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

Westcott, Nathan ; Boesch, Austin; Rybakin, Vasily ; Hwang, Ji Young ; Jørgensen, Kira ; Lassen, Rasmus ; Kræmer, Martin ; Bak, Martin; Veiga, Gael; Bruun, Jonas

Total number of authors:
15

Publication date:
2019

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

B66

Presented at the
AACR 2019
Tumor Immunology and Immunotherapy
November 17-20, 2019 • Boston, MA

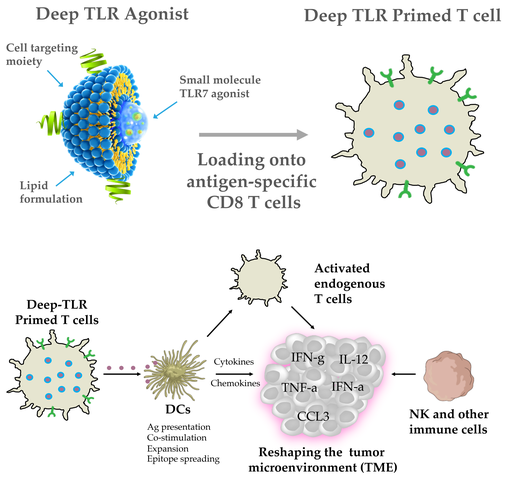
Deep TLR Primed™ T cells induce potent anti-tumor activity without systemic toxicity

Nathan Westcott[1], Austin Boesch[1], Vasily Rybakin[1], Ji Young Hwang[1], Kira Jørgensen[2], Rasmus Lassen[1], Martin Kraemer[2], Martin Bak[2], Gael Veiga[2], Jonas Bruun[1], Carlos Tassa[1], Harrison Rodts[1], Manny Sequeira[1], Karsten Sauer[1], Thomas L. Andresen[1]
[1]Torque Therapeutics, Cambridge, MA [2]Technical University of Denmark, Lyngby, Denmark

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.[1,2] However, multiple TLR agonists, including TLR7/8 agonists, have displayed considerable toxicities upon systemic administration.[3] 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



Results

1. TLR agonists 1 and 2 are specific for TLR7

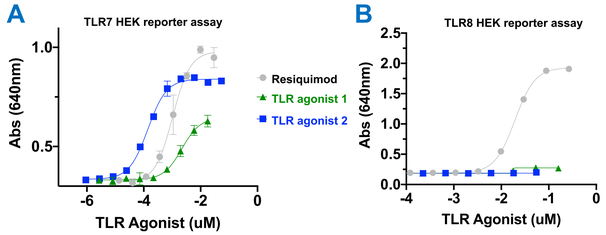


Figure 1. HEK cells expressing human TLR7 or TLR8 were cultured in media without selection agent for at least 2 generations. After adding different concentrations of TLR agonist 1, TLR agonist 2, or the known TLR7/8 agonist Resiquimod for one day, reporter gene activation was visualized by absorbance for **(A)** TLR7 and **(B)** TLR8. TLR7 EC_{50}s were 154 nM for Resiquimod, 1700 nM for TLR agonist 1, and 210 nM for TLR agonist 2. TLR agonists 1 and 2 did not activate TLR8.

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

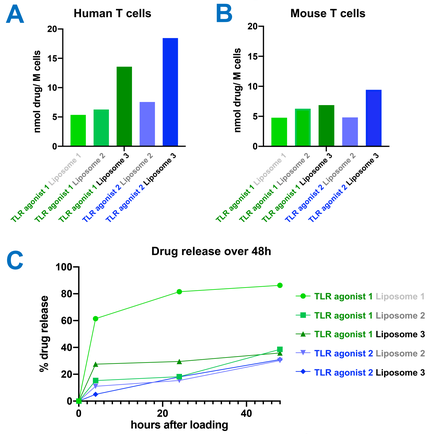


Figure 2. **(A)** Human T cells were anti-CD3/CD28 bead stimulated and then grown in media containing IL-2. The cells were incubated with different liposome formulations and washed with media. The cells were then lysed and the protein was precipitated. Drug content was measured by HPLC. **(B)** The same loading experiment executed with T cells isolated from PMEL TCR transgenic mice after anti-CD3/CD28 plate stimulation. **(C)** PMEL T cells were activated and loaded with liposomes. After 1 h incubation, the cells were washed and cultured with media containing IL-2. Cells were lysed and drug content was measured at 4 h, 24 h, and 48 h post loading to determine drug release.

