

Novel AND-gated LINK CAR T cells demonstrate improved safety compared to 2nd-generation CA9 CAR T cells for the treatment of clear cell renal cell carcinoma

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LINK exploits downstream TCR signaling machinery to optimize and customize CAR T cell responses

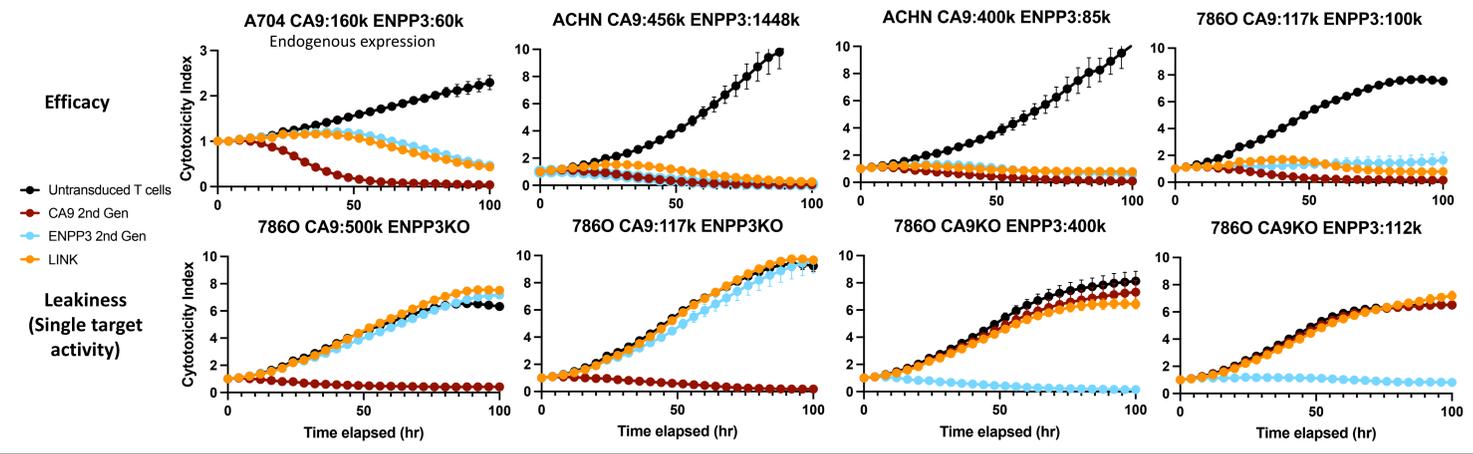
Problem Limited number of tumor-specific antigen targets

Solution **LINK**

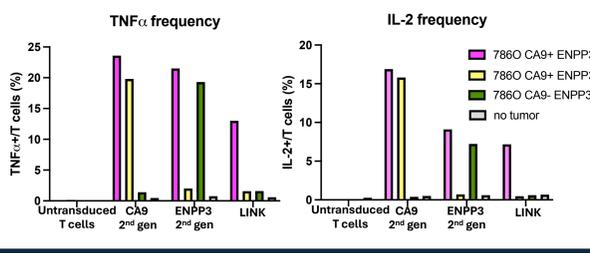
Rapid and direct Boolean logic AND-gating

Safety of chimeric antigen receptor (CAR) targets, such as CA9, in solid tumors can be compromised by target expression in normal tissues (Lamers et al., 2013 Mol Ther). High-fidelity AND-gated CAR T cells (LINK CAR T) can overcome this issue by co-opting the combined downstream T cell signaling proteins, SLP76 and LAT (Tousley et al., 2023 Nature). LINK CARs will only exhibit cytotoxic activity when both antigens are present, sparing healthy tissue with single target expression. Here we de-risk CA9, a target for clear cell renal cell carcinoma (ccRCC) by identifying a second target antigen ENPP3 which displays high tumor-specific expression, with a different subset of healthy tissue liabilities. Combined, co-expression of these antigens is limited to ccRCC tumor. Targeting both antigens with LINK CAR T cells eliminates on-target, off-tumor toxicity and potentially could be a safe and efficacious therapeutic.

