly differentiated humanized anti-CD47 antibody, preferentially binds to tumor vs normal cells; 2) To determine whether AO-176 shows efficacy alone or in combination with a variety of approved anti-cancer drugs in solid tumors.
Methods: We investigated AO-176 as a single agent and in combination with chemotherapies and targeted antibodies, utilizing standard *in vitro* phagocytosis assays, tumor cell killing assays, and *in vivo* xenograft models.
Results: AO-176 is a highly-differentiated anti-CD47 antibody that has previously been shown pre-clinically to not only block the CD47/SIRPα interaction to stimulate phagocytosis of tumor cells, but also to exert direct killing activity on tumor cells (non-ADCC), induce immunogenic cell death, and exhibit preferential binding to tumor cells compared to normal cells. Here we show that AO-176 binds tumor and normal cells in a unique pattern on the cell surface, co-localizing with integrin β1. As RBCs do not express integrin β1, AO-176 has negligible binding to RBCs. Knockout of integrin β1 in tumor cells abrogated AO-176 binding. As a single agent, AO-176 induced cell killing (8-25% Annexin V+ at 100 μg/ml) and phagocytosis (20%, 0.3-10 μg/ml) in ovarian, gastric, and head and neck solid tumor cell lines. When combined with tumor-targeted antibodies (cetuximab in head and neck, or the checkpoint inhibitor avelumab in ovarian cancer cell lines), AO-176 significantly enhanced phagocytosis of the tumor cells *in vitro*. In combination with cisplatin, AO-176 also potentiated direct tumor killing of gastric cancer cells in vitro (3-6 fold increase). *In vivo*, AO-176 showed potent single-agent anti-tumor activity against ovarian and gastric tumor xenografts. These data add to previous pre-clinical anti-tumor activity of AO-176 reported in breast cancer, multiple myeloma, and non-Hodgkin's lymphoma xenografts. When cisplatin was added to the AO-176 treatment regimen, significant combination anti-tumor activity was observed in ovarian and gastric xenografts. We further extended our *in vitro* findings of AO-176 combined with a checkpoint inhibitor to an *in vivo* model. As AO-176 recognizes only human CD47, we utilized a tool murine-reactive anti-CD47 blocking antibody and combined it with a murine-reactive anti-PD1 or PDL1 antibody to treat MC38 murine tumors established *in vivo*. Combination of the two antibodies significantly improved anti-tumor efficacy compared to single agents alone.
Conclusions: AO-176 is an anti-CD47 antibody with direct killing activity that is highly differentiated from current clinical agents targeting the CD47 axis**.** AO-176 is the first anti-CD47 agent that targets CD47 in a context-dependent manner. AO-176 binds CD47 complexed with integrin β1; this explains the negligible binding to RBCs as they do not express integrin β1. AO-176 exhibits single-agent and combination efficacy with chemotherapy and targeted antibodies. AO-176 is currently being evaluated in a Phase 1 clinical trial (NCT03834948) for the treatment of patients with select solid tumors.

AO-176 Preferentially Binds Tumor vs. Normal Cells, and Has Negligible Binding to RBCs



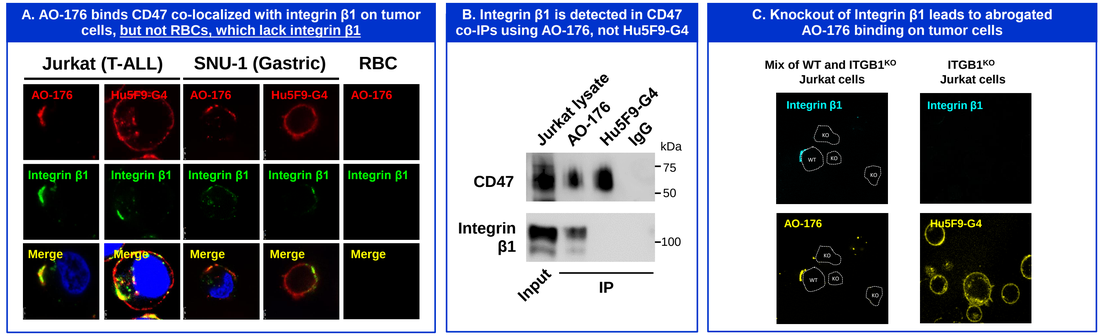
(A) Binding of AO-176 to tumor cells (Jurkat) with reduced binding to RBCs and other normal cells. **(B)** Negligible binding of AO-176 to human RBCs compared to Hu5F9-G4.

