An mRNA-encoded Adjuvant-enzyme Activates STING to Enhance the Immunogenicity of Vaccines Targeting Human Papillomavirus Positive Cancers

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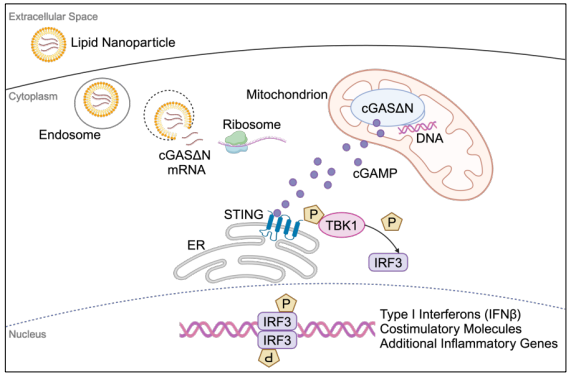
Abstract # 3552

Enhancing the immunogenicity of lipid nanoparticle-based vaccines using an mRNA-encoded adjuvant



- Human papillomavirus (HPV) infection can lead to oropharyngeal and anogenital cancers. HPV16 is a particularly prevalent cancer-causing strain, driving approximately 60% of cervical cancers.
- As a vaccination strategy, lipid nanoparticles (LNPs) can carry mRNA encoding antigens but can also encode immunostimulatory proteins that can help enhance responses.
- cGASΔN mislocalizes to mitochondria and is thus an active form of cGAS, an enzyme that produces the cyclic dinucleotide cGAMP upon binding to DNA.
- Cyclic dinucleotides such as cGAMP activate the transmembrane protein STING, initiating a signal transduction cascade that results in the production of type I interferons (IFN), costimulatory molecules and additional inflammatory genes.
- **Our objective is to test the feasibility of combining cGASΔN with the delivery of mRNA-encoded antigens such as E6 and E7 from HPV16 to induce T cell responses against this cancer-causing virus.**

Constitutively active cGAS is encoded as mRNA and encapsulated in LNPs for translation

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mRNA	Length (nt)	Particle Size (nm)	Polydispersity Index	pH	Osmolality (mOsm/kg)	Zeta Potential (mV)
GFP	997	96.41	0.065	7.73	693	-1.74
cGASΔN	1360	92.86	0.094	7.61	834	-2.76
cGASΔN^{mut}	1359	90.96	0.027	7.64	883	31.3

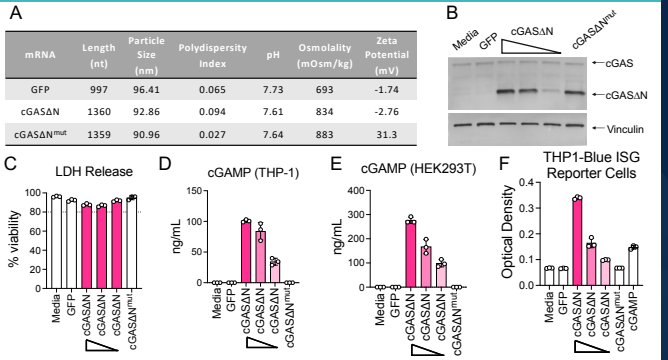

Figure 1: (A) mRNA encoding GFP, cGASΔN, and cGASΔN^{mut} were formulated in LNPs containing SM-102 ionizable lipid and characterized for their physical attributes. (B) THP-1 cells were incubated with 1 μg/mL LNPs for 1 day. Cells were additionally treated with lower doses of cGASΔN at 0.5 and 0.1 μg/mL. Protein lysates were used for western blotting of cGAS expression. (C and D) THP-1 cells were treated with 1 μg/mL LNP for 1 day or 0.5 or 0.1 μg/mL cGASΔN LNP. Cell viability was measured for lactate dehydrogenase (LDH) release in the cell culture supernatant. Cells were harvested and lysed to measure cGAMP production. (E) HEK293T cells which naturally lack cGAS expression were similarly treated with LNPs. Cells were lysed to measure cGAMP production. (F) THP-1-Blue ISG reporter cells were treated with 1 ng/mL LNPs or 0.5 or 0.25 ng/mL cGASΔN LNP. As a positive control, cells were treated with 10 μg/mL cGAMP. Alkaline phosphatase reporter gene activity was measured one day later.