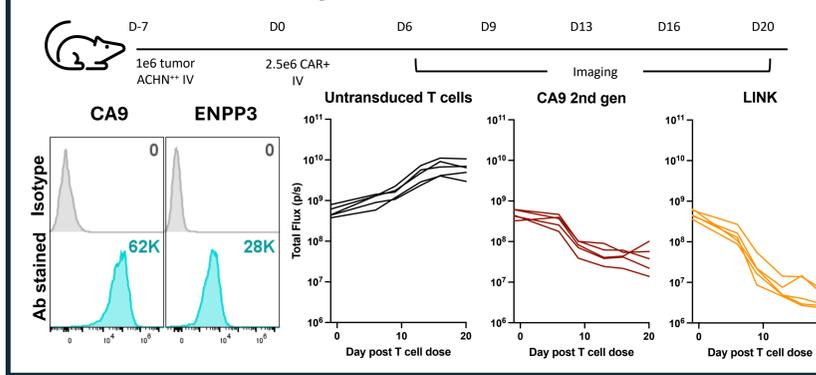
LINK has selective cytotoxic activity against dual positive tumors at various antigen densities



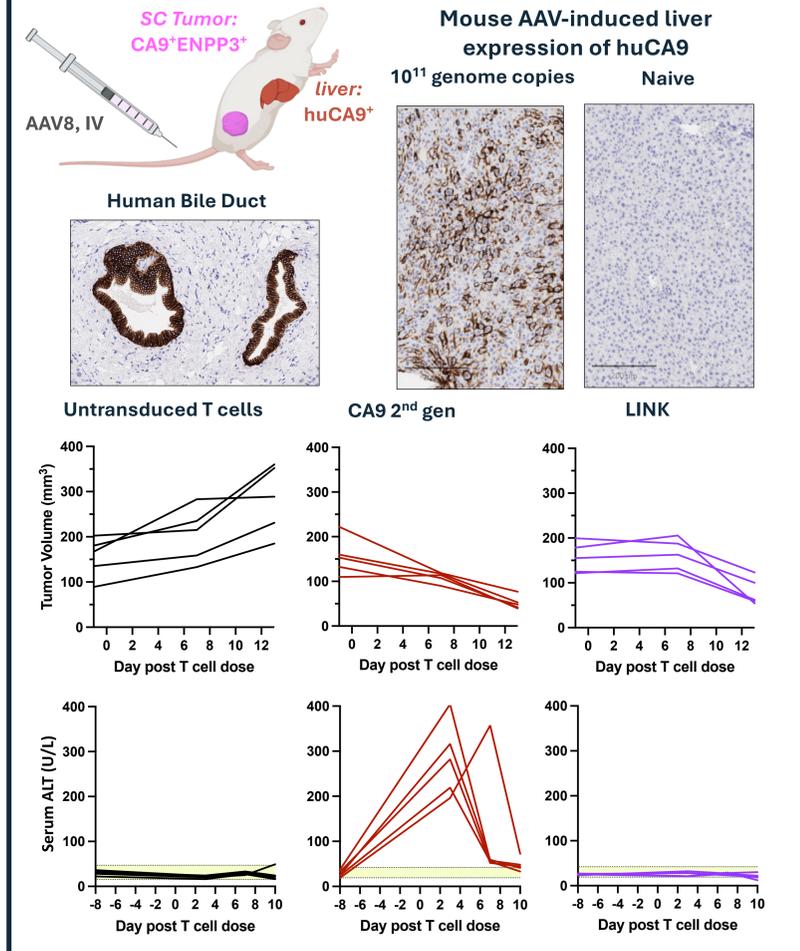
LINK has selective cytokine release



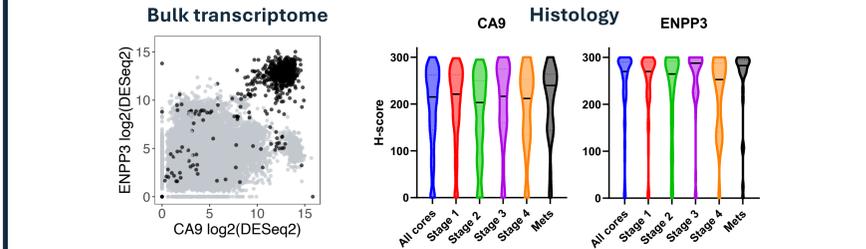
LINK has efficacy in a metastatic model of RCC



CA9 2nd gen CARs induce liver injury in an AAV safety model while LINK CARs do not

