ONCT-808 ROR1 CAR T Cells Induce Significant Cancer Cell Death in Mantle Cell Lymphoma Cells Derived CDX & PDX Models and In Vitro Killing Assays

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Background: Type 1 transmembrane protein receptor tyrosine kinase-like orphan receptor 1 (ROR1) is expressed at high levels on many hematologic and solid malignancies, but minimally in adult tissues. ROR1 expression on tumor cells increases post-chemotherapy or cell therapy, making it an attractive target for immunotherapy. Oncternal's ONCT-808 autologous CAR T cells, currently in a Phase 1/2 clinical trial for aggressive B cell lymphoma, are genetically modified via ex vivo transduction with a self-inactivating lentivirus vector to express a ROR1-directed CAR containing a single chain variable fragment derived from Oncternal's investigational anti-ROR1 monoclonal antibody, zilovertamab.

Mantle cell lymphoma (MCL) is an aggressive non-Hodgkin lymphoma (NHL) with a short remission observed after treatment with standard therapies. In this study, ONCT-808 activity against MCL was investigated as a representative preclinical model of ONCT-808 treatment for NHL.

Methods: ONCT-808 ROR1 CAR T cells were manufactured using apheresis material from healthy donors using the CliniMACS Prodigy, an automated cell processing platform. At completion of the manufacturing, T cell transduction rate, purity and T cell differentiation were analyzed with flow cytometry. For in vitro killing assays, ONCT-808 cells were incubated overnight with Jeko-1 (ROR1 expression high MCL cell line), Raji (ROR1 dim) and K562 (ROR1 negative) cells. Cancer cell death was analyzed with flow cytometry. Killing assay cell culture supernatant was analyzed for ONCT-808 cytokine release upon interaction with target cells using multiplex cytokine assays or ELISA.

To explore the in vivo cancer treatment efficacy, cell line-derived xenograft (CDX) immunodeficient NOD scid gamma (NSG) models were established with Jeko-1 and Raji cells via subcutaneous or intravenous inoculation, MCL patient-derived xenograft (PDX) NSG models were established with primary MCL cells via intravenous inoculation. ONCT-808 and untransduced T cells were then intravenously injected into the mice. Tumor growth was monitored via measuring tumor volume with calipers or non-invasive luciferase-based bioluminescence imaging using in vivo imaging system (IVIS) as well as flow cytometry.

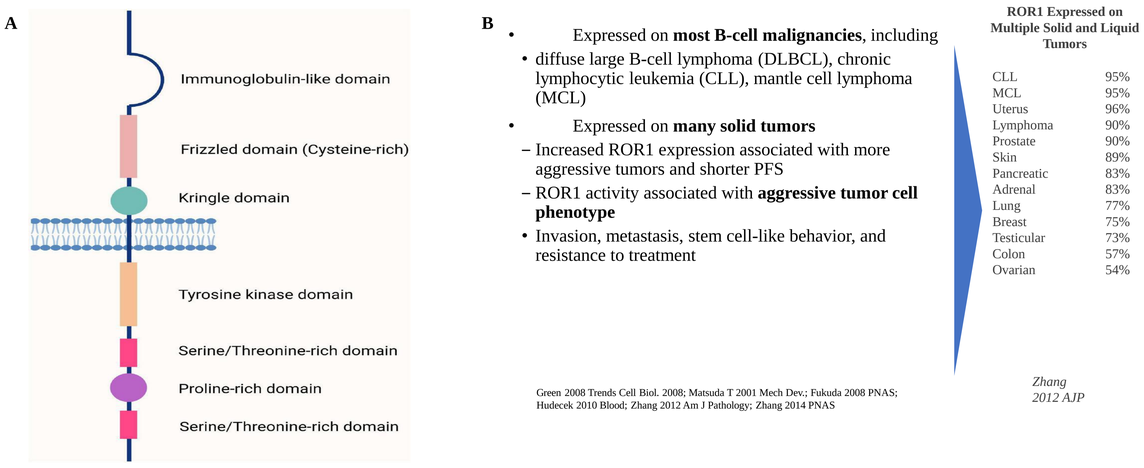

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Figure 1: ROR1 (Receptor Tyrosine Kinase-like Orphan Receptor 1) is a compelling tumor specific target for cancer therapy. A: The basic structure of the human ROR1; B: ROR1 expression in various types of cancers.

