Developing CRISPR-Cas13 antiviral therapeutics for respiratory pathogens of pandemic potential

*Moe EI Mohamad, Rob De Rose, Stanislav Kan, Wenxin Hu, Joshua Casan, Honglin Chen, Paula Cevaal, Danielle Fong, Nadia Saraya, Ajantha Rhodes, Michael Roche, Damian Purcell, Matthew McKay, Joseph A Trapani, Mohamed Fareh, Sharon R Lewin, Wei Zhao**
792 Elizabeth Street
The Peter Doherty Institute for Infection and Immunity
Melbourne, Vic, Australia
Moe.elmohamad@unimelb.edu.au, Wei.zhao@unimelb.edu.au

There is an urgent need for effective antiviral therapeutics against respiratory viruses, particularly in the face of new pandemics. We previously demonstrated the ability of CRISPR-Cas13 to inhibit SARS-CoV-2 viral replication *in vitro*[1]. The main goal of this work was to determine if CRISPR-Cas13 antiviral therapeutics could be delivered as mRNA packaged in lung-tropic lipid nanoparticles (LNPs) delivered intravenously.

We used a novel LNP formulation, LNP X, to first optimise the delivery of CRISPR Cas13b to a Vero cell line that constitutively expressed green fluorescent protein (GFP; Vero-GFP), and quantified GFP expression by flow cytometry. We co-encapsulated mRNA encoding for Cas13b together with a guide RNA (crRNA) that recognises the coding sequence in the gene for GFP. Using 250 ng of RNA, we achieved a 90% reduction in GFP expression (n=3). We then used LNP X to deliver mRNA encoding for Cas13b together with a crRNA that recognises the nucleocapsid gene in SARS-CoV2. Following 24 hours of mRNA delivery to Vero cells, the Vero cells were infected with either the ancestral SARS-CoV2 or Omicron BA.1. After 24 hours, we observed a 3.8 and 2.4-log reduction in infectious virus in supernatant respectively, compared to an identical LNP which carried a non-targeting crRNA as a negative control (n=2). To ultimately test for antiviral efficacy in a more physiological model, we tested a modified LNP X, LNP XL, in Calu-3 cells grown in air-liquid interface (ALI) cultures. We encapsulated the LNPs with a nanoluciferase tagged-CRISPR Cas13b mRNA (Cas13b-NanoLuc) and transfected the Calu-3 cells with 1000 ng mRNA. The best performing LNP induced a 3-fold increase in Nanoluc expression compared to the other LNPs. To evaluate *in vivo* delivery, we encapsulated Cas13b-NanoLuc mRNA in an LNP that contains the cationic lipid DOTAP and is known to target the lungs following intravenous administration. Following the intravenous administration of 15 µg Cas13b-Nanoluc in mice, the Nanoluc expression was mainly observed in lung tissue, followed by the spleen and nasal tract (n=4).

Cas13b and crRNA can be delivered as mRNA to cell lines and organoid models *in vitro*, and *in vivo* in mice using a range of novel LNPs. *In vitro*, Cas13b mRNA and crRNA targeting nucleocapsid can potently inhibit replication of SARS CoV2. This approach could be a promising platform for therapeutics for pathogens of pandemic potential spread by the respiratory route.

References:
1 Fareh M; et al. Nature comm **2021**, 12, 4270.