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

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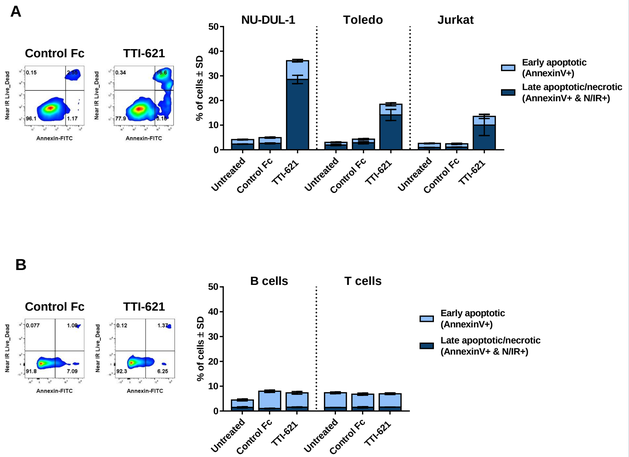
Introduction

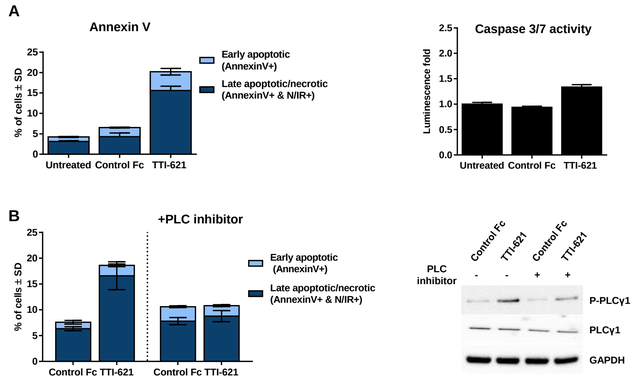
- CD47 binds to SIRPα on the surface of macrophages and delivers a "do not eat" signal to suppress phagocytosis.
- Tumor cells frequently overexpress CD47 and exploit this pathway to evade macrophage-mediated destruction.
- TTI-621 is a soluble SIRPα recombinant fusion protein with an IgG1 Fc tail that triggers macrophage phagocytosis of tumor cells *in vitro* and potently inhibits tumor growth *in vivo*.
- TTI-621 is currently being evaluated in two clinical studies in patients with hematologic and solid cancers (NCT02663518 and NCT02890368).
- 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 Induces Apoptosis in DLBCL and T-ALL Cells but not in Normal Cells



Tumor cells (NU-DUL-1, Toledo and Jurkat) (A) or normal B and T lymphocytes (B) were cultured on immobilized TTI-621 or control Fc for 48 hr (A) and 18 hr (B). Apoptosis was determined by flow cytometry. T cells are defined as the PBMCs that are singlet, live, CD14-CD16- and CD3+CD20-. B cells are defined as the PBMCs that are singlet, live, CD14-CD16- and CD3-CD20+. Representative flow cytometry histograms of Toledo cells (A) and normal B cells (B) are shown.

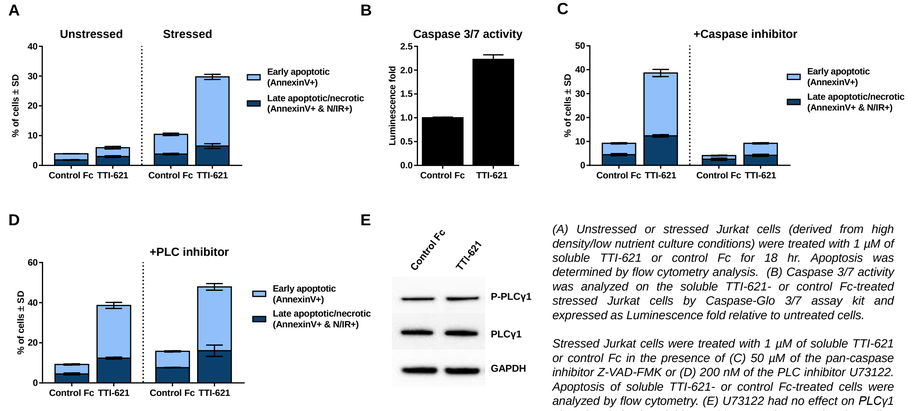
Immobilized TTI-621 Promotes Caspase-Independent and PLCγ-1-Dependent Apoptosis in Jurkat Cells



(A) Jurkat cells were cultured for 18 hr on immobilized TTI-621 or control Fc. Apoptosis was determined by flow cytometry analysis (left). Caspase 3/7 activity was measured by Caspase-Glo 3/7 assay kit and expressed as Luminescence fold relative to untreated cells (right).