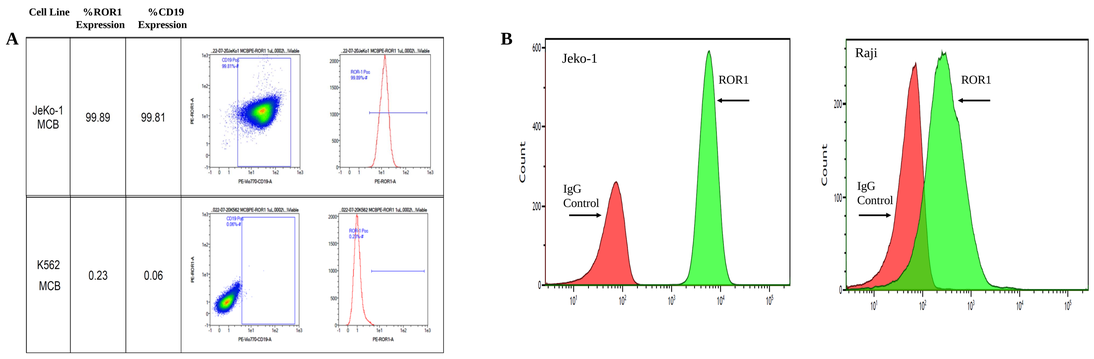

Figure 2: Flow cytometry analysis of ROR1 expression in Jeko-1, Raji and K562 cells. A: ROR1 expression in Jeko-1 and K562 cells; B: ROR1 expression in Jeko-1 and Raji cells.

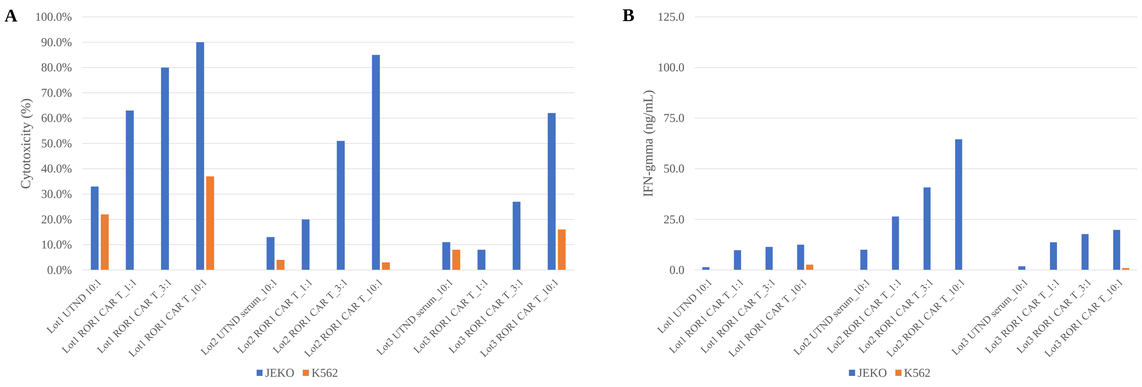

Figure 3: ONCT-808 induced cell death and IFN-ɤ release upon interaction with Jeko-1 and K562 cells. A:ROR1 CAR T treatment led to significant cell death in ROR1 expressing Jeko-1 cells, but not ROR1 negative K562 cells; B: Dose-dependent IFN-ɤ secretion by ROR1 CAR T cells was induced upon interaction with ROR1 expressing Jeko-1 cells, but not ROR1 negative K562 cells. $1x10^4$ Jeko or K562 cells were incubated with $1x10^4$, $3x10^4$, $10x10^4$ ROR1 CAR T cells or untransduced T cells (UNTD) overnight before cytotoxicity of Jeko or K562 cells was analyzed using flow cytometry or IFN-ɤ in cell culture supernatant was measured with MACSPlex Cytokine Standard Kit. Lot 1, 2 and 3 are manufacturing process batches using serum-containing complete medium. Lot 1 is a 12-day process, while Lot 2 and 3 are 8-day processes. Lot 2 and 3 manufacturing process is the same. This process has been adopted for ONCT-808 clinical manufacturing.

