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 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 development as therapeutic agents. Torque’s Deep Primed™ T cell technology enhances T cell function by tethering T cells directly to antigen presenting cells (APC) through the use of chimeric antigen receptors (CAR), enabling 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 immuno-modulators to the T cell to enable adoptive cell transfer (ACT), and by using Torque’s multi-targeted T cell (MTC) platform that primes the T cell 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

Deep TLR Primed T cell

Loading onto antigen-specific CD8 T cells

1. TLR agonists 1 and 2 are specific for TLR7

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

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

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

Results

Figure 1. (A) Cells expressing human TLR7 or TLR8 over-expressed in mice without any adjuvant alone at least 3 generations. After adding different concentrations of TLR7 agonist 1 and 2, in the absence or presence of 640 nmol Resiquimod (R3), we observed agonist release by TLR7 agonist 1 and 2 in the presence of Resiquimod, and TLR7 agonist 1 and 2 did not release TLR agonist 2. (B) TLR agonist release from Deep TLR Primed MTCs in vitro. MTCs were stimulated using APCs and cytokines after 16 h of TLR agonist 1 and 2 release. The next day, the cells were collected and subjected to flow cytometry. (C) Agonist release from Deep TLR Primed MTCs in vitro. MTCs were stimulated using APCs and cytokines after 16 h of TLR agonist 1 and 2 release. The next day, the cells were collected and subjected to flow cytometry.

Figure 2. (A) Human T cells were anti-CD3/CD28 bead stimulated and then expanded for 10 days. Human T cells from 10M PMEL T cells + Resiquimod (R3) Gdq liposome 1, liposome 2, and liposome 3 were measured using flow cytometry for various therapy groups. (B) T cells supplemented with systemic TLR agonist, Deep TLR Primed PMEL T cells, or buffer for various therapy groups. (C) T cells supplemented with systemic TLR agonist, Deep TLR Primed PMEL T cells, or buffer for various therapy groups. P values for comparisons between the indicated groups were calculated using Student’s t test for various therapy groups. • = p < 0.05, •• = p < 0.01 and ••• = p < 0.001.

Figure 3. (A) Human T cells were anti-CD3/CD28 bead stimulated and then expanded for 10 days. Human T cells from 10M PMEL T cells + Resiquimod (R3) Gdq liposome 1, liposome 2, and liposome 3 were measured using flow cytometry for various therapy groups. (B) T cells supplemented with systemic TLR agonist, Deep TLR Primed PMEL T cells, or buffer for various therapy groups. P values for comparisons between the indicated groups were calculated using Student’s t test for various therapy groups. • = p < 0.05, •• = p < 0.01 and ••• = p < 0.001.

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 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 stimulatory potential of TLR7 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 immunotherapeutic approach.

References


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