T-regulatory cells impair CAR T cell-mediated antitumor activity in a murine solid tumor model

Michael S. Leibowitz[1,2], Nicholas A. Olimpo[1], Liqing Wang[1,2], Aparna Jorapur[3], Deepa Pookot[3], Maria Liousia[2], Evguenia Arguiri[2], Jing Sun[2], Astero Klampatsa[4], Dirk G Brockstedt[3], Wayne W. Hancock[1,2], Steven M. Albelda[2]

[1]Children's Hospital of Philadelphia, Philadelphia, PA, [2]University of Pennsylvania, Philadelphia, PA, [3]RAPT Therapeutics, Inc., South San Francisco, CA, [4]The Institute of Cancer Research, London, United Kingdom

INTRODUCTION

Chimeric antigen receptor (CAR) T cell immunotherapy has had only modest success in solid malignancies due in part to tumor antigen heterogeneity and immune evasion. We have previously demonstrated that administration of cyclophosphamide (CTX) prior to CAR T infusion promoted the eradication of solid tumors that lacked homogenous expression of the CAR target antigen. Although CTX has multiple effects, we hypothesized that one potential mechanism was depletion of T-regulatory (Treg) cells. To test this, we used a genetic model of Treg depletion involving mice with FOXP3-driven diphtheria toxin receptor expression (FOXP3-DTR) and an orally administered chemokine receptor 4 (CCR4) antagonist (CCR4-351). Tregs express CCR4 and migrate toward CCL17 and CCL22 which are frequently upregulated by tumors. We have previously shown that CCR4-351 decreases adoptively transferred iTreg accumulation in tumors. Herein, we tested whether T-reg depletion via systemic depletion or inhibition of T-reg trafficking resulted in augmentation of the antitumor effects of CAR T cells in our pre-clinical, immunocompetent solid tumor model.

METHODS

An anti-human mesothelin M11 scFv was fused to murine CD8a hinge, CD8a transmembrane domain and 41BB-CD3z intracellular signaling domains. The CAR was subcloned into a GFP-expressing MIGR1 retroviral vector. Phoenix cells were transfected with the M11 CAR construct and helper plasmids using lipofectamine. CD3+ T cells were isolated from murine splenocytes and cultured with IL-2 and anti-CD3/28 beads for 48 hours. Activated T cells were retrovirally transduced with the infective M11 viral particles using retronectin. Transgenic FOXP3-DTR C57BL/6 mice were injected s.c. in the flank with a syngeneic murine mesothelioma cell line (AE-17) in which 90% of the tumor cells expressed human mesothelin. When tumors reached ~150 mm^3, 50 ng of diphtheria toxin (DT) was given i.p. Two doses of 10^7 transduced M11 CAR T cells were injected i.v. 2 days apart. In our pharmacologic study, wild type, tumor-bearing C57BL/6 mice were given CCR4-351 daily at 50 mg/kg by oral gavage when tumors reached 100-150 mm^3. Mice were treated with a single dose of M11 CAR T cells starting one day after CCR4-351 therapy.

RESULTS

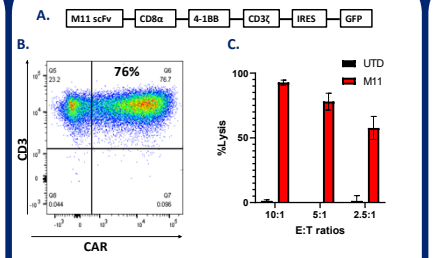


Figure 1. **Mouse mesothelin-specific CAR T cells with high activity were generated.** We were able to achieve a >70% transduction efficiency using our M11 CAR construct (A, B). Our CAR T cells killed meso-expressing tumor cell targets specifically at various E:T ratios compared to untransduced (UTD) T cells (C).

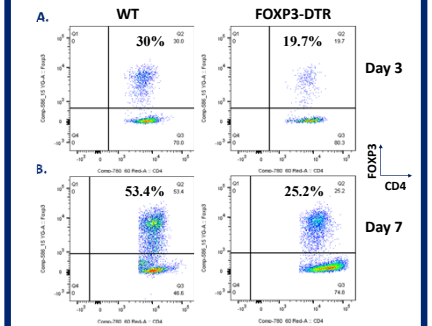


Figure 2. **Intra-tumoral Tregs were reduced significantly by administration of DT to FOXP3-DTR** mice. Wild-type and FOXP3-DTR mice injected with 90% expressing human meso-expressing AE17M tumor cells. Tumors were digested and stained for Tregs (CD4+FOXP3+) 3 days (A) and 7 days (B) after DT injection. FOXP3-DTR mice treated with DT had >30% reduction in Tregs by day 3 (A) and >50% reduction in T-regs by day 7 (B) compared to wild-type (WT) control mice.

