Delivery of TLR7 agonists by Deep-Primed™ T cells induces immune activation and improves anti-tumor activity in mice while circumventing systemic toxicity

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Torque Deep TLR Primed™ T cell product

Deep TLR Primed T cell

1. Deep TLR Primed T cells contain TLR7 agonist loaded liposomes and display slow payload release over time

2. Deep TLR Priming increases T cell expansion in vivo and reduces PD-1 expression

3. TLR7 agonist delivery enriches pDCs in draining LNs as well as endogenous CD8 T cells and MDCs in tumors

4. Deep TLR Primed T cells promote tumor growth inhibition and extend host survival

5. Deep TLR shows low potential toxicity

Key findings
- Torque’s Deep TLR Primed T cells release a potent small molecule agonist of TLR7 over an extended period of time.
- ACT of Deep TLR Primed T cells strongly improves tumor growth inhibition and host survival over controls in the murine B16-F10 model.
- Deep TLR Primed T cell expansion exceeds that of CD8 T cells alone or co-administered with the TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls.
- Deep TLR-mediated agonist delivery increased pDCs in draining lymph nodes and endogenous CD8 T cells in tumors, consistent with the known effects of TLR7 agonist in vivo (Mouries et al. 2003).
- Deep TLR-mediated agonist delivery increased MDCS content in the TME which may be beneficial given TLR7 agonist is known to convert MDCS into functional APCs (Spinetti et al. 2016).
- Deep TLR Primed T cells caused no significant weight loss. Transient plasma levels of pro-inflammatory cytokines (TNFα and IL-6 not shown) remained below 5% of known toxic levels (Sorenson et al. 2014, Baud et al. 2009, Tateda et al. 1996). This suggests that our Deep TLR Primed T cell therapy has the potential to be efficacious and well tolerated.

Materials and experimental design
- TLR7 agonist and several liposome formulations were screened for optimal T cell tethering and release, measured by HPLC.
- Formulations with desired characteristics were tethered to murine optimal T cell tethering and release, measured by HPLC.
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Introduction
TLR7 agonists boost immune responses in the tumor microenvironment (TME), primarily through dendritic cell (DC) engagement, enhancement of antigen presentation and T cell co-stimulation. However, multiple TLR agonists have displayed unfavorable PK/ PD profiles and considerable toxicities upon systemic administration. To overcome these limitations, we designed a T cell mediated delivery system for TLR agonists that targets the TME and the lymphatic system to maximize efficacy while avoiding systemic toxicities. Torque’s Deep-Primed™ T cell technology enhances T cell function through tethering of immune modulators to the T cell before adoptive cell transfer (ACT) and uses Torque’s multi-targeted cell (MTC) platform to prime the T cells against multiple tumor antigens. By transporting the immunomodulators to antigen-expressing tissues, Deep-Primed™ MTCs focus their effect on desired locations. Hence, we show that Deep TLR Primed T cells delivering TLR7 agonists induce potent immune cell activation in the TME and elicit exquisite anti-tumor efficacy without overt toxicity.

Figure 1. Deep TLR Primed T cells release a potent TLR7 agonist over an extended period of time. (A) Torque TLR7 agonist A displays a slow trajectory in eliciting CD8+ expansion and immune antitumor responses in vivo. Each deep-primed group is agitated for 3x 10^6 cells with either HPLC: agonist-free or agonist-containing liposome. (B) MTCs were administered with soluble TLR7 agonist. PD-1 expression was assessed by flow cytometry. Data are shown as fold change over the MTC T cell control group. Deep-primed groups were also observed in tumors (2x and 3x 10^6 cells) for 3 days. Percentage of PD-1 expressing T cells in peripheral blood and in tumors. P values are comparisons between the indicated groups were calculated using Student’s t-test.

Figure 2. Deep TLR Primed T cells display accelerated expansion and reduced PD-1 in tumor-bearing, lymphodepleted mice. (A) Deep TLR Primed or control PMEL T cells were injected into lymphodepleted B16 TME mice and expansion was measured by HPLC. Deep-primed groups were compared to the indicated liposomes. (B) Percentage of PD-1 expressing T cells in peripheral blood and in tumors. P values are comparisons between the indicated groups were calculated using Student’s t-test.

Figure 3. Deep TLR Primed T cells promote tumor growth inhibition and extend host survival. (A) PMEL T cell expansion is measured by peripheral blood PMEL+ content at the indicated time points. (B) Tumor growth inhibition is assessed by tumoricidal volume at 2x, 1x and 0.5x of ACT dose at 8x TME. (C) A-A. PMEL and MTCs were administered with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls. (D) Tumor burden is measured by tumor burden volume at 2x, 1x and 0.5x of ACT dose at 8x TME. (E) A-A. PMEL and MTCs were administered with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls. (F) Tumor burden is measured by tumor burden volume at 2x, 1x and 0.5x of ACT dose at 8x TME. (G) A-A. PMEL and MTCs were administered with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls. (H) Tumor burden is measured by tumor burden volume at 2x, 1x and 0.5x of ACT dose at 8x TME. (I) A-A. PMEL and MTCs were administered with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls. (J) Tumor burden is measured by tumor burden volume at 2x, 1x and 0.5x of ACT dose at 8x TME. (K) A-A. PMEL and MTCs were administered with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls. (L) Tumor burden is measured by tumor burden volume at 2x, 1x and 0.5x of ACT dose at 8x TME. (M) A-A. PMEL and MTCs were administered with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls. (N) Tumor burden is measured by tumor burden volume at 2x, 1x and 0.5x of ACT dose at 8x TME. (O) A-A. PMEL and MTCs were administered with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls. (P) Tumor burden is measured by tumor burden volume at 2x, 1x and 0.5x of ACT dose at 8x TME. (Q) A-A. PMEL and MTCs were administered with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls. (R) Tumor burden is measured by tumor burden volume at 2x, 1x and 0.5x of ACT dose at 8x TME. (S) A-A. PMEL and MTCs were administered with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls. (T) Tumor burden is measured by tumor burden volume at 2x, 1x and 0.5x of ACT dose at 8x TME. (U) A-A. PMEL and MTCs were administered with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls. (V) Tumor burden is measured by tumor burden volume at 2x, 1x and 0.5x of ACT dose at 8x TME. (W) A-A. PMEL and MTCs were administered with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls. (X) Tumor burden is measured by tumor burden volume at 2x, 1x and 0.5x of ACT dose at 8x TME. (Y) A-A. PMEL and MTCs were administered with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls. (Z) Tumor burden is measured by tumor burden volume at 2x, 1x and 0.5x of ACT dose at 8x TME. (AA) A-A. PMEL and MTCs were administered with soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls.

Figure 4. Deep TLR Primed T cells mediate superior tumor growth inhibition in the B16 melanoma synergistic mouse model. (A) Tumor burden is measured by HPLC. Deep-primed groups were compared to the indicated liposomes. (B) Percentage of PD-1 expressing T cells in peripheral blood and in tumors. P values are comparisons between the indicated groups were calculated using Student’s t-test.

Figure 5. ACT of Deep TLR Primed T cells exhibits low potential toxicity. (A) IFNγ levels in CD8+ T cells were measured by EL-12p/Th7. (B) NK activity was measured by EL-12p/Th7. (C) Cytokine release kinetics. (D) IFNγ peak level with LPS + TLR7 agonist.