# New methods for controlling CAR T cell-mediated cytokine storms

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## Abstract

Use of chimeric antigen receptor (CAR) T cells to eliminate tumors has attracted considerable attention due to their ability to eradicate otherwise difficult to treat leukemias. However, although remarkable success has been reported in CAR T cell treatment of ALL (e.g. up to 70-93% complete responses), the technology is still plagued by the common occurrence of a cytokine storm (a.k.a. cytokine release syndrome) that causes fever, nausea, chills, hypotension, tachycardia, asthenia, headache, rash, dyspnea and sometimes death. Indeed, this inability to control the rate and degree of cytokine release has forced the interruption of several CAR T cell clinical trials, emphasizing the need for development of a reliable method to regulate the activation state of these genetically engineered cells. We have recently designed several novel methods to control the rate and extent of CAR T cell activation by using a bispecific adaptor molecule to mediate engagement of the CAR T cell with the cancer cell. In this strategy, the CAR T cell contains the usual 4-1BB and/or CD28 insert in the cytoplasmic domain of CD3ζ, but with an scFv that binds fluorescein instead of a tumor-specific scFv in the exoplasmic domain. The resulting CAR T cell can then only bind and kill a cancer cell when the bispecific adaptor, formed by linking fluorescein to a tumor-specific ligand, can establish a bridge between the CAR T cell and the cancer cell (Fig. 1 and 2). Using the adaptor, fluorescein-folate, which bridges between our anti-fluorescein CAR T cell and any folate receptor-expressing cancer cell, we explore several novel strategies for regulating a cytokine storm, including: 1) interruption of bi-specific adaptor administration (Fig. 5), 2) injection of excess folate to block/compete for adaptor bridging of the CAR T cell to the cancer cell (Fig. 6), 3) use of a very low or very high dose of adaptor (Fig. 7), 4) and gradual escalation of bi-specific adaptor dose (Fig. 8). We show here that all of the above strategies mitigate/eliminate a cytokine storm, but at different rates and with different potencies. Thus, discontinuation of adaptor administration or injection of excess free folate promotes a rapid decline in cytokine storm with concomitant regain of normal body weight. In contrast, metronomic administration of low adaptor dose or gradual escalation of adaptor dose over several days can totally prevent a cytokine storm under conditions where it would otherwise be prominent. Because the circulation half-life of most bi-specific adaptors is ~30 min, these data demonstrate that unwanted toxicity from CAR T cell-induced cytokine storms can be either pre-emptively prevented or rapidly suppressed following their emergence by use of anti-FITC CAR T cells together with easily adjustable bi-specific adaptor molecules.

## Overview of universal CAR T cell platform

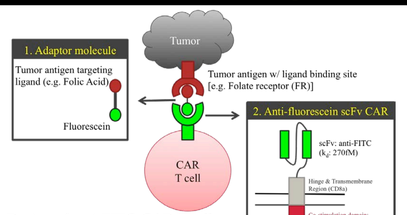



**Figure 1**. Design of anti-FITC CAR T cell that will kill folate receptor over-expressing cancer cells (40% of human cancers).

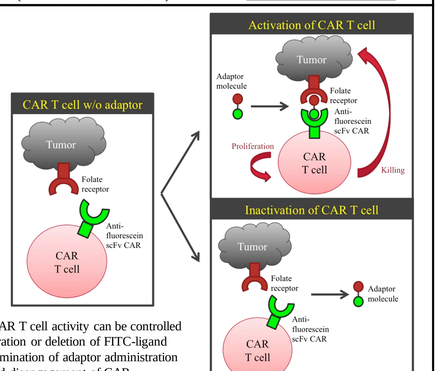



**Figure 2.** CAR T cell activity can be controlled by administration or deletion of FITC-ligand adaptor: Termination of adaptor administration triggers rapid disengagement of CAR.