AO-176 Displays a Unique Binding Pattern to CD47 on the Surface of Tumor and Normal Cells



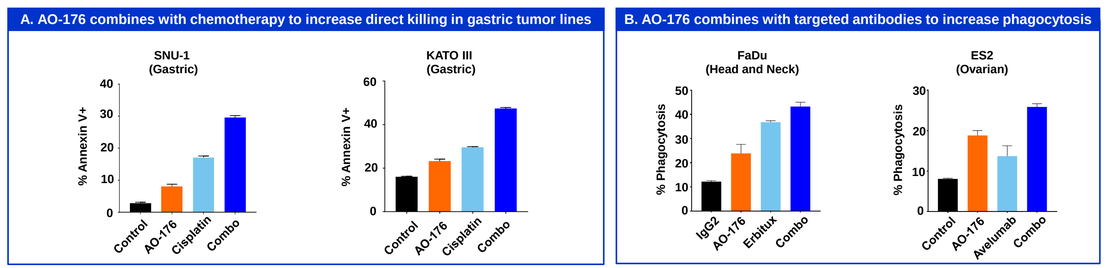
(A) Cartoon illustrating the differences in binding properties between AO-176 and Hu5F9-G4. **(B)** Fluorescent microscopy images showing the distinct binding pattern of AO-176 to discrete regions on the cell surface of tumors, and negligible binding to RBCs. **(C)** Fluorescent microscopy images of AO-176 (Red) binding vs. competitor CD47 targeting therapeutics (Red) binding on RBCs, T cells, and monocytes (Mono).

AO-176 Binds CD47 Complexed with Integrin β1: Negligible RBC Binding (Integrin β1 negative)



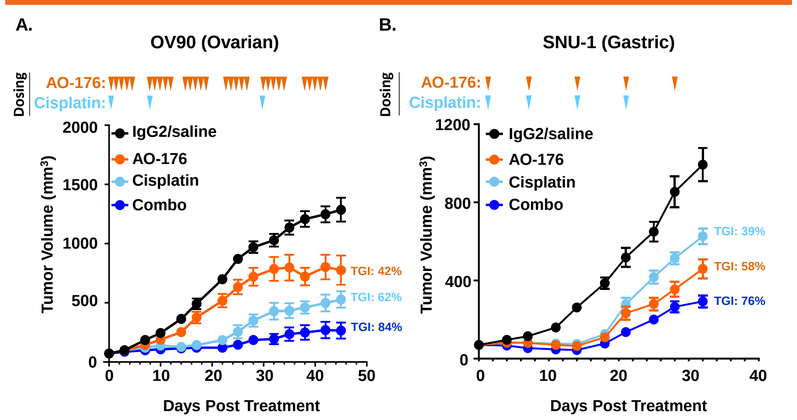
(A) AO-176 binds to CD47 on both hematological (Jurkat) and solid (SNU-1) tumor cells (red, upper panels) which co-localizes with integrin β1 (green, middle panels), shown by overlap of the stainings (yellow, lower panels). AO-176 negligibly binds RBCs, which lack integrin β1. **(B)** Immunoblot of co-IP using either AO-176, 5F9, or human IgG2 control antibody to pull down CD47 from Jurkat lysates. **(C)** Integrin β1-KO and control Jurkat cells were stained with AO-176 or Hu5F9-G4. For AO-176 staining, WT and KO cells were mixed 1:1 to capture both stains in one field of view.

