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Advancing regenerative medicine with RNA nanotechnology for chronic and end organ diseases

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Therapies for chronic and end-organ diseases are often associated with systemic toxicity, and such conditions result in difficulty treating patients. RNA nanomedicine has demonstrated unprecedented advantages in the field of therapeutics through its selective organ targeting, personalized treatment option and safety. In this paper, we extensively discuss the current state of nanotechnology in regenerative medicine and how this unique combination of RNA nanomedicine and regenerative medicine can be leveraged for a successful end-organ and chronic disease therapy. More specifically, we have put forward strategies to specifically deliver RNA nanomedicine to target organs/tissues in chronic disease patients, design considerations for regenerative medicine RNA therapy versus RNA vaccines, and barriers to clinical translation. We further discuss the call to action short- and long-term goals in the field of regenerative RNA nanomedicine that could change the face of regenerative medicine in the coming years.

End-organ diseases refer to the permanent damage to one or several major organs due to varied causes, leading to the shutdown of these organs and the inability to perform adequately for the normal functioning of the body.

The use of RNA-based therapies has encompassed several advantages in the field of regenerative medicine, including specific organ/cell targeting, a dose-dependent response, and ease of production¹⁻³. However, there are significant barriers to the viability of RNA therapies, including nuclease degradation, clearance by the reticuloendothelial system, and inefficient RNA delivery into the cellular cytoplasm due to its negative charge and size⁴⁻⁶. Additionally, RNA molecules trigger an antiviral response and accumulate in off-target sites, decreasing therapeutic efficacy in cell and organ regeneration⁷⁻⁹ (Fig. 1).

To overcome these critical limitations, nanoparticles have been formulated to enable successful RNA delivery. RNA-containing nanoparticles can be designed to avoid macrophage clearance, extend RNA half-life, and target delivery to avoid toxicity at off-target sites^{10,11}. Multiple RNA types can also be potentially incorporated into one nanoparticle to target different genes¹². RNA nanomedicine has emerged in recent years as a vehicle by which genetic instructions can be safely delivered into target cells without being degraded. Such nanoparticles have various formulations and can carry RNA into target cells to reprogram them. For example, one could envision packaging RNA that can block the production of harmful and damaging proteins or could stimulate regeneration.

Additionally, the milestone achieved in RNA therapeutics through the success of Pfizer-BioNTech and Moderna mRNA-LNP COVID-19 vaccines has motivated several researchers to explore this field of research for therapeutics beyond vaccines. Evidently, the safety profile of these mRNA vaccines, showing exceptional efficacy against coronavirus with no adverse reactions except transient local and systemic reactions, exhibits RNA nanomedicine as a safe and effective means of therapeutic delivery¹³⁻¹⁶. Additionally, mRNA vaccines have shown to improve protein translation, regulate innate and adaptive immunogenicity, and enhance cellular delivery¹⁷. Patients with chronic and end-organ disease are a group that would especially benefit from such localized therapy to prevent systemic side effects. End-organ diseases involving the liver, kidneys, heart, lungs, or brain has shown to increase exponentially each year¹⁸. The rate of descent into end-organ disease varies from one organ to another. For example, although chronic liver disease can evolve into cirrhosis and liver failure over time, this accumulates over decades due to the liver's unique capacity to regenerate¹⁹⁻²¹. This allows the liver to heal itself over time despite ongoing injury²⁰⁻²². If we could leverage and harness this unique regenerative capacity of the liver at earlier stages and across organs, we could prevent progression to end-organ disease and associated deaths¹⁹.

Considering the high impact of RNA nanomedicine in the current era, we convened a Medicine by Design Convergent Working group on March 27th, 2023, to discuss the challenges and future perspectives in translating