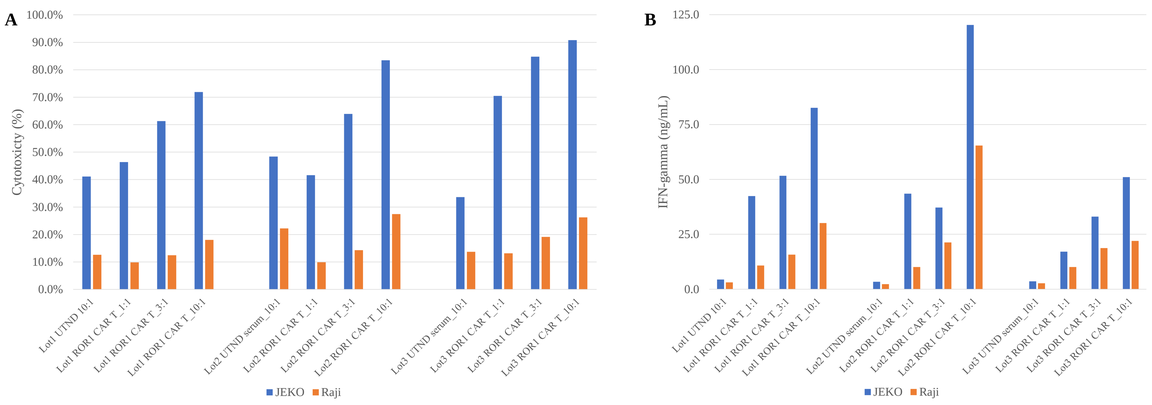

Figure 4: ONCT-808 induced cell death and IFN-ɤ release upon interaction with Jeko-1 and Raji cells. A:ROR1 CAR T treatment led to significant cell death in ROR1^{high} Jeko-1 cells, but at much lower level in ROR1^{dim} Raji cells; B: Dose-dependent IFN-ɤ secretion by ROR1 CAR T cells was induced upon interaction with ROR1^{high} Jeko-1 cells , but at much lower level with ROR1^{dim} Raji cells. $1x10^4$ Jeko or Raji cells were incubated with $1x10^4$, $3x10^4$, $10x10^4$ ROR1 CAR T cells or untransduced T cells (UNTD) overnight before cytotoxicity of Jeko-1 or Raji cells was analyzed using flow cytometry or IFN-ɤ in cell culture supernatant was measured with ELISA.

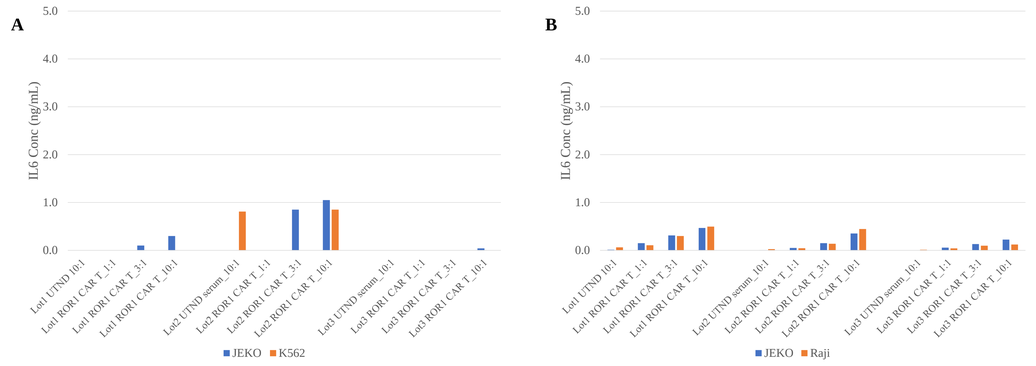

Figure 5: ONCT-808 secreted low and baseline level of IL6 upon interaction with ROR1^{high} Jeko-1. There is no difference in the levels of IL6 secretion from ONCT-808 and untransduced T cells interaction with ROR1^{high} Jeko-1, ROR1^{dim} Raji, ROR1 negative K562 cells. A:Low and comparable level of IL6 secretion was induced upon ONCT-808 interaction with Jeko-1 and K562 cells; B: Low and comparable level of IL6 secretion was induced upon ONCT-808 interaction with Jeko-1 and Raji cells. $1x10^4$ Jeko-1, K562 or Raji cells were incubated with $1x10^4$, $3x10^4$, $10x10^4$ ROR1 CAR T cells or untransduced T cells (UNTD) overnight before IL6 in cell culture supernatant was measured with MACSPlex Cytokine Standard Kit (A) or Inflammation 20-Plex Human ProcartaPlex Panel (B).