AO-176 Exhibits Single-Agent Activity and Combines With Chemotherapies and Targeted Antibodies To Potentiate Direct Tumor Killing and Phagocytosis



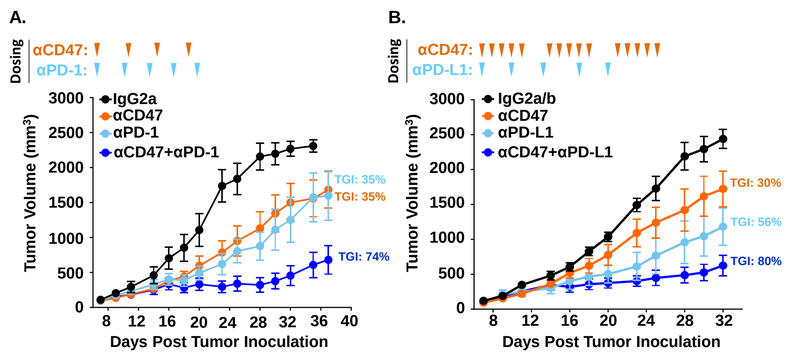
(A) SNU-1, Hs 746T, or KATO III gastric tumor cell lines were treated with AO-176 (μg/mL), Cisplatin (SNU-1:1.3 μM, Hs 746T:11.6 μM, and KATO III: 33.3 μM), or the combination for 24 hours, and stained with Annexin V. **(B)** Left: CFSE-stained FaDu head and neck tumor cells were incubated with human macrophages in the presence of AO-176 (0.3 μg/mL), the anti-EGFR antibody Erbitux (1 μg/mL), or in combination. Right: CFSE-stained ES2 ovarian tumor cells were incubated with human macrophages in the presence of AO-176 (10 μg/mL), the anti-PDL1 antibody Avelumab (10 μg/mL), or in combination.

AO-176 Inhibits Solid Tumor Growth *In Vivo* as a Single Agent and in Combination with Chemotherapeutics



(A) Human OV90 ovarian cancer cells were subcutaneously injected into NSG mice and treatments begun when tumors reached ~100mm^3. AO-176 was dosed (IP) at 25 mg/kg. Cisplatin was dosed (IP) at 5 mg/kg. *Similar was efficacy observed with AO-176 in combination with paclitaxel.* **(B)** Human SNU-1 gastric cancer cells were subcutaneously injected into NSG mice and treatments begun once tumors reached ~100mm^3. AO-176 or IgG2 control antibody were dosed (IP) at 25 mg/kg. Cisplatin or saline was dosed at 3 mg/kg.

Proof of Concept Using a Murine-Reactive CD47 Antibody, Not AO-176: CD47 Blockade Synergizes with Checkpoint Inhibitors in MC38 Model



The mouse MC38 colon cancer cell line was subcutaneously injected into C57BL/6 mice for these studies. **(A)** anti-CD47 (10mg/kg) or anti-PD-1 (100μg) antibodies were dosed interperitoneally or intratumorally, respectively. **(B)** Anti-CD47 (10mg/kg) or anti-PD-L1 (250μg) antibodies were dosed interperitoneally.

Conclusions

AO-176 is the only clinical stage anti-CD47 antibody with direct killing activity. It is highly differentiated from current clinical agents targeting the CD47 axis:

- **AO-176 is the only anti-CD47 antibody to selectively bind CD47 complexed to integrin β1, allowing context-dependent preferential binding to tumors**
- **RBCs do not express integrin β1, explaining the negligible binding by AO-176**
- **AO-176 exhibits single-agent and combination efficacy with chemotherapy and targeted antibodies**
- **AO-176 is currently being evaluated in a Phase 1 clinical trial (NCT03834948) for the treatment of patients with select solid tumors**

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