RESULTS

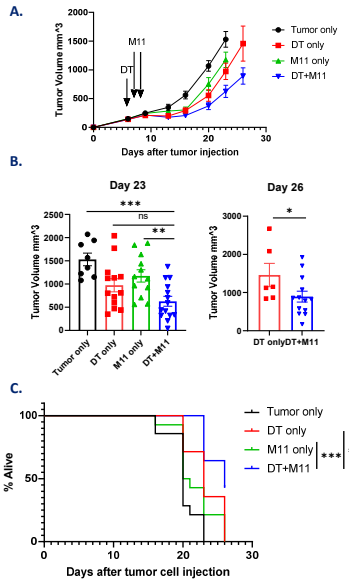


Figure 3. **DT-induced T-reg reduction augments the *in vivo* anti-tumor efficacy of CAR T cells.** AE17M tumor bearing mice were injected with one dose of DT on day 6 and given 10^7 M11 CAR T cells on days 7 and 9 via tail vein alone or in combination with DT (n=14 mice/group). Tumor growth was assessed twice/weekly (A). On day 23, the mice that received the combination of M11 CAR T cells and T-reg depletion (DT+M11) had significantly smaller tumors compared to the M11 only (p=0.0054) and tumor only (p=0.0002) groups but not the DT only group (p=0.0683) (B). On day 26, the DT+M11 tumors were significantly smaller than the DT only group (p=0.0462) (B). The DT+M11 combination significantly improved survival (C) (log-rank test). Mice were euthanized based on standard humane endpoints.

RESULTS

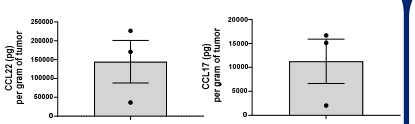
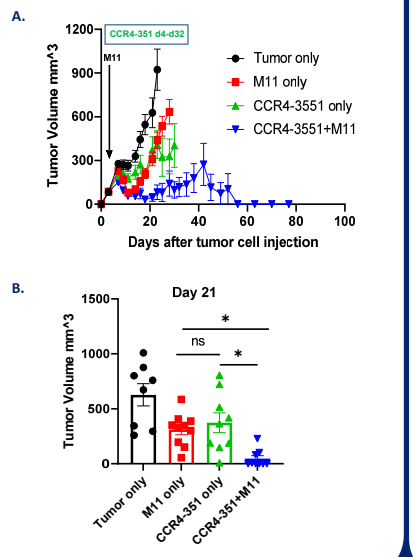


Figure 4. **AE17 mesothelioma tumors produce the CCR4 ligands CCL17 and CCL22.** AE17M subcutaneous tumors were resected, digested and assayed for the presence of CCL17 and CCL22.



RESULTS

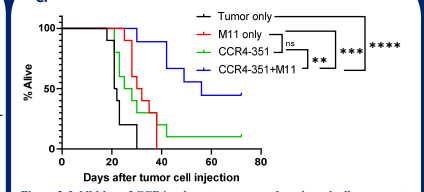


Figure 5. **Inhibition of CCR4 reduces tumor growth and markedly augments the antitumor efficacy of CAR T cells.** AE17M tumor bearing mice began treatment with CCR4-351 on days 4 through 32. One dose of 7M M11 CAR T cells was given on day 5 via tail vein alone or in combination with CCR4-351 (n=10 mice/group). Tumor growth was assessed twice weekly (A). By day 21, mice that received the compound and M11 CAR T cells (CCR-351+M11) had significantly smaller tumors compared to the M11 only (p=0.046), CCR4-351 only (p=0.0122) and tumor only (p<0.0.0001) groups (C). The CCR4-351+M11 combination significantly improved survival (log-rank test). Mice were euthanized based on standard humane endpoints

CONCLUSIONS

- **Systemic depletion of Tregs significantly improves CAR T cell mediated tumor growth inhibition and survival in a syngeneic mouse tumor model**
- **Systemic CCR4 inhibition significantly decreases tumor growth and enhances survival through augmenting the efficacy of CAR T cells**
- **Studies are ongoing to determine the mechanisms by which CCR4 inhibition may alter the tumor microenvironment and whether these findings are generalizable to other solid tumor models**

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