Deep TLR Primed™ T cells induce potent anti-tumor activity without systemic toxicity

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Deep TLR Primed™ T cells induce potent anti-tumor activity without systemic toxicity

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Abstract
TLR® agonists have been shown to augment immune responses in the tumor microenvironment (TME). The agonists work primarily through two mechanisms: antigen presenting cell (APC) engagement and enhancement followed by T cell co-stimulation. However, multiple TLR agonists, including TLR7/8 agonists, have displayed considerable toxicities upon systemic administration. To circumvent this problem, we developed a T cell mediated delivery system of TLR7 agonists that can target the TME and lymphoid organs to maximize efficacy while avoiding systemic toxicities. Torque’s Deep Primed™ T cell technology enhances T cell function by tethering immune modulators to the T cell before adoptive cell transfer (ACT) and, by using Torque’s multi-targeted T cell (MTC) platform that primes the T cells against multiple tumor antigens. Herein, we screened several liposome formulations containing two different TLR7 agonists for both in vitro agonist loading and release in mouse and human T cells followed by in vivo testing in a mouse melanoma model.

Introduction
Deep TLR Agonist
Loading onto antigen-specific CD8 T cells

Results

1. TLR agonists 1 and 2 are specific for TLR7

Figure 1: A. 48T cells expressing human TLR7 or TLR8 over-expressed without agonist addition at least 2 generations. After adding different concentrations of TLR agonist 1, TLR agonist 2, or the known agonist 3, the cells were incubated with different liposome formulations and washed with media.

2. Optimal liposome formulation maximizes agonist loading and extends drug release

Figure 2: A. Human T cells were loaded with TLR agonists 1 and 2 in liposome formulations and harvested after 48h. B. The cells were incubated with different liposome formulations and washed with media.

3. Deep TLR loaded T cells retain viability and extend TLR agonist release

Figure 3: Agonist release from Deep TLR Primed MTCs in vitro. MTCs were loaded with Deep TLR Priming protocols and washed with media. The next day the cells were re-added and incubated EIL Ag. MTCs. Deep TLR therapy, viability and cell count were determined compared in a 72-hour co-culture. IFN γ was measured using ELISA after cell lysis and protein precipitation. TLR agonist 1 was incubated with cells and performed into the media was measured by ELISA.

4. Deep TLR Primed™ T cells increase cell expansion and tumor control in vivo

Figure 4: A. Both human T cells and CD45 KRJ2-110 melanoma cells were co-cultured and drug-release for 48h. B. The cells were incubated with different liposome formulations and washed with media. The next day the tumor and the protein was precipitated. Drug content was measured by HPLC. C. Two T cell supplemented with systemic agonist 1 and 2 (PMEL) or buffer supplemented with systemic agonist 1 and 2 (PMEL) was injected i.v. into B16 tumor-bearing 110 mice. Tumor volumes were measured using the formula (Tumor Volume in mm3 = L x W x D / 2). Drug content was measured using flow cytometry for TLR agonist 1 and 2 (HBSS) tumor volumes post i.CT for 3 variations between the evaluated groups were calculated using Student’s t test. *p < 0.05 and **p < 0.01.

Conclusions
• Torque’s Deep TLR Primed T cells released a potent small molecule agonist of TLR7 over an extended period of time.
• Two TLR7-specific agonists capable of liposome encapsulation were identified.
• Formulation optimization enabled high concentrations of two different TLR7 agonists to be loaded on both mouse and human T cells with extended release.
• The optimal liposomal formulation enabled encapsulation of high concentrations of TLR7 agonists loaded onto MTCs with minimal effect on viability and proliferative capacity.
• Deep TLR Primed T cells remain viable and release TLR agonist slowly over 10 days.
• Deep TLR Primed T cell expansion exceeds that of CDB T cells alone or co-administered with systemic TLR7 agonist.
• ACT with Deep TLR Primed T cells provides a novel avenue to leverage the immune stimulating potential of TLR agonists for superior anti-tumor efficacy while avoiding systemic exposure and toxicities - key current bottlenecks to successful TLR therapy.
• In the future, agonist delivery via Deep-Primed™ tumor antigen-specific autologous T cells could target a wide variety of tumors and their distant metastases, enabling a new immunotherapy option.

References
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