Hijacking Suppression: Anti-CD40 Converts Regulatory T Cells Into Type I Effectors
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Background
Pancreatic ductal adenocarcinoma (PDA) is an aggressive and heterogeneous cancer that is often refractory to current treatments. We found that a combination of anti-PD1, anti-CTLA-4, and anti-CD40 results in tumor regression. Thus, the aim of our study is to gain mechanistic understanding into this therapeutic combination. To that end, we leverage quantitative high multiplex microscopy, enabling us to decipher the complexities of cellular behaviors, interactions, and phenotypes in an intact TME.

Results and Conclusions
In the TME of responsive tumors, our therapy severely depleted regulatory T cells. We found this Treg loss to be ADCC–independent, but anti-CD40–dependent, a surprising result given that anti-CTLA-4 was thought to mediate Treg depletion. Residual Tregs were restricted to the tumor periphery by 48 hours post anti-CD40 administration. We used tamoxifen-inducible Foxp3 lineage tracing mice to label all Foxp3–expressing cells prior to therapy initiation. Strikingly, we found that many of the lineage marked Tregs no longer expressed Foxp3. These ExTregs now had high levels of Tbet and IFNg, and had evidence of cognate antigen recognition, as assessed via imaging of NFAT1 nuclear translocation in situ. Single and combination treatments in the absence of anti-CD40 failed to induce this ExTreg phenomenon. Blockade of MHC-II, IL-12, or IFNg or tumor implantation into Batf3 KO, IL12p40 KO, or IFNg KO mice also ablated this phenomenon. Combined, these data reveal a unique mechanism by which the clinically relevant agonistic anti-CD40 amplifies the anti-tumor immune compartment through the conversion of immunosuppressive Tregs to an effector population within the tumor microenvironment.