A constitutively active form of cGAS (cGASΔN) can be encoded in mRNA for delivery and expression via LNP. cGASΔN expression is sufficient for cGAMP production, enabling STING signaling.

cGASΔN LNPs activate human monocyte-derived dendritic cells

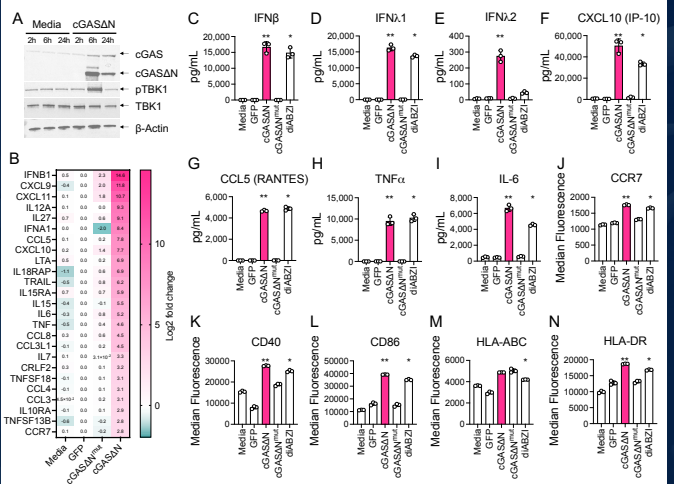

Figure 2: (A) Human monocyte-derived dendritic cells (moDCs) from one donor were treated with 1 μg/mL LNP. After 2, 6, and 24 hours of treatment, cells were lysed and analyzed via western blot for expression of endogenous and recombinant cGAS and TBK1 phosphorylation. (B) moDCs from four donors were treated with 1 μg/mL LNP or media control for 6 hours. RNA was isolated for sequencing. Listed are the top 25 cytokines, chemokines, and cytokine receptors significantly upregulated by cGASΔN treatment. (C-N) Human moDC were treated with 1 μg/mL LNP or 0.5 μM of the small molecule STING agonist diABZI for 1 day. (C-I) Cytokine expression was quantified from culture supernatant. (J-N) moDC were fluorescently stained for flow cytometry analysis. Triplicates from one of three representative donors are shown. One-way ANOVA was used to compare groups. *p<0.0001 for diABZI compared to media control. **p<0.0001 for cGASΔN in comparisons to GFP and cGASΔN^{mut}.

cGASΔN mRNA can be delivered to human primary dendritic cells for translation, initiating a signaling cascade. Many genes are transcribed, including inflammatory cytokines and chemokines. Further analyses of protein production determined that cGASΔN significantly upregulated cytokines, chemokines, and cell surface activation markers.

cGASΔN-treated dendritic cells improve the human CD8 T cell IFNγ response

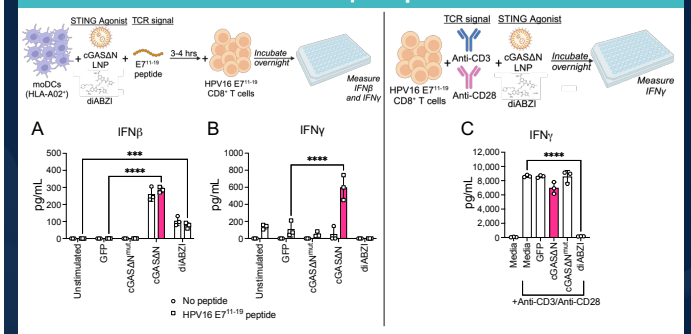

Figure 3: (A and B) HLA-A*02:01+ moDC were incubated with the HPV16 E7$^{11-19}$ minimal epitope and were activated using 1 μg/mL cGASΔN LNP or 0.5 μM diABZI. After a 3-hour incubation, allogeneic E7-specific CD8+ T cells were added at 1:3 (APC:T cell) ratio. Next day, supernatants were analyzed for IFNβ production by moDC and IFNγ production by T cells. ***p<0.001; ****p<0.0001 using two-way ANOVA multiple comparisons. (C) E7-specific CD8+ T cells were activated using 10 μg/mL anti-CD3 and 10 μg/mL anti-CD28. Cells were additionally incubated with 1 μg/mL LNPs or 0.5 μM diABZI. After an overnight incubation, IFNγ production was quantified. ****p<0.0001 using one-way ANOVA, comparing anti-CD3/anti-CD28 treatment with and without diABZI treatment.

