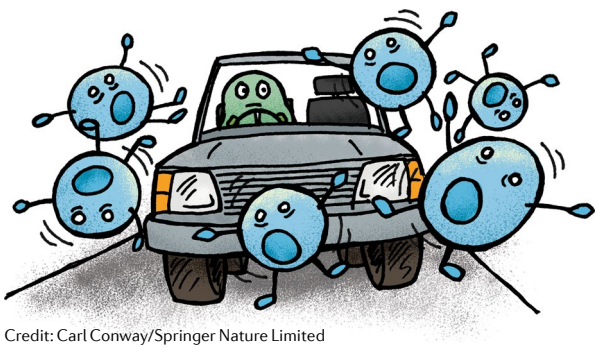
CANCER IMMUNOTHERAPY

Steering CARs in the right direction

Immunotherapy with chimeric antigen receptor (CAR) T cells has proven effective in the treatment of patients with B cell malignancies. Yet, challenges remain that prevent their broader appeal as a therapy for more patients and those with different types of cancer. These include limited efficacy against solid tumours, resistance through antigen loss, and severe toxicities. Three new studies sought to address each of these deficits of CAR T cell therapy with unique approaches.

CAR T cells directed against an oncogenic EGFR mutation, EGFR variant III (EGFRvIII) (CART-EGFRvIII), have demonstrated efficacy by decreasing numbers of EGFRvIII-expressing glioblastoma cells in patients. Yet, the remaining tumour tissue in these patients maintains expression of wild-type EGFR, which is not expressed in normal brain tissue but is amplified in 80% of glioblastomas. Attempting to overcome the heterogeneity in target antigen expression, Choi et al. modified the CART-EGFRvIII construct to express a bispecific T cell engager (BiTE) antibody against wild-type EGFR (CART.BiTE) to achieve multi-antigen targeting. Intraventricular delivery of these CART.BiTE cells into immunodeficient mice harbouring intracerebral heterogenous EGFRvIII and wild-type EGFR-expressing glioblastomas led to complete and durable tumour regressions.

Whilst the tumour antigen-binding arm of the BiTE is directed against EGFR, the other arm, by binding CD3, engages T cells.



Credit: Carl Conway/Springer Nature Limited

> “vaccine boosting donor cells through the CAR”

This BiTE feature will not only direct CAR T cells to tumours but also function to recruit and activate bystander T cells. Indeed, evaluating this aspect in vitro, the authors found that EGFR-specific BiTEs bound to unmodified T cells as well as CART.BiTE-expressing cells. Moreover, both the proliferation and antigen-specific cytotoxicity of the bystander T cells were increased.

A potential concern with targeting wild-type EGFR is that its expression on normal tissues might lead to on-target, off-tumour toxicity. However, in a skin graft toxicity model wherein EGFR-expressing human skin is implanted into immunodeficient mice, CART.BiTE-treated mice showed no signs of the cutaneous graft-versus-host disease observed in CART-EGFR-treated mice.

To enhance CAR T cell responses to solid tumours, irrespective of the antigen specificity, Ma et al. took the approach of vaccine boosting donor cells through the CAR. This was achieved by taking a ligand for a CAR and attaching it to albumin-binding phospholipid polymers to form amphiphile ligands (amph-ligands). The design has the dual advantage of targeting the ligands to the lymph nodes via albumin, which constitutively traffics to these sites, and insertion into the membranes of antigen-presenting cells. By priming CAR T cells in their native environment, injected amph-ligands mediated proliferation and expanded polyfunctionality of CAR T cells as well as improved tumour control and prolonged survival in several different syngeneic solid tumour mouse models.

Mestermann et al. reasoned that side effects of CAR T cell therapy, such as cytokine release syndrome (CRS), which arises through the secretion of pro-inflammatory cytokines from CAR T cells and, consequently, innate immune cells, might be resolved by controlling the activity and function of CAR T cells without permanent termination — a so-called universal pharmacological on–off switch. This idea led the investigators to the tyrosine kinase inhibitor dasatinib. Although originally designed as an inhibitor of the BCR–ABL fusion protein, dasatinib also inhibits ATP binding sites of the SRC family tyrosine kinase LCK. Autophosphorylation of LCK is part of the signalling cascade downstream of CAR as well as endogenous T cell receptor engagement, suggesting that dasatinib treatment could provide a means to transiently exert control over CAR T cell function.

Co-culturing $CD4^+$ and $CD8^+$ T cells expressing a CD19-CAR and 41BB co-stimulation (CD19-CAR/41BB cells) with $CD19^+$ target cells in the presence or absence of dasatinib revealed that treatment with 100 nM dasatinib was sufficient to completely block cytolytic activity, cytokine secretion and proliferation of the CAR T cells. In addition, titrating the dose resulted in partial inhibitory effects. Importantly, dasatinib outperformed dexamethasone, which has been used to prevent toxicities in the clinic previously.

Investigating whether dasatinib could function as a control switch in vivo, the authors used a lymphoma xenograft model in which CD19-CAR/41BB cells were administered at day 0 and dasatinib between days 3 and 5 to mimic a ‘function on-off-on’ scenario in the clinic. Dasatinib treatment rapidly paused activated CAR T cells, an effect that was completely reversible upon dasatinib discontinuation after day 5, and preserved the therapeutic potential of the CAR T cells. Furthermore, a short treatment course of dasatinib could stop the emergence of fatal CRS after CAR T cell transfer in a mouse model of rapid-onset CRS.

The relative feasibility of all three strategies suggests we could see their implementation in the clinic in the near future.

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ORIGINAL ARTICLES Choi, B. D. et al. CAR-T cells secreting BiTEs circumvent antigen escape without detectable toxicity. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-019-0192-1 (2019) | Ma, L. et al. Enhanced CAR-T cell activity against solid tumors by vaccine boosting through the chimeric receptor. *Science* **365**, 162–168 (2019) | Mestermann, K. et al. The tyrosine kinase inhibitor dasatinib acts as a pharmacologic on/off switch for CAR T cells. *Sci. Transl Med.* **11**, eaau5907 (2019)