VIRUS-FREE CRISPR CAR T CELLS INDUCE SOLID TUMOR REGRESSION

Lauren E. Sarko, University of Wisconsin-Madison, Madison, Wisconsin USA
sarko@wisc.edu
Katherine P. Mueller, University of Wisconsin-Madison, Madison, Wisconsin USA
Nicole J. Piscopo, University of Wisconsin-Madison, Madison, Wisconsin USA
Matthew H. Forsberg, University of Wisconsin-Madison, Madison, Wisconsin USA
Louise A. Saraspe, University of Wisconsin-Madison, Madison, Wisconsin USA
Amritava Das, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin USA
Brittany Russell, University of Wisconsin-Madison, Madison, Wisconsin USA
Madeline Smerchansky, University of Wisconsin-Madison, Madison, Wisconsin USA
Lei Shi, University of Wisconsin-Madison, Madison, Wisconsin USA
Adeela Ali, University of Wisconsin-Madison, Madison, Wisconsin USA
Cicera R. Lazzarotto, University of Wisconsin-Madison, Madison, Wisconsin USA
Shengdar Q. Tsai, University of Wisconsin-Madison, Madison, Wisconsin USA
Christian M. Capitini, University of Wisconsin-Madison, Madison, Wisconsin USA
Krishanu Saha, University of Wisconsin-Madison, Madison, Wisconsin USA

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Chimeric antigen receptor (CAR) T cell therapy has shown promising efficacy in treating hematologic malignancies and has led to the FDA-approval of three CAR T cell products. However, there has been little success in treating solid tumors, as clinical trials to date have yielded little to no responses and no improvement in survival. Current methods of CAR T cell production typically involve the use of viral vectors which can give rise to complications such as insertional mutagenesis, leading to gene silencing or oncogene activation. In addition, GMP-grade viral vector manufacturing can be expensive with lengthy wait times for new batches. Here we have developed a virus-free strategy in primary T cells that has eliminated the use of viral vectors through the use of CRISPR-Cas9 to precisely edit the chimeric antigen receptor into the *TRAC* gene[1]. Our method of virus free production begins through the generation of a double stranded DNA (dsDNA) template produced by polymerase chain reaction (PCR). This template is then combined with a *Sp*Cas9-single guide RNA to create a ribonucleoprotein (RNP) complex. Isolated human primary T cells from adult healthy donors are then nucleofected with the RNP and dsDNA template on day 2 of ex vivo expansion. Flow cytometry is then utilized to immunophenotype the cell product and analyze the percent of efficiency of CAR gene transfer. Within the cell product, the editing efficiencies are >95% TCR knockout and 35% CAR+. Transcriptional profiling indicates that the virus-free CART cells have a favorable memory-like phenotype. In addition to our *in vitro* work, *in vivo* mice studies with anti-GD2 CART products demonstrate regression of GD2+ solid tumors upon virus-free CART treatment, showing similar potency and survival to viral-produced CAR T cells. The production of virus-free CAR T cells has high potential to enable the rapid and flexible manufacturing of highly defined and highly potent CAR T cell products for the treatment of solid tumors.

[1] Mueller, K. et al. CRISPR-mediated insertion of a chimeric antigen receptor produces nonviral T cell products capable of inducing solid tumor regression. *bioRxiv* preprint doi: https://doi.org/10.1101/2021.08.06.455489 (2021).