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 Agonist

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 have 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 TME and the lymphatic system to maximize efficacy while avoiding systemic toxicities. Torque’s Deep TLR Primed T cells delivering enhances T cell function through tethering of immune modulators to non-bone marrow derived antigen-presenting cells (aAPCs) in vivo. Each deep T cell expansion is generated for the TME using a site-specific, low potential toxicity TLR7 agonist (Deep TLR) or a commercial agonist (iB) agonist release from Deep TLR Primed MTCs in vivo. MTCs prepared using Torque’s Primed™ MTCs focus their effect on desired antigen Loading onto antigen-specific CD8 T cells

Materials and experimental design
TLR7 agonists and several liposomal formulations were screened for optimal T cell tethering and release, measured by HPLC. Formulations of desired characteristics were further evaluated using PMEL CD8 T cells specific for the B16-F10 melanoma antigen gpl20 to generate Deep TLR Primed T cells. Following ACT into immunocompromised syngeneic B16-F10 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

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 (Sorrentino et al. 2014, Brand et al. 2009, Tatoa et al. 1996). This suggests that our Deep TLR Primed T cell therapy has the potential to be efficacious and well tolerated.

Cytochrome release kinetics

Agonist release from Deep TLR Primed PMEL T cells induced superior tumor outgrowth inhibition in the B16 melanoma syngeneic mouse model. Experimental setup as in Fig. 2. A: Tumor growth inhibition is measured as the relative open space delivery of Deep TLR Primed PMEL T cells compared to all other groups. The curves show tumor burden kinetics with tumor volume at volume at day 22 and tumor volume at day 26 at an ACT dose of 1*10^7 PMEL T cells with or without TLR7 agonist. No tumor growth inhibition was observed beyond that caused by lymphodepletion ( grou) or tumor-bearing tissue alone. Evaluation of plasma IFNγ and IL-12 is in contrast to ACT of 1*10^7 PMEL T cells alone which results in substantially lower tumor free times.

Figure 1. Deep TLR Primed T cells release a potent TLR7 agonist over an extended period of time. (A) Torque TLR agonist A displays slight potency in stimulating CD8+ splenocytes ex vivo antigen presenting cells in vivo. Each deep T cell expansion is generated for the TME using a site-specific, low potential toxicity TLR7 agonist (Deep TLR) or a commercial agonist. (B) Agonist release from Deep TLR Primed MTCs in vivo. MTCs prepared using Torque’s Primed™ MTCs focus their effect on desired antigen.

Figure 2. Deep TLR Primed T cells show the abundance of pDCs in draining lymph nodes, endogenous CD8s and splenocytes and synovial MDCS in mice. Abundance of cell subsets is assessed as described in Fig. 1. Deep TLR Priming was assessed in B16-D1 TLR7 agonist A or a commercial agonist A.

Figure 3. Deep TLR Primed MTCs focus their effect on desired antigen Loading onto antigen-specific CD8+ T cells

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 measured as the relative open space delivery of Deep TLR Primed PMEL T cells compared to all other groups. The curves show tumor burden kinetics with tumor volume at volume at day 22 and tumor volume at day 26 at an ACT dose of 1*10^7 PMEL T cells with or without TLR7 agonist. No tumor growth inhibition was observed beyond that caused by lymphodepletion ( grou) or tumor-bearing tissue alone. Evaluation of plasma IFNγ and IL-12 is in contrast to ACT of 1*10^7 PMEL T cells alone which results in substantially lower tumor free times.

Figure 5. Act1 of Deep TLR Primed T cells mediates low potential toxicity. Deep TLR Primed PMEL T cells were prepared as described in Materials and experimental design. (A) Agonist release from Deep TLR Primed MTCs in vivo. MTCs prepared using Torque’s Primed™ MTCs focus their effect on desired antigen.

Figure 6. Deep TLR Primed T cells promote tumor growth inhibition and extend host survival

Figure 7. Deep TLR Primed T cells exhibit low potential toxicity. Deep TLR Primed PMEL T cells co-cultured with B16-F10 tumor cells stopped tumor growth beyond that caused by lymphodepletion ( grou) or tumor-bearing tissue alone. Evaluation of plasma IFNγ and IL-12 is in contrast to ACT of 1*10^7 PMEL T cells alone which results in substantially lower tumor free times.

Figure 8. 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 (Sorrentino et al. 2014, Brand et al. 2009, Tatoa et al. 1996). This suggests that our Deep TLR Primed T cell therapy has the potential to be efficacious and well tolerated.