Background: Although co-stimulation of T cells with agonist antibodies targeting 4–1BB (CD137) improves antitumor immune responses in preclinical studies, clinical development has been hampered by on-target, off-tumor toxicity. To overcome this, there has been a large effort to restrict activity of these agonist antibodies to the tumor microenvironment using a wide range of strategies. Recently, we have demonstrated that fusions of cytokines with collagen anchoring proteins enables retention of these cytokines in the tumor microenvironment, simultaneously leading to better efficacy and reduced toxicity. Here, we apply that strategy to 4–1BB agonists and report the development of a tumor-anchored 4–1BB agonist (4–1BB-LAIR), which consists of an 4–1BB antibody fused to the collagen binding protein LAIR.

Methods: While combination treatment of 4–1BB-LAIR with an antitumor antibody (TA99) displayed only modest efficacy in curing B16F10 tumor-bearing mice, simultaneous depletion of CD4+ T cells boosted cure rates to over 90% of mice. However, mice failed to form immunological memory against secondary tumor rechallenge. Using high dimensional flow cytometry and RNA-sequencing we probed the mechanism of this synergy to design more clinically relevant combination therapies with improved memory responses.

Results: We elucidated two mechanisms of action for this synergy: αCD4 eliminated tumor-draining lymph node Tregs, enhancing priming and activation of CD8+ T cells, and TA99 + α4–1BB-LAIR supported the cytotoxic program of these newly primed CD8+ T cells within the tumor microenvironment. Exclusive depletion of Tregs, rather than the whole CD4+ T cell compartment, was achieved by treating Foxp3-DTR mice with diphtheria toxin and confirmed that Tregs were responsible for constraining the efficacy of 4–1BB-LAIR. FTY720 treatment further highlighted the importance of the observed therapy-induced priming wave for therapeutic efficacy. Replacement of αCD4 with αCTLA-4, a clinically approved antibody that enhances T cell priming, produced equivalent cure rates while additionally generating robust immunological memory against secondary tumor rechallenge.

Conclusions: In this study, we demonstrate that collagen anchoring is a widely applicable strategy to restrict activity of immunomodulators to the tumor microenvironment, specifically demonstrating its utility in the context of 4–1BB-LAIR agonists. Furthermore, we uncover a fundamental two-step approach to combination immunotherapy: 1) prime CD8+ T cells by regulatory T cell depletion/inhibition; followed by 2) tumor-localized immune agonism (e.g. by 4–1BB) of infiltrating CD8+ T cells.

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http://dx.doi.org/10.1136/jitc-2023-SITC2023.1224