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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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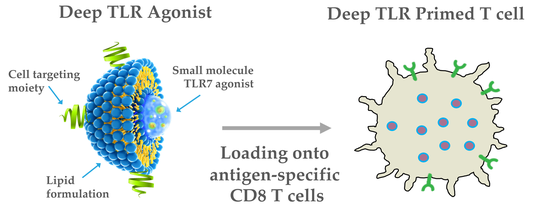
Torque Deep TLR Primed™ T cell product



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 TLR7 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 cells 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 elicit 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 tethered to murine 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.

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

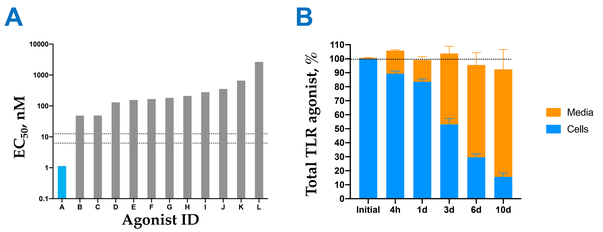


Figure 1. Deep TLR Primed T cells release a potent TLR7 agonist over an extended period of time. **(A)** Torque TLR7 agonist A displays a high potency in stimulating CD86 upregulation on mouse antigen presenting cells *in vitro*. Each column represents an agonist from the Torque library, and dashed lines represent EC50 of two commercial agonists. **(B)** Agonist release from Deep TLR Primed MTCs *in vitro*. MTCs prepared using Torque's Deep Priming process were loaded with Deep TLR and then frozen. The next day, the cells were thawed and cultured for 10 d. TLR agonist retained within cells (blue) and released into the media (orange) was assessed by HPLC to determine the mass balance over time. The metabolization of the agonist remained negligible.

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

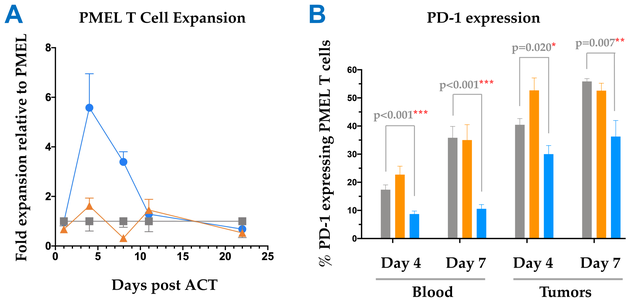


Figure 2. Deep TLR Primed™ PMEL T cells display accelerated expansion and reduced PD-1 expression in tumor-bearing, lymphodepleted mice. **(A)** Deep TLR Primed or control PMEL T cells were i.v. injected into lymphodepleted B16-F10 tumor bearing mice. Peripheral blood PMEL T cell content at the indicated time points was assessed by flow cytometry. Data are shown as fold change over the PMEL T cell control group. Enhanced expansion was also observed in tumors (not shown). **(B)** Percentage of PD-1 expressing T cells in peripheral blood and in tumors. P values for comparisons between the indicated groups were calculated by Student's t-test.

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

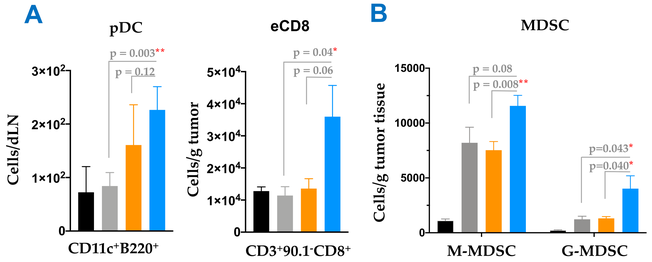


Figure 3. Deep TLR Primed™ PMEL T cell ACT increased the abundance of pDCs in draining lymph nodes, endogenous CD8s and myeloid and granulocytic MDSC in tumors. Abundance of cell subsets of interest in dissociated B16-F10 tumor tissue determined by flow cytometry 7 days after ACT. **(A)** pDC in draining lymph nodes (dLN) (left) and endogenous CD8 T cells in tumor (right). **(B)** M-MDSC and G-MDSC densities in tumors. P values for comparisons between the indicated groups were calculated using Student's t-test.

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

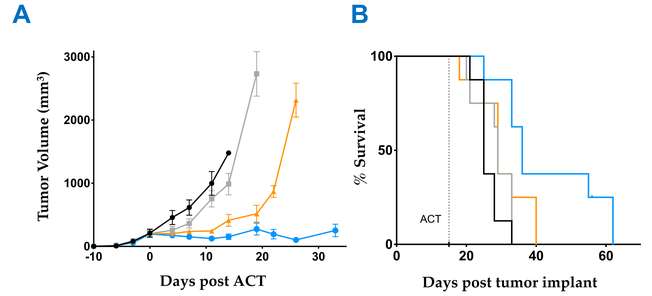


Figure 4. Deep TLR Primed™ PMEL T cells mediate superior tumor growth inhibition in the B16 melanoma syngeneic mouse model. Experimental setup as in Fig. 2. **(A)** Tumor growth inhibition is maximal upon delivery of Deep TLR Primed PMEL T cells compared to all other groups. The curves show tumor growth kinetics in mice with tumor start volumes of ~200 mm^3 at ACT, 8 mice/group. **(B)** Deep TLR Primed PMEL T cells improve host survival.

5. Deep TLR shows low potential toxicity

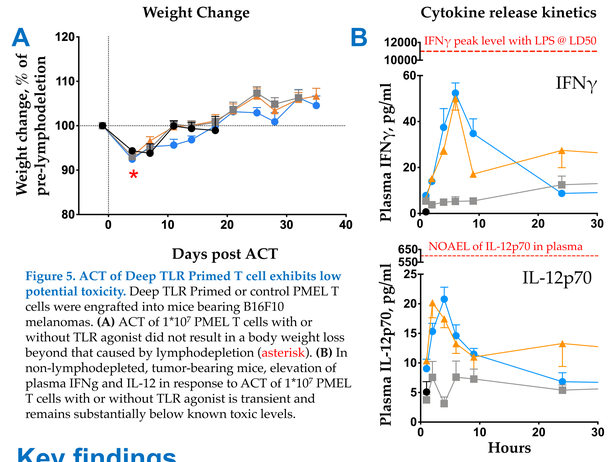


Figure 5. ACT of Deep TLR Primed T cell exhibits low potential toxicity. Deep TLR Primed or control PMEL T cells were engrafted into mice bearing B16F10 melanomas. **(A)** ACT of $1*10^7$ PMEL T cells with or without TLR agonist did not result in a body weight loss beyond that caused by lymphodepletion (asterisk). **(B)** In non-lymphodepleted, tumor-bearing mice, elevation of plasma IFNg and IL-12 in response to ACT of $1*10^7$ PMEL T cells with or without TLR agonist is transient and remains substantially below known toxic levels.

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 soluble TLR7 agonist. PD-1 downregulation suggests reduced exhaustion of Deep TLR Primed T cells vs. controls.
- Deep TLR-mediated agonist delivery increased pDC in draining lymph nodes and endogenous CD8 T cells in tumors, consistent with the known effects of TLR7 agonism in vivo (Mouries et al. 2008).
- Deep TLR-mediated agonist delivery increased MDSC content in the TME which may be beneficial given TLR7 agonism is known to convert MDSCs 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 (Soromou et al. 2014, Beurel 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.

TORQUE

■ Buffer ■ PMEL T cells ■ PMEL T cells + systemic TLR7 agonist ■ Deep TLR Primed T cells