(B) Jurkat cells were cultured for 18 hr on immobilized TTI-621 or control Fc followed by a 2 hr treatment with 200 nM of the PLC inhibitor U73122 or vehicle. Apoptosis was determined by flow cytometry (left) and changes in P-PLCγ1 and PLCγ1 expression were observed by immunoblot analysis (right).

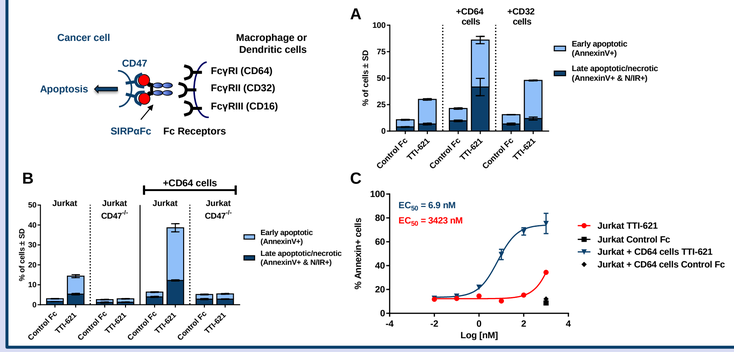
Cellular Stress Enables Soluble TTI-621-Mediated Caspase-Dependent and PLCγ-1-Independent Apoptosis



(A) Unstressed or stressed Jurkat cells (derived from high density/low nutrient culture conditions) were treated with 1 μM of soluble TTI-621 or control Fc for 18 hr. Apoptosis was determined by flow cytometry analysis. (B) Caspase 3/7 activity was analyzed on the soluble TTI-621- or control Fc-treated stressed Jurkat cells by Caspase-Glo 3/7 assay kit and expressed as Luminescence fold relative to untreated cells.

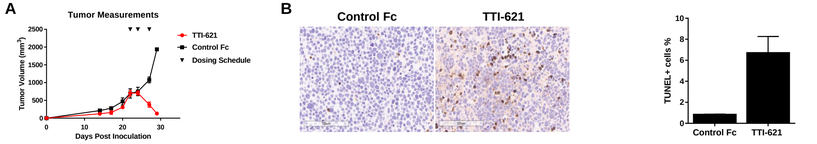
Stressed Jurkat cells were treated with 1 μM of soluble TTI-621 or control Fc in the presence of (C) 50 μM of the pan-caspase inhibitor Z-VAD-FMK or (D) 200 nM of the PLC inhibitor U73122. Apoptosis of soluble TTI-621- or control Fc-treated cells were analyzed by flow cytometry. (E) U73122 had no effect on PLCγ1 phosphorylation in soluble TTI-621-treated stressed Jurkat cells.

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



(A, B) Stressed or CD47 knockout (CD47-/-) Jurkat cells were co-cultured with cells overexpressing CD64 (Fcγ receptor I) or CD32 (Fcγ receptor IIa) in the presence of 1 μM of soluble TTI-621 or control Fc for 18 hr. Apoptosis of Jurkat cells was determined by flow cytometry. (C) Apoptosis in stressed Jurkat cells alone or in a co-culture with CD64 cells was assessed by flow cytometry in the presence of titrated amounts of TTI-621 or control Fc. EC_{50} values were generated by linear regression using a sigmoidal dose-response curve.

TTI-621 Induces Tumor Regression and Apoptosis in the Toledo (DLBCL) Xenograft Tumor Model



(A) Toledo cells were implanted subcutaneously into the right flank of NOD.SCID mice on day 0. Mice were dosed (3x/week) intratumorally with TTI-621 at 10 mg/kg or control Fc when the mean tumor volumes were approximately 700 mm^3. (B) Tumors were harvested 2 days following the last treatment. TUNEL assay staining was performed on formalin fixed tumor tissues and the representative histology images are shown. The percentage of TUNEL-positive cells was calculated by dividing the number of TUNEL-positive cells by the total number of cells.

Conclusions

- *In vitro*, ligating CD47 with immobilized TTI-621 efficiently induced apoptosis in malignant DLBCL and T-ALL cell lines 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 FcγR-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 on tumor cells and activating FcγR on macrophages, binding of TTI-621 to FcγRs on tumor-infiltrating immune cells may provide a cross-linking scaffold to enhance TTI-621-mediated apoptosis via CD47 on cancer cells.