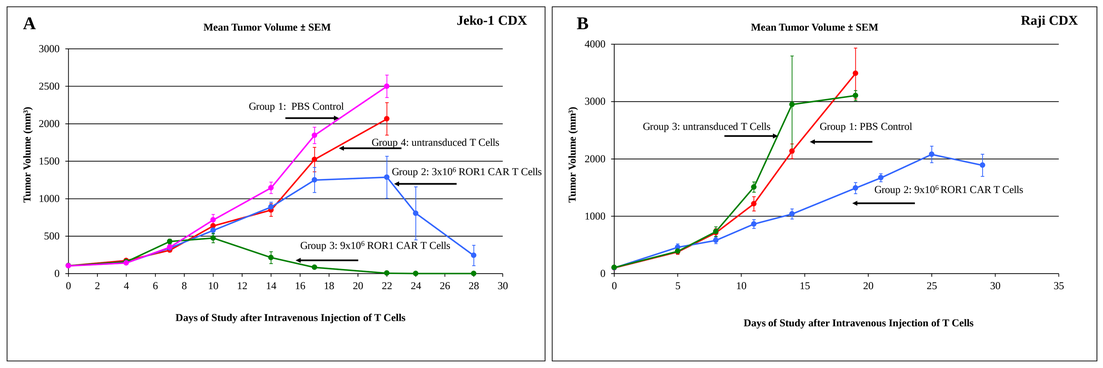

Figure 6: ONCT-808 efficiently cleared or reduced tumor cells in CDX model NSG mice. A: NSG mice were subcutaneously inoculated with $3x10^6$ Jeko-1 cells. $3x10^6$ or 9 $x10^6$ ROR1 CAR T Cells manufactured in Lot 3 were injected intravenously after the average tumor volume reached 150 mm^3. 9 $x10^6$ ROR1 CAR T Cells (E/T=3) cleared tumors in all of the mice, while $3x10^6$ ROR1 CAR T cells (E/T=1) significantly reduced tumor volume in the mice. In contrast, vehicle control (PBS) or untransduced T cells did not inhibit tumor growth; B: NSG mice were inoculated with $3x10^6$ Raji cells. 9 $x10^6$ ROR1 CAR T Cells manufactured in Lot 3 were injected intravenously after the average tumor volume reached 150 mm^3. 9 $x10^6$ ROR1 CAR T Cells (E/T=3) slowed tumor growth in the mice, vehicle control (PBS) or untransduced T cells did not inhibit tumor growth.

