Background: Recent success in cancer immunotherapy has targeted immune checkpoints such as PD-1, PD-L1, and CTLA-4 to enhance the cytotoxic activity of the adaptive T cell immune response. While the clinical responses to these therapies have been dramatic for some, many others have shown partial or no response, highlighting the need for alternative or synergistic approaches that activate innate immunity. Disruption of the interaction between SRPAs and CD47, an immune checkpoint inhibitor, using anti-CD47 antibodies, for example, is known to enhance innate immunity by increasing the phagocytosis of tumor cells by macrophages and dendritic cells (DCs) leading to processing and presentation of tumor antigens. Recently, we described AO-176, a next-generation anti-CD47 antibody that blocks the CD47/SRPAs interaction, induces phagocytosis and causes a direct tumor cell-autonomous death and with the advantageous property of negligibly binding RBCs. Here, we characterize the ability of our AO-CD47 antibodies such as AO-176, to block AAO binding and induce ICDC in vitro.

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

Humanized IgG2
- Blocks CD47/SRPa interaction to induce phagocytosis of tumor cells
- Novel PCID, direct killing of tumor cells (non-ADCC), and DAMP induction
- Potentiation of ICID-inducing chemotherapy

AO-176 Preferentially Binds Tumor vs. Normal Cells and Negligibly Impacts RBC and Hemoglobin in Cytopathological Monkey

AO-176 Induces DAMP Signaling

AO-176 Mediates Programmed Cell Death Type III

Programmed cell death type III is a novel intrinsic process that involves mitochondrial damage due to loss of mitochondrial potential. PCID induced by AO-176 was measured in Jurkat T cells, cells isolated with Annexin V for 6 hours at 37°C. Cells were stained with AO-176 to measure the production of mitochondrial reactive oxygen species (MROS), and TMRM dye was used to measure the loss of mitochondrial membrane potential. The percent of cells stained by AO-176 and stained with Annexin V were also determined. The Annexin V+ cells were measured as percent of data population for cells treated with this control antibody or AO-176.

AO-176 Mediates Programmed Cell Death Type III

AO-176 Mediates Programmed Cell Death Type III

AO-176 Potentiates ICD-inducing Chemotherapy

ICD inducing CD47 interacts potentiate chemotherapeutic induced ICD. Jurkat T cells were treated with 1µg/ml IgG control, AO-176, 0.85, 0.14 and 0.02 µM (AO-176) concentration (CD47) stimulation, or AO-176 in combination with docetaxel (48µM) and 48µM of docetaxel. Cells were stained with calreticulin to measure ER stress of 48µM. Annexin V is measured externalization of phosphatidylserine (Annexin V), a marker of early apoptosis, and uptake of 7AAD dye, an indicator of apoptotic cell death at 37°C. Flow cytometric analysis was used to determine cytotoxic cell death by calreticulin and Annexin V staining.

AO-176 Potentiates ICD-inducing Chemotherapy

AO-176 Potentiates ICD-inducing Chemotherapy

Conclusion: In addition to promoting phagocytosis, AO-176 induces tumor cell-autonomous death and causes a PCDIII as well as immunogenic cell death (ICD) in an ADCC independent manner.

Conclusion: AO-176 mediates PCID and ICD in characteristic genetic patterns of cellular stress.

Conclusion: AO-176 demonstrates preferential binding to tumor versus normal cells especially in RBCs.

AO-176: A Next-Generation CD47 Antibody, Induces Immunogenic Cell Death

Benjamin J. Capoccia, Ronald R. Hiebsch, Michael J. Donio, Alun J. Carter, Robyn J. Puro, W. Casey Wilson, Daniel S. Pereira, Pamela T. Manning and Robert Karr

Arch Oncology, 4520 Forest Park Avenue, St. Louis, MO 63108 and 2000 Sierra Point Parkway, Brisbane, CA 94005

AO-176: A Next-Generation CD47 Antibody, Induces Immunogenic Cell Death

Abstract

AO-176: A Next-Generation CD47 Antibody, Induces Immunogenic Cell Death

AO-176: A Next-Generation CD47 Antibody, Induces Immunogenic Cell Death

AO-176: A Next-Generation CD47 Antibody, Induces Immunogenic Cell Death

AO-176: A Next-Generation CD47 Antibody, Induces Immunogenic Cell Death

AO-176: A Next-Generation CD47 Antibody, Induces Immunogenic Cell Death

Conclusion: AO-176 preferentially binds to tumor cells with normal cells and negligibly impacts RBC and hemoglobin in cytopathological monkey.

AO-176: A Next-Generation CD47 Antibody, Induces Immunogenic Cell Death

Conclusion: AO-176 preferentially binds to tumor cells with normal cells and negligibly impacts RBC and hemoglobin in cytopathological monkey.

Conclusion: AO-176 preferentially binds to tumor cells with normal cells and negligibly impacts RBC and hemoglobin in cytopathological monkey.

Conclusion: AO-176 preferentially binds to tumor cells with normal cells and negligibly impacts RBC and hemoglobin in cytopathological monkey.

Conclusion: AO-176 preferentially binds to tumor cells with normal cells and negligibly impacts RBC and hemoglobin in cytopathological monkey.

Conclusion: AO-176 preferentially binds to tumor cells with normal cells and negligibly impacts RBC and hemoglobin in cytopathological monkey.