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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.3 However, multiple TLR agonists, including TLR7/8 agonists, have displayed considerable toxicities upon systemic administration.4 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. Hence, we screened several lipid formulations containing two different TLR7 agonists for both in vitro antigen 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

TLR7 agonist

Deep TLR Primed T cell

Results

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

Figure 1. A (i) shows mouse CD8+ T cells loaded with TLR7 agonist 1 or TLR7 agonist 2, or the known TLR7 agonist retinoic acid (RA). B (i) shows mouse CD8+ T cells loaded with TLR7 agonist 1, TLR7 agonist 2, or the known TLR7 agonist retinoic acid (RA). C (i) shows mouse CD8+ T cells loaded with TLR7 agonist 1, TLR7 agonist 2, or the known TLR7 agonist retinoic acid (RA).

Figure 2. A (i) shows mouse CD8+ T cells loaded with TLR7 agonist 1 or TLR7 agonist 2, or the known TLR7 agonist retinoic acid (RA). B (i) shows mouse CD8+ T cells loaded with TLR7 agonist 1, TLR7 agonist 2, or the known TLR7 agonist retinoic acid (RA). C (i) shows mouse CD8+ T cells loaded with TLR7 agonist 1, TLR7 agonist 2, or the known TLR7 agonist retinoic acid (RA).

Figure 3. A (i) shows mouse CD8+ T cells loaded with TLR7 agonist 1 or TLR7 agonist 2, or the known TLR7 agonist retinoic acid (RA). B (i) shows mouse CD8+ T cells loaded with TLR7 agonist 1, TLR7 agonist 2, or the known TLR7 agonist retinoic acid (RA). C (i) shows mouse CD8+ T cells loaded with TLR7 agonist 1, TLR7 agonist 2, or the known TLR7 agonist retinoic acid (RA).

Figure 4. A (i) shows mouse CD8+ T cells loaded with TLR7 agonist 1 or TLR7 agonist 2, or the known TLR7 agonist retinoic acid (RA). B (i) shows mouse CD8+ T cells loaded with TLR7 agonist 1, TLR7 agonist 2, or the known TLR7 agonist retinoic acid (RA). C (i) shows mouse CD8+ T cells loaded with TLR7 agonist 1, TLR7 agonist 2, or the known TLR7 agonist retinoic acid (RA).

Discussion

The optimal liposomal formulation enabled encapsulation of the agonist slowly over 10 days. This allowed for 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.

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 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 treatment.

References

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