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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TLR7 agonists induce potent immune cell activation in the TME and locations. Here, we show that Deep TLR Primed T cells delivering tumor antigens. By transporting the immunomodulators to antigen the T cell before adoptive cell transfer (ACT) and uses Torque’s multi-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 T cell (MTC) platform to prime the T cell against multiple tumor antigens. By transporting the immunomodulators to antigen expressing tissues, Deep-Primed™ MTCs focus their effect on desired locations. Here, we show that Deep TLR Primed T cells delivering TLR7 agonists induce potent immune cell activation in the TME and exhibit exquisite anti-tumor efficacy without overt toxicity.

Materials and experimental design
TLR7 agonists and several liposomal formulations were screened for optimal T cell tethering and release, measured by HPLC. Formulations with desired characteristics were formulated to contain PMEL CD8 T cells specific for the B16-F10 melanoma antigen gp100 to generate Deep TLR Primed T cells. Following ACT into immunocompetent syngeneic tumor-bearing animals, the T cell product was evaluated for efficacy and immune cell activation.

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. Multiple TLR agonists have displayed engagement, enhancement of antigen presentation and T cell co-stimulation, and improves anti-tumor activity in the tumor microenvironment (TME), primarily through dendritic cell (DC) engagement, enhancement of antigen presentation and T cell co-stimulation. Multiple TLR agonists have displayed high potency in stimulating CD86 upregulation on mouse antigen presenting cells (M-MDSC) in tumors, consistent with the known effects of TLR7 agonism in vivo. Deep TLR shows low potential toxicity and improves anti-tumor activity in mice while circumventing systemic toxicity (et al. 2014, 2016).

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 agonism in vivo (et al. 2008).
• Deep TLR-mediated agonist delivery increased MDCS content in the TME which may be beneficial given TLR7 agonism is known to convert MDCS into functional ACPs (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 (et al. 2014, 2016).

Figure 1. ACT of Deep TLR Primed T cells exhibits low potential toxicity. Deep TLR Primed MTCs were prepared using Torque’s proprietary process were loaded into Deep TLR Primed T cells and injected systematically into SCID mice bearing B16-F10 murine melanoma. (A) ACT of 1*10^9 PMEL T cells or with or without TLR7 agonist shows normal tumor growth kinetics in mice beyond that caused by lymphodepletion (GenEng). (B) Reduced splenomegaly, tumor bearing size, elevation of plasma TNFα and IL-6 is superior to ACT of 1*10^9 PMEL T cells alone. (C) Mice engrafted with PMEL+R3 KRJ2-110 cells were treated with 1*10^7 PMEL T cells loaded with agonist and monitored for 90 days.

Figure 4. ACT of Deep TLR Primed T cells mediate superior tumor growth inhibition in the B16 melanoma syngeneic mouse model. Experimental setup as in Fig. 1. (A) Tumor growth inhibition is illustrated as a bar chart showing open delivery of Deep TLR Primed TMCs, T cells compared to controls. The curves show tumor burden kinetics with tumor burden volumes at 1 week after ACT, tumor volume 4 weeks after ACT, Deep TLR Primed T cell expansion (Et al. 2016).

Figure 2. Deep TLR Primed T cells contain TLR7 agonist loaded liposomes and display slow payload release over time.

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