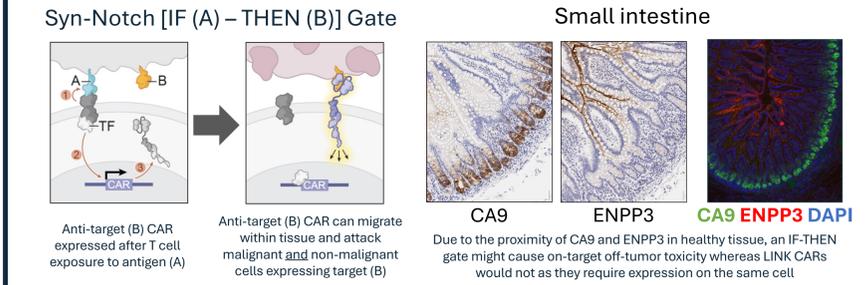


LINK targets CA9 and ENPP3 are highly and selectively co-expressed in ccRCC

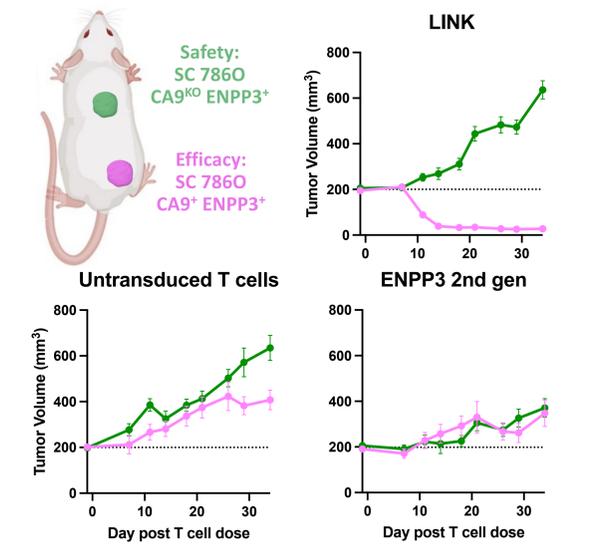


CA9 2nd generation CARs have previously shown toxicity in clinical trial due to expression in liver bile duct (Lamers et al., 2013 Mol Ther). We identified a second antigen, ENPP3 which has exclusive co-expression with CA9 on ccRCC (black dot = individual ccRCC sample; grey dot = individual normal tissue sample). Both CA9 and ENPP3 contribute to tumor survival, reducing the possibility of escape via downregulation (Mardjuki et al., 2024 Cell Reports; Chiche et al., 2010 J Cel Mol Med).

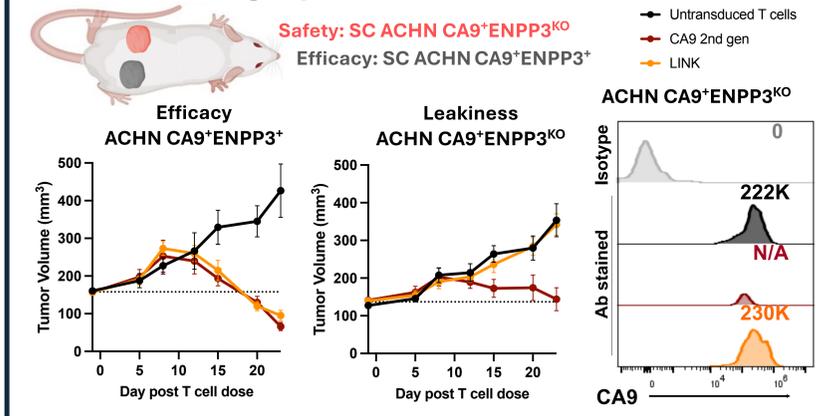
LINK's AND-gate opens the door to compelling targets that may be challenging for other AND-gated technologies



LINK CARs exhibit safety against ENPP3 single positive tissue in vivo



LINK CARs target double positive, but not CA9+ single positive tumor in vivo



LINK CARs are efficacious and safe in pre-clinical models of ccRCC

- CA9-ENPP3 targeting LINK CARs are efficacious in vitro and in multiple in vivo models
- LINK CARs spare healthy single-expressing tissue while targeting dual-positive tissue, demonstrating safety in vitro and in vivo
- 2nd gen clinically relevant CARs liver injury in a model of AAV-induced liver expression of CA9 while LINK CARs do not exhibit this elevated ALT toxicity
- CA9-ENPP3 LINK CARs are a promising therapeutic for ccRCC

Disclosures
All authors are employees of Link Cell Therapies and hold equity in the company. Many authors are on patents related to this work.