VZV mRNA vaccine with Cap5 (ENE) showed stronger immune response in mice

SYNTHGENE

BACKGROUND

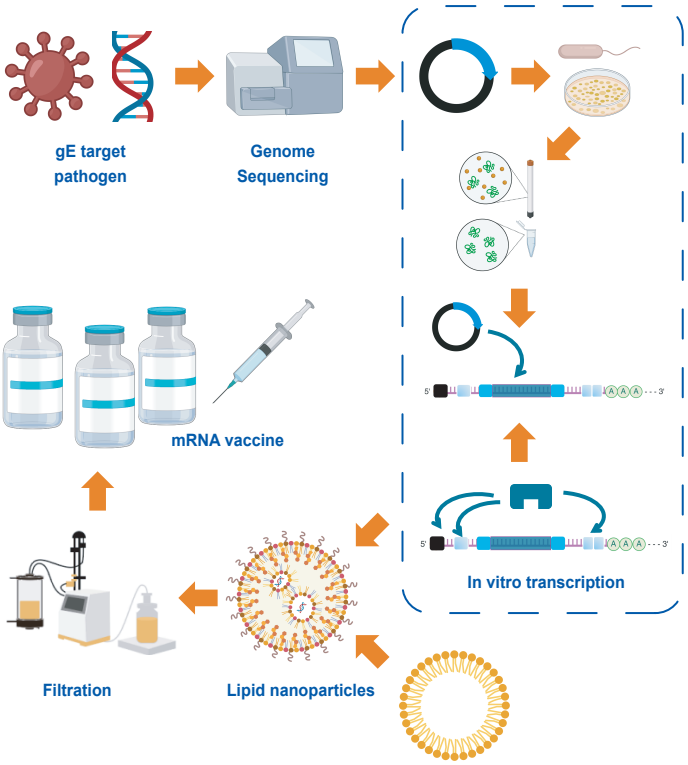
Herpes zoster (HZ), commonly known as shingles, occurs when the latent varicella-zoster virus (VZV), which causes chickenpox in childhood, reactivates from its latent state. Over 90% of adults carry this virus, putting them at risk for reactivation. Shingrix®, a recombinant subunit vaccine, has shown an overall vaccine efficacy of 97.2% among individuals aged 50 and above. With the success of mRNA vaccines for COVID-19, there's keen interest in developing an mRNA-based shingles vaccine as this approach offers notable benefits, including a shorter design and testing timeline, as well as easier manufacturing processes.

METHOD

Synthgene worked with a collaborator on a VZV vaccine program in which we designed the mRNA vaccine, manufactured the mRNA, packaged it into LNP and then injected it into mice to test immune response.

To start, we searched the VZV genome to identify a suitable antigen, and in this case we decided on glycoprotein E (gE), a 623-amino-acid protein that is a major component of the VZV capsid. Then, we designed multiple mRNA sequences and synthesized them by in-vitro transcription (IVT). Two of these sequences, GND0251 and GND0254, showed promising results and we decided to advance both for animal testing.

When synthesizing the GND0251 and GND0254 mRNA, we used both our novel cap analog Cap5 (ENE) and the benchmark m7GpppAmG.



RESULTS

mRNA-LNP Prep.

Table 1. Cap5(ENE) vs. CAP m7GpppAmG mRNA Prep.
CGE purity ≥ 82%; Cap rate ≥ 96%

mRNA	Con.(ng/μl)	260/280	CGE purity	Capping
GND0251(CAP m7GpppAmG)	1039.4	1.93	91.4%	96.6%
GND0254(CAP m7GpppAmG)	1018.0	1.94	84.7%	99.2%
GND0251(Cap5(ENE))	1033.7	1.92	87.1%	98.8%
GND0254(Cap5(ENE))	1039.3	1.94	82.9%	99.5%

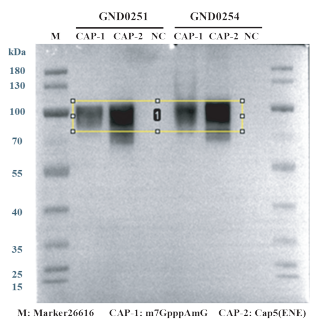
Table 2. Cap5(ENE) vs. CAP m7GpppAmG mRNA-LNP
Vaccine particle size 90-100nm; PDI ≤ 0.08; Encapsulation rate ≥ 95%

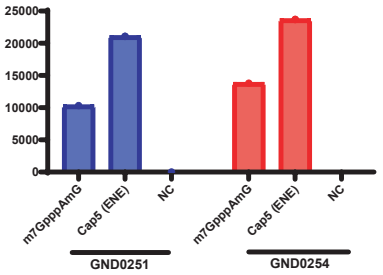
mRNA-LNP	Size nm	PDI	mRNA Con. μg/ml	Free mRNA Con. μg/ml	encapsulation %
GND0251-LNP (CAP m7GpppAmG)	92.83	0.048	101.10	3.74	96.30
GND0254-LNP (CAP m7GpppAmG)	92.41	0.037	125.01	4.66	96.28
GND0251-LNP (Cap5(ENE))	93.68	0.038	110.38	4.44	95.98
GND0254-LNP (Cap5(ENE))	93.53	0.074	114.40	5.04	95.59

Target Protein Expression

After transfecting 10μg of GND0251 and GND0254 into HEK293T cells for 48 hours, the expression of gE-specific protein (ab272686) was verified by Western blotting (WB). The expression level of gE protein with Cap5(ENE) was found to be significantly higher compared to m7GpppAmG, showing approximately 2.04-fold and 1.72-fold increases for GND0251 and GND0254After 10μg GND0251 and GND0254 transfected into HEK293T cells 48hr, gE specific protein (ab272686) verified by WB. The expression level of gE protein in Cap5(ENE) is significantly higher than m7GpppAmG, about 2.04 folds and 1.72 folds.