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

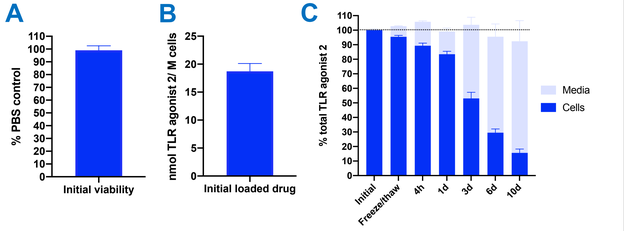


Figure 3. Agonist release from Deep TLR Primed MTCs *in vitro*. MTCs prepared using Torque's Deep Priming process were loaded with Deep TLR and then frozen. The next day, the cells were thawed and cultured for 10 d. **(A)** After Deep TLR loading, viability and cell count were assessed compared to a PBS treated control. **(B)** After media washes, TLR agonist loading was measured using HPLC after cell lysis and protein precipitation against a standard curve. **(C)** TLR agonist retained within cells and released into the media was assessed by HPLC to determine the mass balance over time. The metabolization of the agonist remained negligible.

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

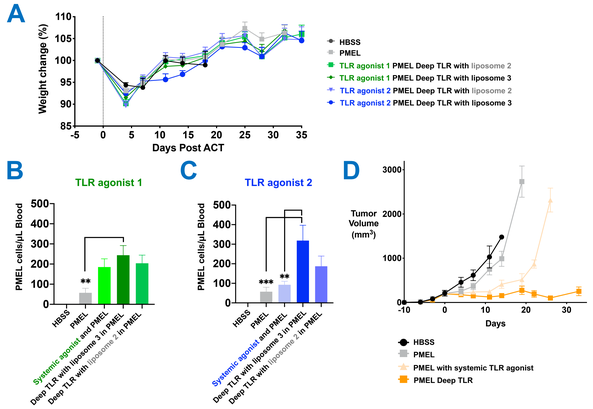


Figure 4. B6D2F1 mice were injected with B16-F10 tumor cells and 4 mg/kg of cyclophosphamide as intraperitoneal injection 8 days later. The following day, mice received *in vitro* expanded PMEL T cells alone, PMEL T cells supplemented with systemic TLR7 agonist, Deep TLR Primed PMEL T cells, or buffer i.v. Tumor volume was measured twice weekly along with weight. **(A)** ACT PMEL T cells with or without Deep TLR did not result in a body weight loss beyond that caused by lymphodepletion. On day 4, PMEL T cell numbers in the peripheral blood were measured using flow cytometry for **(B)** TLR agonist 1 and **(C)** TLR agonist 2. **(D)** Tumor volumes post ACT for various therapy groups. P values for comparisons between the indicated groups were calculated using Student's t-test. ** = $p < 0.01$ 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 TLR 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 immunotherapy treatment.

References

1. Cen X. at al. The Role of Toll-Like Receptor in Inflammation and Tumor Immunity. Front. Pharmacol. 2018.00878
2. Smith M. et al. Trial Watch: Toll-like receptor agonists in cancer immunotherapy. OncoImmunology 2018.e1526250
3. Dowling D. Recent Advances in the Discovery and Delivery of TLR7/8 Agonists as Vaccine Adjuvants. ImmunoHorizons 2018 2: 185-197

Acknowledgments

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

TORQUE