CO-1: A NOVEL POTENT DUAL FUNCTION ANTI-CD47 ANTIBODY FOR CANCER THERAPY

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**Background**

CD47 is a central part of the innate immune system through its interaction with signal regulatory protein alpha (SIRPα). This interaction is known as the "don't eat me" signal which inhibits cell phagocytosis by innate immune cells.1,2 Cancer cells often evade recognition by the hosts’ immune cells through overexpression of CD47, and several anti-CD47 monoclonal antibodies (mAbs) are undergoing development to inhibit this interaction.3 Some anti-CD47 mAbs have been shown to have potential as treatment of human malignancy, but generally only in combination with other chemotherapeutic agents as their functionality primarily is due to the inhibition of the phagocytic signal.1,4 Here, we characterize CO-1, a novel bifunctional anti-CD47 mAb that is capable of eradicating cancer cells through direct induction of programmed cell death (PCD) as well as by enhancing cancer cell phagocytosis.

**Methods**

The binding properties of CO-1 to a wide range of cancer cell lines derived from solid and hematological tumors was determined by incubating the cells to FITC-conjugated antibodies, followed by flow cytometry analysis. The induction of PCD after treatment with different anti-CD47 mAbs at different time points was determined by Annexin V staining.5

The enhancement of tumor cell phagocytosis induced by CO-1 was determined by co-culturing DiO-labelled RAW264.7 macrophages with CFSE-labelled target cell lines. Double-positive DiO-RAW264.7 and CFSE-target cells indicated phagocytosed cells.

Xenograft models were established by injecting lentivirally transduced cancer cells into NOD-scid IL2Rγnull (NSG) mice. The lentiviral vector contained sequences for firefly luciferase and enhanced green fluorescent protein.6

**Results**

CO-1 binds to cancer cells with high efficiency and induces rapid (within 3 hours) and profound cell death in several cell lines derived from human hematological and solid tumors. No direct correlation was evident between CD47 expression and PCD response. In a xenograft model of B cell precursor acute lymphoblastic leukemia (BCP-ALL) in NSG mice, the mice were rapidly cured of their tumor even after only two injections of CO-1 as monotherapy. This demonstrates the PCD potency of CO-1 towards human tumor cells directly. Next, we determined the ability of CO-1 to block the "don’t eat me" interaction and found that CO-1 potently and significantly enhanced tumor cell phagocytosis, even in cell lines that previously had responded less favorably with PCD to CO-1 treatment.

**Conclusions**

CO-1 is a new and unique bifunctional anti-CD47 mAb that eradicates cancer cells by two mechanisms of action: 1)by direct induction of PCD, and 2)by enhancing cancer cell phagocytosis.

**REFERENCES**


**Ethics Approval**

The study was conducted under adherence with the Declaration of Helsinki. Animals were kept under appropriate housing conditions with food and water ad libitum. All animal experiments were approved by the Norwegian Food Safety Authorities.