## Universal CAR T cells eradicate solid tumors upon adaptor addition

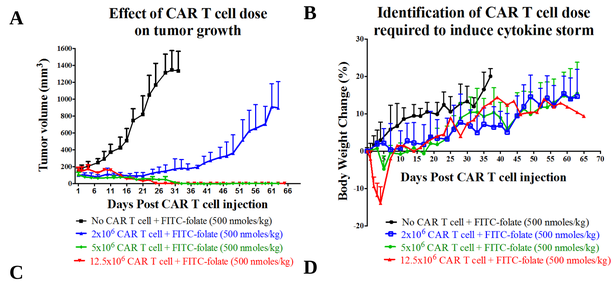
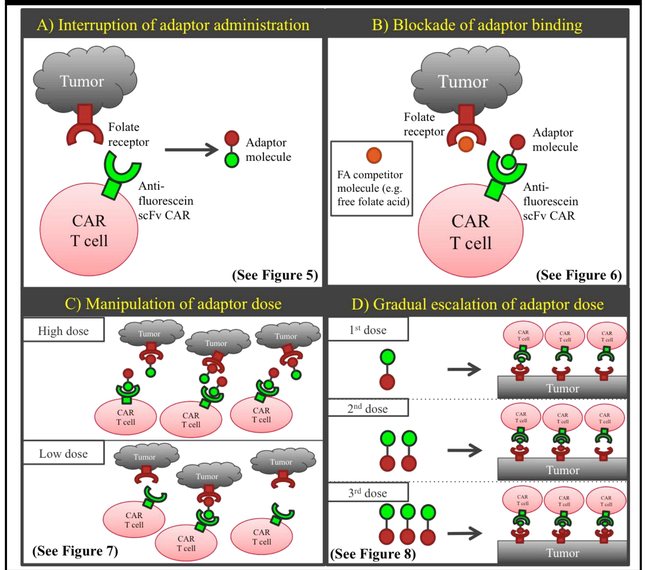



**Figure 3. Anti-tumor efficacy of anti-FITC CAR T cell in the presence of correct FITC-ligand adaptor molecule.** Increasing numbers of anti-FITC CAR T cells (A, B) or 15x10$^6$ CAR T cells (C, D) were injected i.v. on day1 when MDA-MB-231 cell tumor volume was ~100mm$^3$, after which PBS or FITC-folate (500nmole/kg) was injected i.v. every other day. **A.** Tumor growth. **B.** Mouse body weight. **C.** CAR T cell numbers on day 6. and **D.** Serum cytokines on day 6.

## Methods for regulating universal CAR T cell activity




## A. Control of cytokine production via interruption of adaptor administration

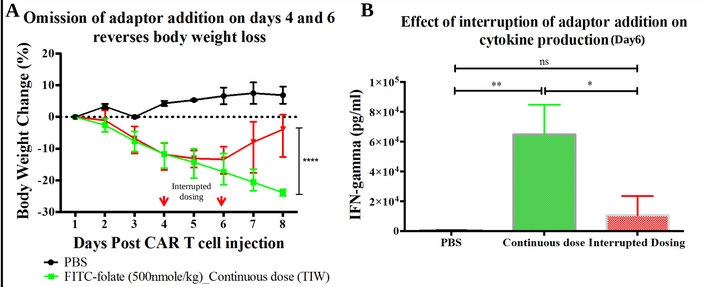



**Figure 5. Manipulation of adaptor administration schedule can reduce CAR T cell mediated toxicity.** To assure occurrence of a potent cytokine storm, all tumor-bearing mice were treated with a very high dose of CAR T cells (15x10$^6$). Mice were then treated every other day with either PBS, FITC-folate or FITC-folate where dosing was omitted on days 4 & 6 (Interrupted Dosing). The following parameters were then measured: **A.** Bodyweight change **B.** Cytokine production. **C.** Tumor volume.

## B. Control of cytokine production via blockade of adaptor binding

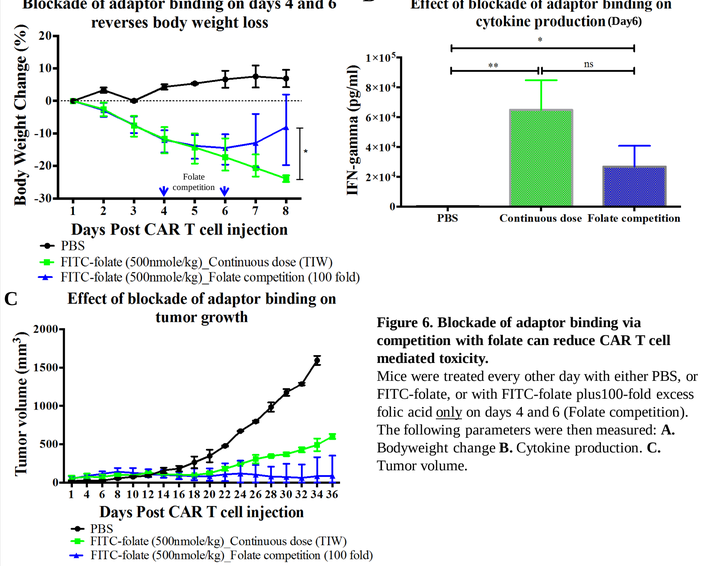



**Figure 6. Blockade of adaptor binding via competition with folate can reduce CAR T cell mediated toxicity.**
Mice were treated every other day with either PBS, or FITC-folate, or with FITC-folate plus100-fold excess folic acid only on days 4 and 6 (Folate competition). The following parameters were then measured: **A.** Bodyweight change **B.** Cytokine production. **C.** Tumor volume.

