The CD47-Blocking Innate Immune Checkpoint Inhibitor TTI-621 Triggers CD47-Mediated Tumor Cell Apoptosis

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Introduction

- CD47 binds to SIRPα on the surface of macrophages and delivers a "do not eat" signal to suppress phagocytosis.
- Tumor cells frequently overexpress CD47 and exploit this pathway to evade macrophage-mediated destruction.
- TTI-621 is a soluble SIRPα reconstituent fusion protein with an IgG1 Fc tail that triggers macrophage phagocytosis of tumor cells in vitro and potentially inhibits tumor growth in vivo.
- TTI-621 is currently being evaluated in two clinical studies with patients with hematologic and solid cancers (NCT03635158 and NCT03993596).
- Engagement of CD47 has been shown to directly induce apoptosis in tumor cells.
- The objective of this study was to examine the pro-apoptotic potential of TTI-621.

Immobilized TTI-621 Promotes Caspase-Independent and PLCγ-1-Dependent Apoptosis in Jurkat Cells

- Jurkat cells were cultured for 18 hr on immobilized TTI-621 or control Fc. Apoptosis was determined by flow cytometry analysis. CD47-positive cells were isolated from Jurkat cells treated with/without TTI-621 on day 3 and analyzed by immunofluorescence assay relative to unstressed cells.
- TTI-621 induced TUNEL+ cells compared to control Fc.

Cellular Stress Enables Soluble TTI-621-Mediated Caspase-Dependent and PLCγ-1-Independent Apoptosis

- Jurkat cells were cultured for 18 hr on immobilized TTI-621 or control Fc, followed by treatment with 20 nM TTI-621 for 1 hr. Caspase 3/7 activity was determined by flow cytometry analysis. CD47-positive cells were isolated from Jurkat cells treated with/without TTI-621 on day 3 and analyzed by immunofluorescence assay.
- TTI-621 induced TUNEL+ cells compared to control Fc.

Fcy Receptor Overexpressing Cells Enhance TTI-621-Induced Apoptosis in a CD47-Dependent Manner

- In vitro, CD47-positive cells were cultured with TTI-621 and recombinant Fc. Apoptosis was determined by flow cytometry analysis of annexin V+ and TUNEL+ cells.
- TTI-621 induced TUNEL+ cells compared to control Fc.

Conclusion

- In vitro, labeling CD47 with immobilized TTI-621 efficiently induced apoptosis in malignant DLBCL and T-ALL cells but had no effect on apoptosis in normal B and T cells.
- Soluble TTI-621 induced apoptosis under conditions of cellular stress.
- Anchoring the IgG1 Fc tail of TTI-621 to FcyR-expressing cells enhanced tumor cell apoptosis in a dose-dependent and CD47-dependent manner.
- An increase in tumor cell apoptosis was observed in vivo following intratumoral injection of TTI-621 in a DLBCL xenograft model.
- In addition to blocking the anti-phagocytic "do not eat" signal and activating FcγR on macrophages, binding of TTI-621 to FcγR on tumor-infiltrating immune cells may provide a cross-linking scaffold to enhance TTI-621-mediated apoptosis via CD47 on cancer cells.