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
TLR7 agonists have been shown to augment immune responses in the tumor microenvironment (TME). The agonist work primarily through two mechanisms: antigen presenting cell (APC) engagement and enhancement followed by T cell co-stimulation.1 However, multiple TLR agonists, including TLR7/8 agonists, have displayed considerable toxicities upon systemic administration.2 To circumvent this problem, we developed a T cell mediated delivery system of TLR7 agonists that can target the TME and lymph 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

Deep TLR Primed T cell

Figure 1. 1A: Cells expressing human TLR7 or TLR8 over-expressed mCherry without agonist addition at least 3 generations. After adding different concentrations of TLR agonist 1 and 2, the expression remained constant as determined by flow cytometry (FL1, Resiquimod). 1B: In vitro agonist loading measured using HPLC after cell lysis and protein precipitation against a PBS control. TLR agonist loading was measured with liposomes of agonist 1 and 2 did not release TLR agonist.

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

Figure 2. 1A: Human T cells were incubated with liposomes containing 1 microM Resiquimod and 3 microM R848 loaded and then grown in media containing 200 ng/ml IFN-α, 10 ng/ml TNF-α, and 20 ng/ml IL-12 for 4 days. A, B: Media analysis to measure drug release over 48h. Liposome 1 and 2 had less drug release than liposome 3. Legend: Liposome 1, 2, and 3. Initial viability was 85. ** = p < 0.01 and *** = p < 0.001.

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

Figure 3. A: Agonist release from Deep TLR (Primed MTC) in vitro. MTCs were primed using Torque’s Deep Priming protocol. Cells were loaded with Deep TLR and then cultured. The next day, the cells were harvested and subjected to flow cytometry for TLR expression. 1B, A: MTC TLR loading, viability and cell count were measured compared to a PBS treated control. TLR agonist loading measured with liposomes of agonist 1 and 2 did not release TLR agonist. 1C: TLR agonist retained within cells and released into the media was assessed by HPLC to determine the mass balance over time. The metabolization of the agonist remained negligible. After Deep TLR loading, viability and cell count were assessed compared to a PBS treated control.

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

Figure 4. 1A: MTDs were used to model E8 and E13-CT2 cells and drug type of cyclophosphamide at equivocal tumor exposure and toxicities. (A) Tumor volume was monitored using (H&E) deep TLR agonist loaded onto MTD cells compared to E8 or E13-CT2 tumor volume alone or co-administered with systemic TLR agonist. (B) Tumor volume was monitored using (H&E) deep TLR agonist loaded onto MTD cells compared to E8 or E13-CT2 tumor volume alone or co-administered with systemic TLR agonist. (C) Tumor volume was monitored using (H&E) deep TLR agonist loaded onto MTD cells compared to E8 or E13-CT2 tumor volume alone or co-administered with systemic TLR agonist. 1D: Drug release over 48h. Liposome 1 and 2 had less drug release than liposome 3.

Results

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 different TLR7 agonist 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 CD8 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 treatment.

References

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