VZV gE protein expression (WB)





Lane	Area
GND0251(CAP m7GpppAmG)	10317.882
GND0251(Cap5(ENE))	21081.104
GND0254(CAP m7GpppAmG)	13797.225
GND0254(Cap5(ENE))	23704.790

Fig 1. Target protein expression level in HEK293T cells

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SYNTHGENE

Evaluation of Humoral Immunity

Six-week-old female BALB/c mice were divided into six groups and immunized with the vaccines. The mice received intramuscular (i.m.) immunizations with 10μg of the vaccine administered at days 0 and 21. Blood samples were collected weekly, and serum samples were utilized to measure the gE-specific IgG titer. In comparison to the CAP m7GpppAmG vaccine, the Cap5(ENE) GND0254 mRNA vaccine showed a 5.26-fold increase in IgG titer on day 7 and a 2.24-fold increase on day 35.

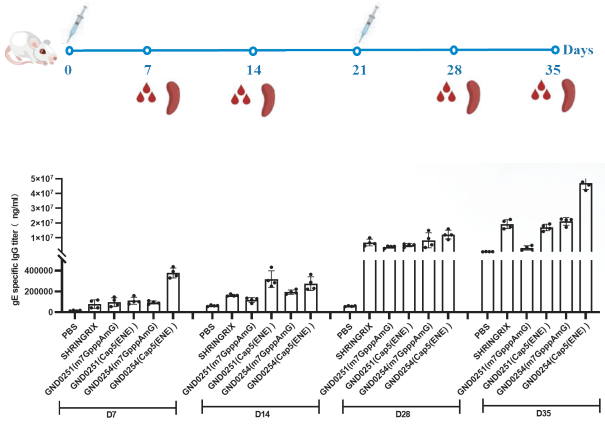


Fig 2. Evaluation of humoral immunity in Balb/c mice

Evaluation of Cellular Immunity

Mice received immunizations via the intramuscular (i.m.) route with a 10μg vaccine administered at days 0 and 21. Splenocytes were collected weekly post-immunization for enzyme-linked immune spot assay (ELISPOT). With GND0254 vaccine, mRNA capped with Cap5 (ENE) demonstrated higher IFNγ secretion than mRNA capped with m7GpppAmG, showing 1.07-fold increase on Day 14 and 1.84-fold increase on Day 35.

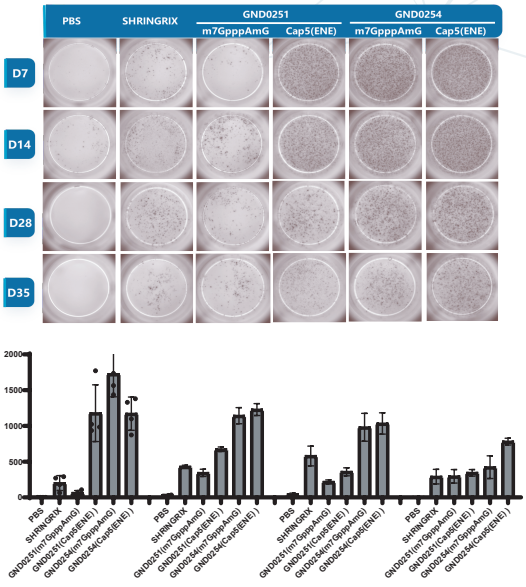
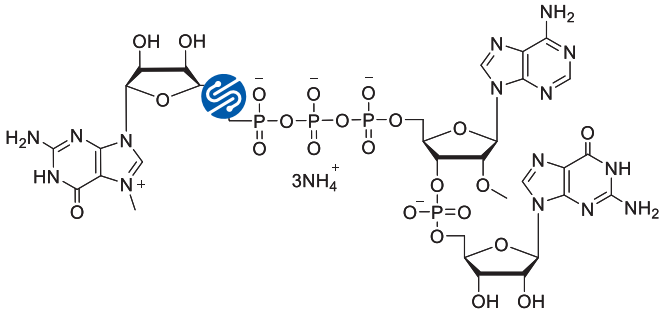


Fig 3. Evaluation of cellular immunity in Balb/c mice

CONCLUSIONS

GND0254 showed better vaccine performance than GND0251. With both GND0254 and GND0251, Cap5 (ENE) outperformed benchmark m7GpppAmG.

GND0254(Cap5(ENE)) provides excellent antigenicity against VZV, inducing not only stronger gE-specific IgG antibody responses but also higher levels of VZV–specific IFNγ compared with other vaccine strategies in mice.



CAP 5 m7G(5')vppp(5')(2'OMeA)pG
100mM Ammonium Solution
CAP5011

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