274 LCK KNOCKOUT CAR-T CELLS AS A NOVEL ALLOGENEIC PLATFORM

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Background Chimeric antigen receptor (CAR) T therapy has shown remarkable success in treating liquid tumours but current approved therapies rely on autologous T cells which are expensive, difficult to manufacture and not readily available for patients whose disease progress rapidly. The production of safe and effective allogeneic CAR-T cells is needed to increase accessibility of CAR-T therapy and broaden its application. The main approach to generate allogeneic CAR-T therapy is by disrupting T cell receptor (TCR) expression to minimize Graft-versus-Host Disease (GVHD) mediated through the TCR of donor cells against the recipient's major histocompatibility complex (MHC). However, the TCR disruption approach has shown limited persistence *in vivo* [1] and in clinical trials [2] unlike the long term durability of autologous CAR-T cells. Here, we propose a novel platform for allogeneic CAR-T therapy that retains the TCR but inhibits TCR signalling by knocking out the Lymphocyte-specific protein tyrosine kinase (LCK) – a well-established kinase for proximal TCR activation. This builds on the discovery that our second generation CD28-CAR can be activated independently of LCK unlike the endogenous TCR.

Methods We utilise the CRISPR-Cas9 system to knockout LCK and TCR in both mouse and human primary T cells. We show how this difference in CAR and TCR signalling can be exploited to generate LCK knockout CAR-T cells that showed similar or enhanced *in vitro* and *in vivo* efficacy against tumour cells compared to conventional CAR-T cells of both human and mouse T cell origin.

Results LCK knockout T cells have reduced proliferation and intracellular cytokine staining against allogeneic PBMCs compared to T cells, suggesting comparable suppression of TCR-mediated alloreactivity to TCR knockout T cells. In the immunodeficient mouse model where human T cells cause xenogeneic GVHD, LCK knockout T cells showed reduced xenogeneic GVHD comparable to TCR knockout T cells. Murine LCK knockout T cells showed the same suppression of TCR signalling as TCR knockout T cells in vitro. In murine major mismatched allogeneic models, murine LCK knockout T cells showed a reduction in GVHD symptoms compared to wild-type T cells. Compared to TCR knockout T cells, the LCK knockout T cells showed superior persistence and higher engraftment in allogeneic recipient mice.

Conclusions Our study suggests LCK knockout CAR-T cells inhibits TCR-mediated alloreactivity and retains the TCR for improved persistence while maintaining CAR activation potential which results in a superior allogeneic CAR-T therapy compared to TCR knockout CAR-T cells.

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Ethics Approval Animal Protocols were approved by NUS IACUC (R20-1303). Use of human primary T cells was approved by Institutional Review Board (IRB) (H-19-026).

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