SIRPaFc, a CD47-Blocking Cancer Immunotherapeutic, Triggers Phagocytosis of Lymphoma Cells by Both Classically (M1) and Alternatively (M2) Activated Macrophages

**Introduction**

- CD47 binds to SIRPa 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.
- Blocking CD47 with a soluble decoy receptor (SIRPaFc) has emerged as a promising strategy to neutralize the suppressive effects of CD47 and promote the eradication of tumor cells.
- In this study, we have examined the ability of SIRPaFc to trigger phagocytosis of lymphoma cells by cells in vitro polarized macrophage populations.

**TTI-621 (SIRPaFc): A Novel Biologic that Blocks the CD47 “Do Not Eat” Signal**

- TTI-621 is a SIRPaFc fusion protein.
  - Human SIRPα linked to a human IgG1
  - Disrupts the interaction of CD47 with cell surface SIRPα and enables macrophage-mediated killing of tumor cells in vitro and in vivo
  - Currently in a Phase I clinical trial for lymphomas and other hematological malignancies

**Macrophage Subsets Vary in Expression of M1 and M2 Surface Markers and Cytokine Production**

- M1 Macrophages
- M2a (IL-4)
- M2b (IL-1β + HAGG, IL-10, TGF-β1)
- M2c (LPS + TGF-β1)

**Macrophage Expression of the High-Affinity FcγR (CD64) Correlates with TTI-621-Triggered Phagocytosis**

- All FcγRs (CD16, CD32, CD64) contribute to TTI-621-mediated phagocytosis.
- On CD64+ macrophages, the high-affinity FcγR CD64 is the main contributor.
- On CD64+ macrophages, the low-affinity CD16 and CD32 FcγRs play a bigger role.

**TTI-621-Mediated Phagocytosis and FcγR Expression by M0, M2a and M2b Macrophages Can Be Further Increased by Re-Polarization with Cytokines and Toll-Like Receptor Agonists**

- TTI-621 treatment increases phagocytosis of lymphoma tumor cells by all macrophage subsets, with M1 and M2c MDMS showing superior phagocytic properties.
- Macrophage subsets with slightly lower phagocytic capabilities (M0, M2a, M2b) were readily repolarized into highly phagocytic MDMS using cytokines or TLR agonists.
- Macrophage expression of the high-affinity FcγR (CD64) correlated with phagocytic activity following TTI-621 treatment.
- All FcγRs (CD16, CD32, CD64) can contribute to TTI-621-mediated phagocytosis. On CD64+ macrophages, the high-affinity FcγR CD64 is the main contributor, whereas on CD64- macrophages, the low-affinity CD16 and CD32 FcγRs play a bigger role.
- A Phase I clinical trial of TTI-621 in patients with advanced hematological malignancies is currently underway (ClinicalTrials.gov # NCT02665318).

**Conclusions**

- TTI-621 increased phagocytosis of lymphoma tumor cells by all macrophage subsets, with M1 and M2c MDMS being superior at phagocytosis.
- Macrophage subsets with slightly lower phagocytic capabilities (M0, M2a, M2b) were readily repolarized into highly phagocytic MDMS using cytokines or TLR agonists.
- Macrophage expression of the high-affinity FcγR (CD64) correlated with phagocytic activity following TTI-621 treatment.
- All FcγRs (CD16, CD32, CD64) can contribute to TTI-621-mediated phagocytosis. On CD64+ macrophages, the high-affinity FcγR CD64 is the main contributor, whereas on CD64- macrophages, the low-affinity CD16 and CD32 FcγRs play a bigger role.
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