



Commentary

mRNA rescues neonatal acidemia while mice report no aftereffects

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Complete control of genetic diseases requires the manipulation of protein expression. Recently, mRNA-based therapy has blossomed into a potential therapeutic agent to prevent and treat a broad range of diseases. This is fundamentally a new way of treating rare inherited genetic diseases with significant pediatric morbidity and mortality. mRNA replacement therapy is also a potential alternative to treat diseases that are incompatible with protein replacement therapies [1]. Delivering mRNA rather than proteins could potentially help eliminate the costly and lengthy steps for protein development and manufacture. Exogenously delivered mRNA only has to cross the plasma membrane and does not alter the host genome and therefore does not have the safety concerns associated with genome editing.

This rapidly growing field has spawned numerous commercial endeavors with several potential therapies currently being evaluated in Phases II and III clinical trials. These efforts have mainly been focused on mRNA-based cancer immunotherapies and cancer vaccines, while treatments for rare genetic diseases are still either in the preclinical stage or just starting Phase I trials [2,3]. Nevertheless, extensive preclinical evaluation of mRNA therapy especially with respect to their long-term efficacy, safety and tolerability remains a critical knowledge gap towards clinical translation. In this article of *EBioMedicine*, Ding An and colleagues completed a comprehensive preclinical evaluation of mRNA therapy in treating methylmalonic acidemia (MMA), and showed this therapy has sustained functional benefit and is well tolerated systemically [4].

A main delivery vehicle for mRNA is a synthetic lipid nanoparticle (LNP). Typically, multiple mRNA molecules are sterically caged within a sub 100 nm biodegradable, ionizable lipid membrane. When systemically injected, these complex molecules are intrinsically targeted to the liver *in vivo*. MMA is a rare genetic disease that can lead to liver and

kidney failure as well as neurological abnormalities [5]. Consequently, An et al [4] chose MMA as a model disease to study the long-term pre-clinical efficacy and toxicity of mRNA therapeutics.

MMA occurs in ~1:100,000 live births and is caused by deleterious mutations of the human methylmalonyl-coenzyme A (CoA) mutase gene (hMUT) that leads to a toxic accumulation of methylmalonic acid in the blood [5]. The ideal treatment is to supplement the patient with fully functional hMUT enzyme. An et al. [4] extended their previous work [6] in murine models of MMA by expanding the treatment period from 2-weeks to 12-weeks. They evaluated a severe model that only expresses hMUT in muscle-tissue to rescue neonatal lethality ($Mut^{-/-}$; $Tg^{INS-MCK-Mut}$) and a hypomorphic model that systemically express a hMut variant with reduced enzymatic activity ($Mut^{-/-}$; $Tg^{INS-CBA-G715V}$).

An et al [4] first studied the dose-response of the mRNA therapeutic *in vivo*. Plasma levels of methylmalonic acid decreased substantially over two weeks before reverting to baseline. For the long-term study bi-monthly injections of 0.5 and 2 mg/kg of hMUT mRNA formulated in LNPs showed a sustained statistically-significant increase in serum hMUT protein and decrease in plasma levels of methylmalonic acid over the 12-week study (with 11–12 mice per group). Tolerability of the mRNA therapeutic was evaluated through frequent clinical observations and toxicity was evaluated both with clinical chemistry as well as liver enzyme levels. The Kaplan-Meier survival curves for the severe murine model showed a dramatic difference between the therapeutic over the PBS control group. The mRNA therapeutic also improved in clinical outcomes and clinical chemistry for the hypomorphic model. The therapeutic was well tolerated in both models and showed minimal toxicity with minimal damage to extrahepatic tissues.

In their mRNA designs, uridines were replaced with 5-methoxyuridine to limit the immunological response. These modified bases are not well recognized by the immune system and limit its activation [7]. *In vivo*, the LNP is thought to be uptaken by the hepatocytes through the endocytosis. hMUT mRNA is translated in the cytoplasm and functional hMUT protein is then trafficked to mitochondria [6]. LNPs containing this hMUT mRNA were assembled using a cartridge based microfluidic systems [8], and they are composed of a complex formulation of ionizable lipid, phosphocholine, cholesterol and

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polyethylene glycol (PEG). The wide-scale manufacturability of these complexes needs to be addressed in the future. The biodistribution of LNPs favors the liver and spleen they are rapidly cleared from the blood and not detectable in the kidney, heart, lung and lymph node [9].

The sustained bioactivity and pharmacology of mRNA therapy observed in this study paves the way for a clinical evaluation. While the translational potential of this work is clear, there remain feasibility gaps in both product development and clinical development that need to be answered in the long term. What percent of mRNA-LNPs are delivered to cells? Can the economics of manufacturing mRNA-LNP and treating thousands of patients scale? If so, will patients be required and willing to receive injections every two weeks for the rest of their lives? The initial promise of mRNA-based therapeutics lay in cancer vaccines and the delivery of cancer immunotherapies that require small numbers of doses, so it remains to be seen whether it can be a long-term palliative therapy. For other diseases, the delivery of mRNA and tropism of LNPs to extrahepatic tissue needs to be addressed [3]. Strategies such as rationally-designed targeting approaches including coupling tissue-specific ligands to the LNPs could be evaluated [10]. In addition, synthetic UTR elements and N1-methyl pseudouridine can be explored to prolong and increase expression, respectively. In conclusion, An et al demonstrated that LNP-delivered mRNA therapy for MMA may be a viable candidate for clinical trials.

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