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

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Deeper 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 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 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

TLR7 agonists

Loading onto antigen-specific CD8+ T cells

Deep TLR Primed T cell

TLR7 agonist

Activated endosomes

Deep TLR Primed T cells

Reaching the tumor microenvironment (TME)

Optimal liposome formulation maximizes agonist loading and extends drug release

Figure 1. (A) Human T cells were loaded with TLR7/8 agonists lipidosomes and then grown in media containing IL-2. T cells were washed and cultured with media containing IL-2 for 4 days. (B) Liposomes were loaded with agonist and then induced with IL-2 for 4 days. (C) CD8 T cells were loaded with agonist and then grown in media for 4 days.

Deep TLR Primed T cells retain viability and extend TLR agonist release

Figure 2. Agonist release from Deep TLR Primed MTCs in vitro. (A) MTCs were loaded using Torque’s Deep Priming process, were washed with Deep TLR and then cultured. The next day, the cells were re-stimulated and harvested. TLR7 agonist 1 and 2 were added and the next day the media was replaced by IL-2. The cells were re-stimulated and harvested. (B) TLR agonist loading was measured using HBSS, after cell lysis and protein precipitation against a standard curve. (C) TLR agonist expanded and cultured the next day. The media was replaced by HBSS, and the agonist level was measured using HBSS, after cell lysis and protein precipitation against a standard curve.

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

Figure 3. (A) B16F10 tumor cells were injected into C57BL/6 mice, and then ten animals were treated with different treatments. (B) Tumor growth was monitored every 2 days. (C) C57BL/6 mice were injected with PMEL and then treated with different treatments. (D) PMEL T cells were injected into C57BL/6 mice and then treated with different treatments. (E) Tumor growth was monitored every 2 days. (F) PMEL T cells were injected into C57BL/6 mice and then treated with different treatments. (G) Tumor growth was monitored every 2 days.

Results

1. TLR agonists 1 and 2 are specific for TLR7

Figure 1. (A) Cells expressing human TLR 7 or 8 were co-cultured in media without agonists against either TLR 7 or 8. (B) Cells were then washed and exposed to a TLR7 agonist or a TLR8 agonist. (C) Cells were then washed and exposed to a TLR7 agonist or a TLR8 agonist.

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

Figure 1. (A) Cells expressing human TLR 7 or 8 were co-cultured in media without agonists against either TLR 7 or 8. (B) Cells were then washed and exposed to a TLR7 agonist or a TLR8 agonist. (C) Cells were then washed and exposed to a TLR7 agonist or a TLR8 agonist.

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

Figure 2. Agonist release from Deep TLR Primed MTCs in vitro. (A) MTCs were loaded using Torque’s Deep Priming process, were washed with Deep TLR and then cultured. The next day, the cells were re-stimulated and harvested. TLR7 agonist 1 and 2 were added and the next day the media was replaced by IL-2. The cells were re-stimulated and harvested. (B) TLR agonist loading was measured using HBSS, after cell lysis and protein precipitation against a standard curve. (C) TLR agonist expanded and cultured the next day. The media was replaced by HBSS, and the agonist level was measured using HBSS, after cell lysis and protein precipitation against a standard curve.

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

Figure 3. (A) B16F10 tumor cells were injected into C57BL/6 mice, and then ten animals were treated with different treatments. (B) Tumor growth was monitored every 2 days. (C) C57BL/6 mice were injected with PMEL and then treated with different treatments. (D) PMEL T cells were injected into C57BL/6 mice and then treated with different treatments. (E) Tumor growth was monitored every 2 days. (F) PMEL T cells were injected into C57BL/6 mice and then treated with different treatments. (G) Tumor growth was monitored every 2 days.

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 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 immunotherapeutic strategy.

References


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