

CARs Deficient in Lck Signaling Require 4-1BB Costimulation to Expand in Vivo, Resist Regulatory T-cell Suppression, and Treat Solid Tumors in Immune-Intact Hosts

Carter M. Suryadevara BS; Rupen Desai MD; Samuel Harrison Farber; Patrick C Gedeon; Adam Swartz; David Snyder; James Herndon PhD; Patrick Healy; Bryan D. Choi MD; Peter Edward Fecci; Luis Sanchez-Perez PhD; John H. Sampson MD, PhD, MHSc, MBA

Introduction

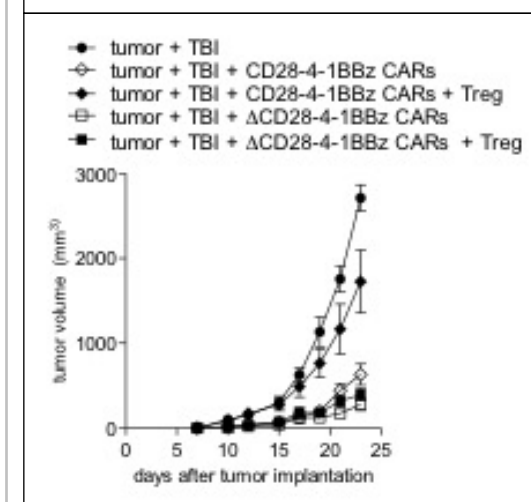
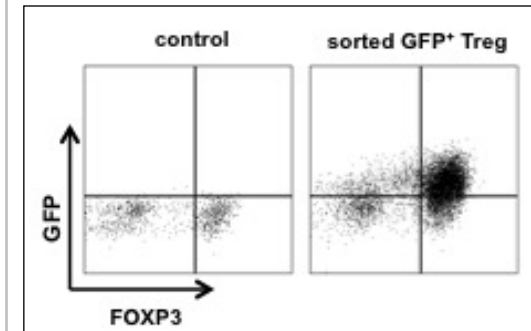
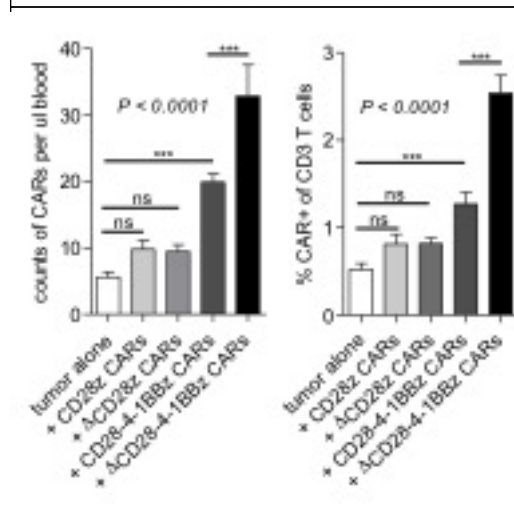
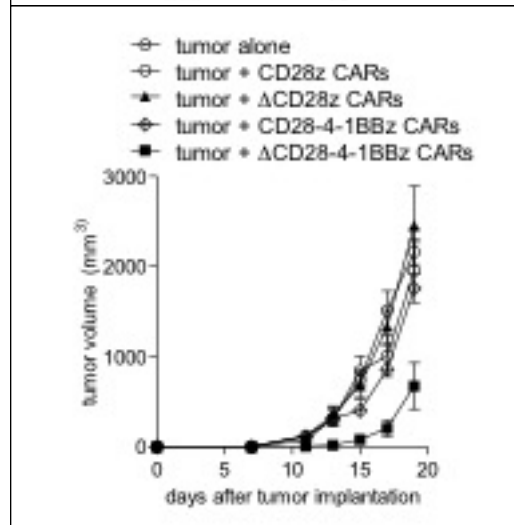
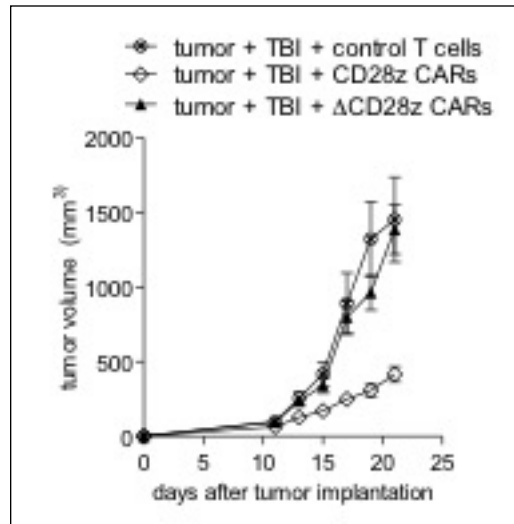
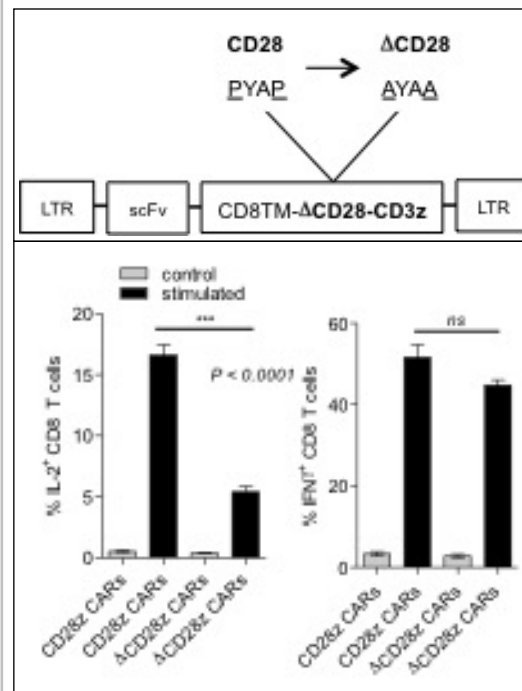
Adoptive transfer of T cells expressing chimeric antigen receptors (CARs) is an effective immunotherapy for hematological cancers but requires a rethinking for clinical efficacy against solid tumors, where CARs have largely failed. Lymphodepletive preconditioning regimens can enhance CAR activity in vivo by promoting T-cell expansion and depleting immunoinhibitory cells that counteract cellular immunity. These nonspecific regimens, however, can be exceedingly toxic and contribute to poor quality of life. Novel strategies are needed to bypass the intratumoral inhibition of CARs. CD4+FoxP3+ regulatory T cells (Tregs) play a critical role in treatment failure, and importantly, CARs have been shown to inadvertently potentiate Tregs by providing a local source of IL-2 for Treg consumption. We explored whether specific disruption of this axis would confer efficacy against solid tumors.

