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

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Publication date: 2019

Document Version
Publisher's PDF, also known as Version of record

Citation (APA):
Deep TLR Primed™ T cells induce potent anti-tumor activity without systemic toxicity

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Introduction

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 agonist 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 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.

Results

1. TLR agonists 1 and 2 are specific for TLR7

Figure 1. A) Cells expressing human TLR7 or mTLR7 were cultured in media without stimulation at least 3 generations. After adding different concentrations of TLR agonist 1 TLR agonist 2 or the Cen X. at al. (2018) agonist, MTCs were loaded with Deep TLR and then frozen. The next day the cells were thawed and stimulated with 1 µg/ml TLR agonist 1 or TLR agonist 2. The cells were then cultured for 1 week and cytokine production was measured. TLR agonist 1 and 2 did not release TLR.

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

Figure 2. A) Human T cells were loaded with CD8 T cells and Deep-TLR agonists and then frozen to maintain T cells in an activated state. The T cells were then thawed and their protein content measured by HPLC. B) Drug release over 60h. Drug release was measured using media washes. TLR agonist loading was measured using HPLC after cell lysis and protein precipitation against a standard curve. An agonist release from Deep TLR Primed MTCs in vitro. MTCs were loaded with Deep TLR, Primed™ tumor cells and then frozen. The next day, the cells were thawed and cultured with 1 µg/ml TLR agonist 1 or TLR agonist 2. The drug release was measured using media washes, TLR agonist loading was measured using HPLC after cell lysis and protein precipitation against a standard curve. TLR agonist retained within cells and released into the media was assessed by HPLC to determine drug release.

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

Figure 3. A) Apoptosis from Deep TLR Primed MTCs in vitro. MTCs were loaded with Deep TLR, Primed™ tumor cells and then frozen. The next day, the cells were thawed and cultured with 1 µg/ml TLR agonist 1 or TLR agonist 2. The drug release was measured using media washes, TLR agonist loading was measured using HPLC after cell lysis and protein precipitation against a standard curve. TLR agonist retained within cells and released into the media was assessed by HPLC to determine drug release.

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

Figure 4. A) Bioluminescence in PMEL T cells + Deep-TLR vs T cells. Drug release over 60h. Drug release was measured using media washes. TLR agonist loading was measured using HPLC after cell lysis and protein precipitation against a standard curve. TLR agonist retained within cells and released into the media was assessed by HPLC to determine drug release.

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 agonists 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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Acknowledgments

We would like to thank our Torque colleagues for productive discussions and criticism.