A cationic nanoemulsion for the delivery of next generation RNA vaccines

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ABSTRACT SUMMARY
We have reinvented the gene vaccine by creating a synthetic self-replicating RNA vaccine platform capable of providing a rapid immune response to prevent and treat current and emerging infectious threats. We have achieved proof of concept for 1st generation self-replicating RNA vaccines in small and large animal models. The vaccine RNA is produced by an enzymatic transcription reaction and formulated with non-viral delivery systems, thereby avoiding the limitations of cell culture production that complicate production of other vectored delivery systems. Given the many positive attributes of nucleic acid vaccines, our results suggest that a comprehensive evaluation of non-viral technologies to deliver self-amplifying RNA vaccines is warranted.

INTRODUCTION
Prophylactic vaccination revolutionized the practice of modern medicine. However, despite advances within the vaccine field over the past 80 years, there are a number of unmet medical needs that current technology has not been able to overcome. Nucleic acid-based vaccines such as viral vectors, plasmid DNA (pDNA), and mRNA are being developed as a means to address the limitations of both live-attenuated and subunit vaccines. Although pDNA vaccines have proven to be safe and broadly effective in small animal models, they often require multiple high doses in larger species and generally lack potency in man. Viral vectors have demonstrated potency in man, but are limited by potential safety issues and interference by anti-vector immunity.

Recently, there has been a focus of mRNA for gene therapy and vaccine development (1). As with other nucleic acid modalities, delivery of the molecule is a major hurdle. Our initial experiments were based on the advances in cytosolic delivery of siRNA with ionizable lipid nanoparticles (LNP). This work illustrated that self-amplifying RNA derived from an alphavirus vector (Figure 1) can be successfully encapsulated and efficiently delivered with a lipid nanoparticle (2).

RESULTS AND DISCUSSION
We optimized a nanoemulsion for delivery of self-amplifying RNA based on the emulsion adjuvant
MF59. The RNA is adsorbed onto the cationic surface of the emulsion prior to administration. Characterization of the particle size before and after RNA complexation shows the emulsion droplets particle size increases slightly from 101nm to 129nm after the complexation with RNA measured by DLS (Figure 2).

Figure 2: Particle size measured by DLS of CNE before (red line) and after (green line) SAM RNA addition. Data are reported as the Z-average (Z-ave) with the polydispersity index (pdi).

It has been demonstrated many times that condensation of DNA with cationic lipids and polymers can protect it from enzymatic degradation. RNase-mediated degradation of RNA in tissues after administration may be a limiting factor in delivering an intact transcript to the cell cytoplasm. To evaluate the protective effect of the emulsion on RNA stability, RNA was incubated with RNase in the presence or absence of CNE. We found that despite being surface absorbed the RNA is able to be protected from an in-vitro RNAse challenge (Figure 3).

Figure 3: RNA agarose gel electrophoresis showing protection of SAM RNA from RNase: Molecular weight ladder (lane 1), SAM RNA (lane 2), SAM RNA after incubation with RNase (lane 3), CNE with SAM RNA after extraction (lane 4), CNE with SAM RNA after RNase exposure, inactivation of RNase and RNA extraction (lane 5).

The cationic nanoemulsion delivery system was evaluated in a variety of animal and disease models. Figure 3 shows representative data from a rabbit study illustrating that self-amplifying RNA (SAM) delivered by CNE generates potent neutralizing antibodies superior to other nucleic acid vaccine technologies, including a viral replicon particle (Figure 4). Similar data was generated in the other species tested.

Figure 4: Neutralization titers of HIV MW965 elicited by 1 x 108 IU VRP, 25 µg HIV gp140-expressing SAM vector in PBS, 25 µg HIV gp140-expressing SAM vaccine in CNE (gp140/CNE), 25 µg HIV CNE-formulated gp140-expressing non-replicating mRNA, and 25 µg HIV gp140-expressing CNE-formulated pDNA

CONCLUSION
We have developed a potent delivery system that can bind RNA and protect it from an RNase challenge. Additionally we have evaluated this emulsion in a number of disease models. The representative data shown here illustrates the delivery system can elicit potent neutralizing antibodies at low doses (25µg) that are comparable to a viral vectored system and superior to alternative nucleic acid technologies in non-rodent species.

REFERENCES

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