Methods

Second- (CD28z) and third- (CD28-4-1BBz) generation CARs were developed against the tumor-specific mutation, EGFRvIII. To eliminate secretion of IL-2, two amino acid substitutions were introduced in the PYAP Lck binding-motif of the CD28 domain (xCD28) of CAR transgenes. CARs were infused IV into mice bearing 7-day established subcutaneous B16.EGFRvIII melanomas. Where indicated, mice were subjected to 5Gy total body irradiation (TBI) immediately prior to CAR infusion. For co-transfer, purified Tregs were infused with CARs at a 1:10 ratio.

Results

Second generation CD28z CARs fail to expand in vivo. Addition of 4-1BB in third generation CARs improves expansion, but this modification alone was insufficient for CD28-4-1BBz CARs to treat tumors without prior host lymphodepletion. CARs deficient in Lck signaling, however, significantly retarded tumor growth in immune-intact mice without prior lymphodepletion, and this was dependent on inclusion of 4-1BB in CAR design. To determine if deficient Lck signaling altered CAR vulnerability to Tregs, we lymphodepleted mice and transferred CARs +/- Tregs. Co-transfer was sufficient to abrogate the efficacy of CD28-4-1BBz CARs, whereas the efficacy of xCD28-4-1BBz CARs remained unperturbed.



Conclusions

xCD28-4-1BBz CARs may be an effective immunotherapy for solid tumors infiltrated with Treg and may mitigate the need for toxic lymphodepletive preconditioning.

References

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2. Sampson JH et al. EGFRvIII mCAR-modified T-cell therapy cures mice with established intracerebral glioma and generates host immunity against tumor-antigen loss. Clin Cancer Res.