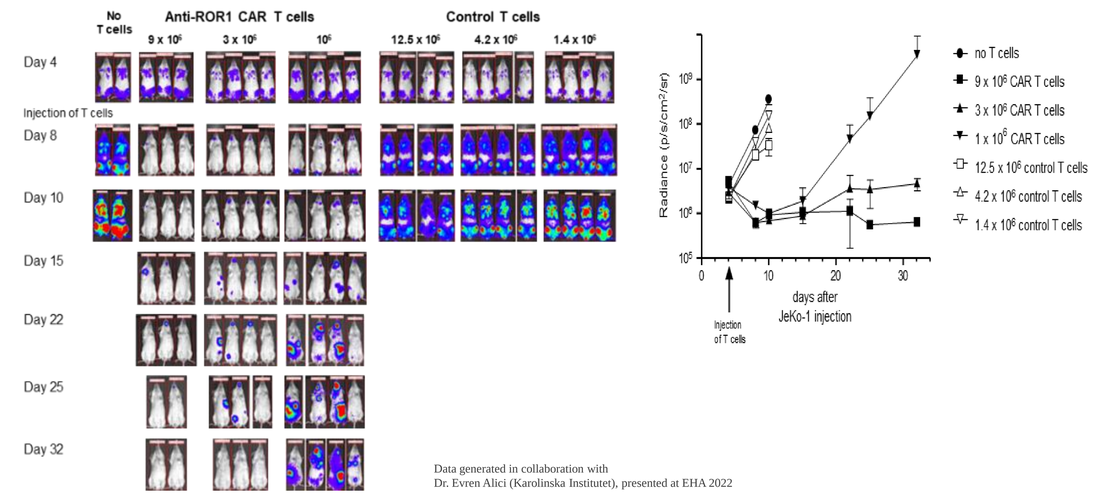

Figure 7: ONCT-808 efficiently cleared or reduced tumor cells in Jeko-1-derived CDX model NSG mice. NSG mice were inoculated with $1x10^6$ luciferase-expressing ROR1^{high} Jeko-1 cells. $1x10^6$, $3x10^6$ or 9 $x10^6$ ROR1 CAR T Cells manufactured in an initial PD run were injected intravenously after tumor was established. Both $3x10^6$ (E/T=3) and 9 $x10^6$ ROR1 CAR T Cells (E/T=9) cleared tumors in all of the mice, while $1x10^6$ (E/T=1) ROR1 CAR T cells controlled the tumor growth in the mice. In contrast, vehicle control (PBS) or untransduced T cells did not inhibit tumor growth.

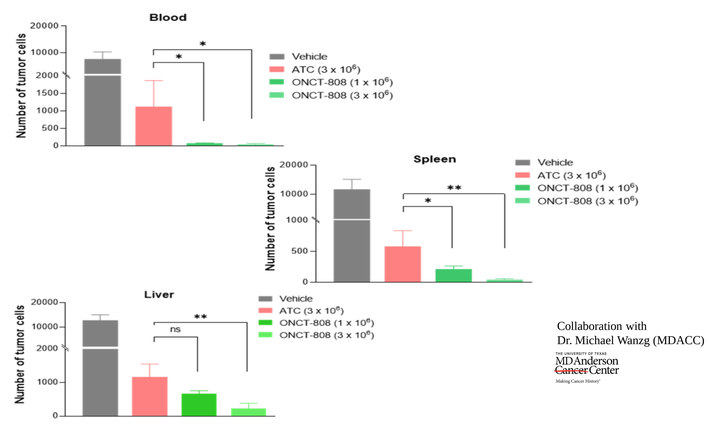

Figure 8: ONCT-808 efficiently reduced tumor burden in PDX model NSG mice. PDX NSG mice models were established with primary cancer cells from an ibrutinib-resistant MCL patient. $1x10^6$ or 3 $x10^6$ ONCT-808 ROR1 CAR T Cells manufactured in Lot 2 were injected intravenously after tumor was established. Cells extracted from blood, spleen and liver were analyzed with flow cytometry. ROR1 CAR T Cells reduced tumor burden in dose-dependent manner. In contrast, vehicle control (PBS) or untransduced T cells (ATCs) did not inhibit tumor growth.

Results: ONCT-808 induced significant ROR1 specific cell death in vitro in ROR1^{high} Jeko-1 cells, but not in ROR1^{dim} Raji or ROR1 negative K562 cells. ONCT-808 triggered the release of specific type 1 cytokines, such as IFN-γ, upon interaction with Jeko-1 cells. In contrast, the release of troublesome cytokine such as IL6, which is the main cause of cytokine release syndrome (CRS), was very low and at baseline.

In cancer therapy efficacy studies using CDX models, treatment with ONCT-808 cells but not untransduced T cells resulted in complete tumor remission in ROR1^{high} Jeko-1 cell-derived CDX mice, and controlled tumor growth in ROR1^{dim} Raji cell-derived CDX mice, reflecting the specificity of the ONCT-808 cells to the ROR1 target. The cancer therapy efficacy was further confirmed using PDX models, treatment with ONCT-808 cells but not untransduced T cells led to significant reduction of tumor burden in various tissue organs, such as blood, spleen and liver, in dose-dependent manner.

Conclusion: ONCT-808 demonstrated high anti-tumor activity in inducing ROR1 specific cancer cell death in both in vivo and in vitro studies, suggesting its potential for treating human cancer patients.

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