## C. Control of cytokine production via manipulation of adaptor dose

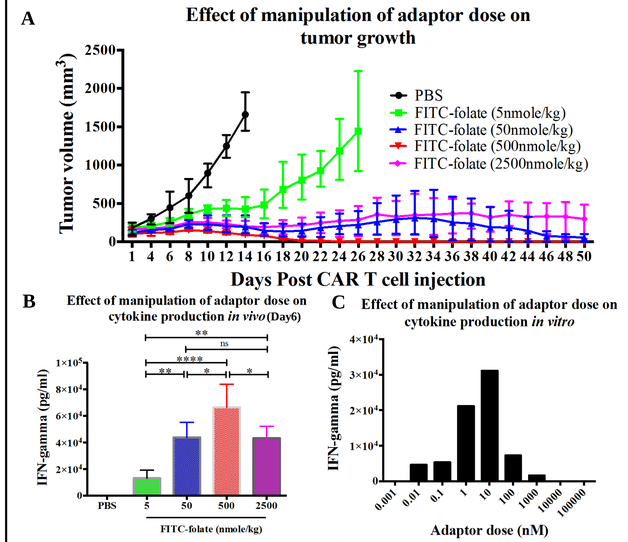



**Figure 7. Adjustment of adaptor dose can control CAR T cell activation and cytokine storm.** Since our CAR T cell activation is determined by the dose of adaptor molecule, we can control cytokine mediated toxicity by manipulating adaptor dose. Therefore, **A:** Tumor volume, **B:** Cytokine production on day 6 in vivo, and **C.** IFNγ in a separate in vitro study were measured as a function of FITC-folate dose.

## D. Control of cytokine production via gradual escalation of adaptor dose

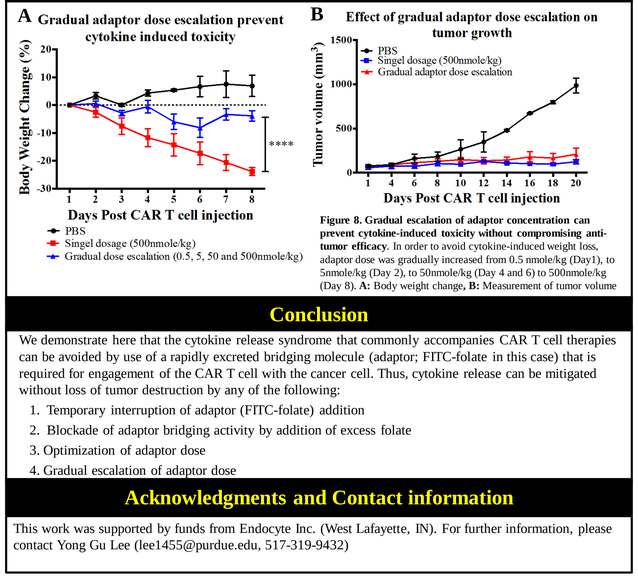



**Figure 8. Gradual escalation of adaptor concentration can prevent cytokine-induced toxicity without compromising anti-tumor efficacy**. In order to avoid cytokine-induced weight loss, adaptor dose was gradually increased from 0.5 nmole/kg (Day1), to 5nmole/kg (Day 2), to 50nmole/kg (Day 4 and 6) to 500nmole/kg (Day 8). **A:** Body weight change, **B:** Measurement of tumor volume

## Conclusion

We demonstrate here that the cytokine release syndrome that commonly accompanies CAR T cell therapies can be avoided by use of a rapidly excreted bridging molecule (adaptor; FITC-folate in this case) that is required for engagement of the CAR T cell with the cancer cell. Thus, cytokine release can be mitigated without loss of tumor destruction by any of the following:

1. Temporary interruption of adaptor (FITC-folate) addition
2. Blockade of adaptor bridging activity by addition of excess folate
3. Optimization of adaptor dose
4. Gradual escalation of adaptor dose

## Acknowledgments and Contact information

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