Restoration of cGAS in tumor cells promotes antitumor immunity *via* transfer of tumor-cell generated cGAMP

Alexander M. Cryer[1,2,3], Pere Dosta[1,2,3#], Michelle Z. Dion[1,2,3#], Leonardo de la Parra Soto[2], Eliz Amar-Lewis[1,2,3], Gabriela Garcia de Leon[2], Alejandro Abraham Espinosa Perez[2], Diego Fernando Ruiz Aguilar[2], Triana Huerta[2], Beatriz Nicolas Ruiz[2], Nathalie Nicole Casteele Hernandez[2], Yael Soria[2], Natalie Artzi*[1,2,3]

. [1]Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA 02139. [2]Department of Medicine, Division of Engineering in Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115. [3]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA, 02215.

Statement of Purpose: Tumor cells are among the most numerous cell types that comprise the tumor microenvironment (TME), but with respect to immune therapy, they are often under-utilized. Stimulator of interferon genes (STING) agonists are a promising class of innate immune agonists and can promote antitumor immunity. However, these molecules are rapidly cleared from the tumor and blood, which contributes to poor efficacy and side effects in humans. We utilize the tumor cells to generate STING agonist molecules whilst simultaneously circumventing commonly found dysfunctional tumor cell STING signaling. We used a lipid nanoparticle (LNP) to deliver cyclic GMP-AMP (cGAMP) synthase (cGAS) mRNA (cGAS LNPs), resulting in production of the endogenous STING agonist cGAMP, that gets released and transferred to neighboring immune cells, and when combined with immune checkpoint blockade, promotes antitumor immunity.

Methods: The cGAS LNPs were prepared using clinically approved components to encapsulate cGAS mRNA. Their size, surface charge and encapsulation efficiency were characterized. cGAS mRNA expression was confirmed by western blot and cGAMP production from tumor cells was quantified by ELISA. The bioactivity and source of tumor-cell cGAMP was assessed using commercially available reporter immune cell assays and flow cytometry analysis primary murine bone marrow cells. The efficacy of cGAS LNPs in combination with immune checkpoint blockade (ICB) was assessed using a syngeneic murine model of melanoma (B16-F10) in which further downstream mechanistic analysis including biodistribution, tissue and plasma cytokine levels, immunophenotyping and cGAMP quantification were performed.

Results: The cGAS LNPs were found to be approximately 200 nm in size, had a neutral surface charge and narrow polydispersity index with high encapsulation efficiency. The LNPs could efficiently transfect and produce cGAS protein in B16-F10 cells which was found to be active in that it could produce cGAMP. This was confirmed by quantifying the cGAMP produced post-transfection both intracellularly and extracellularly. Cells were additionally transfected with gDNA to provide substrate for cGAS, which enhanced its activity. We found that the transfer of cGAMP to murine immune cells *in vitro* predominantly relied on a secreted factor, or direct secretion, although cell-cell contact did play a roll. When evaluated *in vivo* after intratumoral injection, cGAS LNPs were able to control the growth of aggressive B16-F10 melanoma tumors which resulted in significant increases in overall survival compared to untreated mice. Furthermore, combination with ICB further increased survival and resulted in a percentage of tumor free mice. Mechanistically, cGAS LNPs resulted in production of cGAMP in the tumor, leading to release of pro-inflammatory molecules such as CXCL10, IFNα and CCL2. Consequently, there was recruitment of granulocytes to the TME, activation of dendritic cells, macrophages, natural killer cells and $CD8^+$ T-cells.

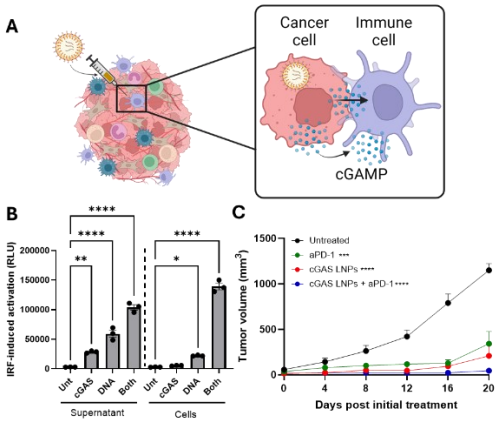

Figure 1. (A) Schematic depiction of cGAS LNPs delivery to tumors and cGAMP transfer. (B) IRF-induced luminescence by either supernatant or cancer cells previously transfected with cGAS LNPs. (C) B16-F10 tumor bearing mice were treated with cGAS LNPs or immune checkpoint blockade (aPD-1) or the combination of both Data are expressed as mean ± SEM ($n = 3$ *in vitro,* n = 8-10 *in vivo*). *P <0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Conclusions: Tumor cells are traditionally thought of as the central protagonist in oncology and while that is somewhat true, attempts to use them in a therapeutic context could prove valuable given their number. In this work, we strive to achieve this by delivering cGAS mRNA to tumor cells so that they may produce cGAMP in response to cytosolic double-stranded (ds)DNA. This cGAMP is then exported into the TME where it can be taken up by immune cells, activate STING and initiate an antitumor response. Utilizing endogenous cGAMP transfer mechanisms mimicking a danger signal from tumor cells has proven an interesting way to promote antitumor immunity, although the exact transfer mechanisms and if other innate immune pathways are at play are still to be determined. The observed benefit with ICB is encouraging and given the "fuel" for cGAS is dsDNA, clinically approved modalities such as radiotherapy or chemotherapy that encourage cytosolic dsDNA production may prove to be a potent strategy.

References: Cryer AM *et al*. PNAS (under revision).