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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 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 liposomal 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 delivery system

TLR7 agonist

TLR Primed T cell

Loading agonist onto antigen-specific CD8 T cells

Activated CD8 T cells

Reaching the tumor microenvironment (TME)

Cell targeting

Results

1. TLR agonists 1 and 2 are specific for TLR7?

Figure 1. A: U937 cells expressing human TLR7 or TLR8 over-expressed mRNA with or without agonists at least 3 generations. After adding different concentrations of TLR agonist 1, TLR7 agonist 2, or the Concan. to U937 cells, we measured TLR7 expression and receptor activation in each group by flow cytometry. B: U937 cells were stimulated with agonist concentrations that induced half-maximal expression of TLR agonist 1 and TLR agonist 2. In (A) TLR agonist 1 and 2 did not act on TLR8.

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

Figure 2. A: Graphs show mean ± SEM of agonist loading and drug release from different liposomes (Liposome 1, Liposome 2, Liposome 3). B: Drug content was measured by HPLC. C: Drug release over 48h.

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

Figure 3. A: Apoptosis assay from Deep TLR-Primed MNCs in vitro. MNCs were exposed to Deep Priming process prior to loading with Deep TLR and then harvested. The next day, the cells were stimulated and harvested. Fig. 3A shows Deep TLR loading, viability and cell count were not compared in a 1:1 matched control in the MNC test. Fig. 3B shows TLR agonist loading remained constant across IFNγ, after cell test and before presentation agonist concentration. ACT TLR agonist encapsulated within cells and released over the course study by IFNγ stimulation. ACT TLR agonist expanded within cells and released over the course study by IFNγ stimulation.

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

Figure 4. A: In vitro T cell responses to different agonist concentrations in different liposomes were measured using IFNγ ELISA. B: TLR7 agonist 1 (C) TLR7 agonist 2 (B) drug release percent (A) TLR agonist 1 and 2 (D) drug release percent (B) TLR agonist 1 and 2. C: Drug release over 48h.

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 TLR 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 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 immunotherapeutic strategy.

References

3. Dowling D. Recent Advances in the Discovery and Delivery of TLR7 Agonists as Vaccine Adjuncts. Immunotherapy 2018;2: 145-197

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