Cell permeable STING agonists such as diABZI are actively being investigated for their ability to initiate STING signaling. While STING signaling in antigen presenting cells is beneficial for enhancing the T cell response, diABZI can directly target T cells, inhibiting their ability to activate and produce IFNγ.

cGASΔN LNPs injected intramuscularly induce acute cytokine secretion that wanes by 24 hours in mice

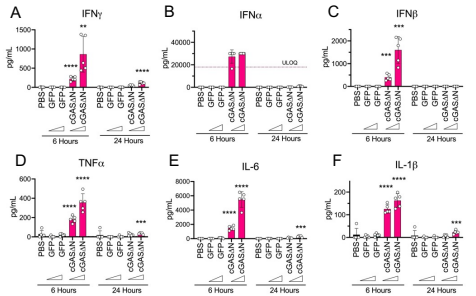

Figure 4: B6 mice were immunized intramuscularly with 1 μg and 5 μg of GFP LNP or cGASΔN LNP. Serum cytokines were assessed at 6 and 24 hours by Legendplex. Means and SD are shown. Each symbol represents one mouse, n=5 mice per group. Data is representative of two experiments. T-test used to compare cGASΔN with equivalent dose of GFP. **p<0.01, ***p<0.001, ****p<0.0001

In comparison to mice immunized with GFP LNPs, mice immunized with cGASΔN LNPs showed elevated serum interferons and pro-inflammatory cytokines at 6 hours post injection which returned to baseline 24 hours post injection, demonstrating intact and transient signaling of cGASΔN in vivo.

When combined with HPV16 LNPs, cGASΔN LNPs enhance T cell responses against E6 and E7

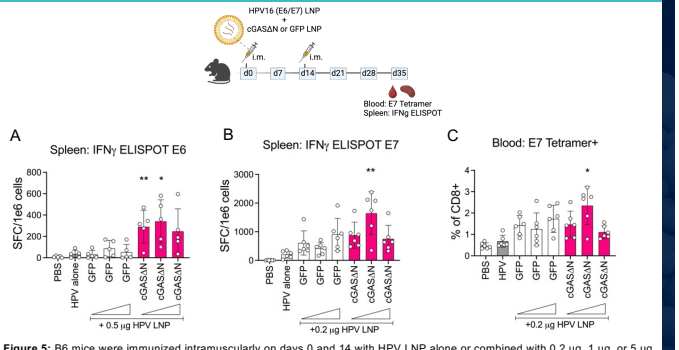

Figure 5: B6 mice were immunized intramuscularly on days 0 and 14 with HPV LNP alone or combined with 0.2 μg, 1 μg, or 5 μg of GFP LNP or cGASΔN LNP. On day 35, antigen-specific T cell responses in the spleen were assessed by IFNγ ELISPOT after restimulation with (A) E6 peptide pool or (B) E7 peptide pool. (C) Antigen specific T cells in the blood were assessed by E7 tetramer staining. Means and SD are shown. Each symbol represents one mouse, n=5-6 mice per group. Data are representative of two experiments. T-test used to compare cGASΔN with equivalent dose of GFP at each dose level. *p<0.05, **p<0.01

Mice immunized with LNPs containing HPV16 E6/E7 antigens combined with cGASΔN LNPs induced stronger T cell responses to both E7 (strongly immunogenic) and E6 (weakly immunogenic) as measured by tetramer staining and IFNγ ELISPOT. A higher dose of HPV antigen LNPs enabled cGASΔN at multiple doses to enhance T cell responses against weakly immunogenic E6 antigen.

Key Takeaways

- cGASΔN can be encoded in mRNA and delivered to cells for translation via lipid nanoparticles.
- cGASΔN LNPs initiate innate immune signaling in human moDCs, leading to upregulation of activation markers and secretion of interferons, inflammatory cytokines, and chemokines.
- When combined with HPV antigen LNPs, cGASΔN LNPs potently adjuvant the T cell response against the weakly immunogenic E6 antigen while also enhancing the response against strongly immunogenic E7 antigen