Enhancing CAR T cell therapy using Constitutively Active Cytokine Receptors

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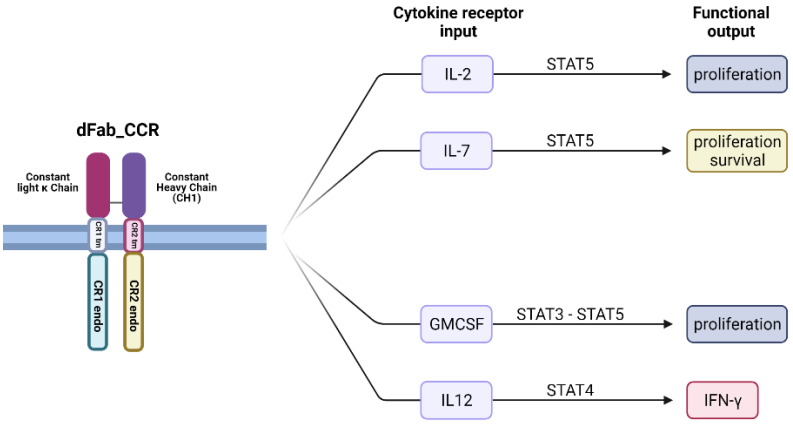
Introduction.

Cytokines are key modulators of adoptively transferred T cell survival and proliferation. Where prolonged trafficking is required to reach the tumour, or in an immunosuppressive microenvironment, paucity of cytokine stimulation can impair T cell engraftment and persistence. Cytokines can be co-administered systemically, or T cells can be engineered to autonomously produce cytokines, however these approaches risk systemic toxicity. An alternative approach is expression of cytokine receptors engineered to be constitutively active. We have developed a versatile constitutive cytokine receptor (CCR) system which recapitulates cytokine signalling by heterodimerization of cytokine receptors. We fully characterize this CCR architecture and identify optimal cytokine signals for use in CAR T cell approaches.

Results.

We generated an IL2-CCR by juxtaposing the IL2 receptor chains, using the antibody CH1 and kappa constant region (Fab) to force heterodimerisation. Under cytokine starvation conditions, T cells expressing the IL2-CCR expanded and had a transcriptional profile similar to that of T cells exposed to exogenous IL2.



We next sought to determine the universality of this FabCCR architecture. Therefore, we tested the common-γ-chain (IL7, IL4, IL9, IL21), common-β-chain (GMCSF, IL3, IL5), IL12 family (IL12, IL23, IL27) and IL17 family (IL17A, IL17C) designed FabCCR. Using a combination of functional and biochemical assays, we demonstrated that each specific cytokine signal could be recapitulated in all FabCCRs tested. Functionally, GMCSF-CCR was particularly effective resulting in 5-fold expansion under starvation condition. IL12 CCR mediated higher basal IFNγ secretion. Following this, we explored the structural constrains of the FabCCR. Substituting the kappa constant region with the light lambda region, maintained comparable expansion. We then investigated whether the quantity of intracellular signal affected the FabCCR output. Progressive truncation of the IL2 β chain resulted a decreasing analogic response, whereas the CγC demonstrated a digital response. Alternatively, truncation of GMCSF endodomain did not modify the CCR functionality. Following this, we explored whether FabCCRs

could enhance CAR function. We co-expressed a subset of FabCCRs with a GD2 CAR. We revealed that under cytokine starvation or chronic antigen stimulation, GMCSF-CCR improved CAR T cell proliferation and protected effector function after serial challenge. We tested the same subset of FabCCRs with GD2 CAR in vivo in two immunocompetent tumour models (B16 F10 and CT26 GD2+ models). Interestingly, we discovered that GMCSF CCR expression boosted CAR T cell expansion (5-fold), persistence and doubled the survival rate of the mice treated. Finally, we examined the transcriptome differences between the CCRs. NanoString analysis revealed complete overlap between IL2 and IL7 CCR. GMCSF CCR displayed differences, with upregulation of genes associated with chemokine/chemokine receptor (e.g. CCR5, CCL18), but also genes involved in control of T cell function (e.g. LAIR1).

Conclusions.

Enhanced CAR T proliferation is essential in solid tumours where the access to antigen is restricted. Our FabCCR allowed constitutive signalling of any cytokine receptors. These FabCCRs could improve T cell therapies (TILs, TCR T cells and CAR T cells), but the versatility may also find broader application in cellular engineering.