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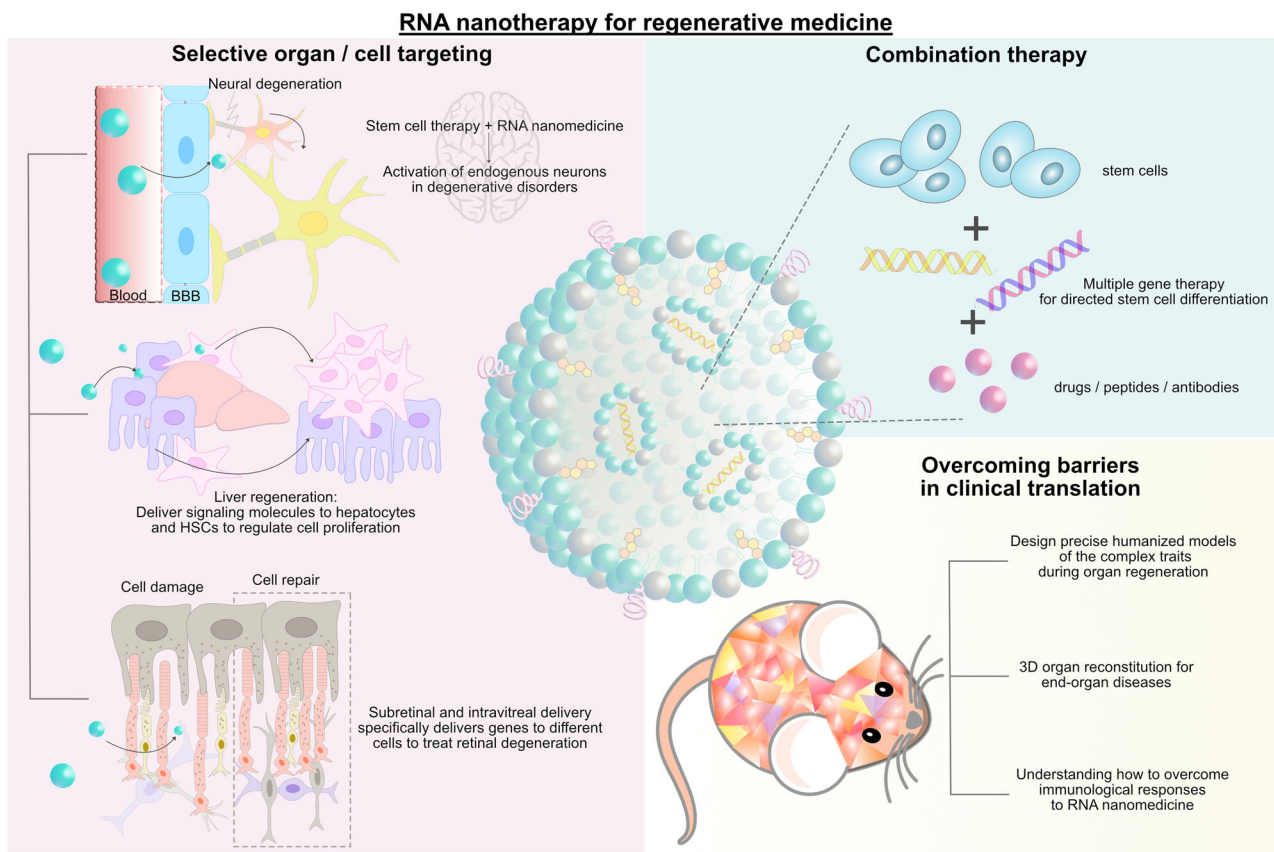


Fig. 1 | Overview of the applications of RNA nanotherapy for regenerative medicine and ways to overcome the barriers in clinical translation.

RNA nanomedicine into the clinic. By inviting local as well as international pioneers in RNA nanomedicine and clinical regenerative medicine, we have drafted this position paper as a call for action on how we can control delivery of RNA-containing nanoparticles into cells of interest, and how we can make these therapies safe for ultimate clinical translation in patients with chronic and end-organ disease along with the advancements, current challenges, and call to action short-term and long-term goals. This is an opportune time to exploit the growing expertise in RNA nanomedicine and bring it to the bedside to stimulate regeneration in patients with chronic diseases.

Current state of nanotechnology in regenerative medicine

Regenerative medicine allows repair, replacement, or restoration of diseased or damaged tissues. Research has evolved from strategies using tissue engineering to tissue reconstruction, further allowing early-intervention treatments for traumatic injury or degenerative diseases. The field of regenerative medicine has shown promise in treating several diseases that are represented with a large unmet medical need, including blindness, neurodegenerative diseases, diabetes, and various organ repairs.

Researchers have harnessed the power of RNA nanomedicine through Patisiran (Onpattro®), a first-in-class, first-in-human FDA-approved siRNA encapsulated lipid nanoparticle (LNP) formulation currently used for the treatment of hATTR in patients with polyneuropathy due to transthyretin (TTR) amyloidosis²³ (<https://www.fda.gov/drugs/spotlight-cder-science/new-class-drugs-fulfills-promise-rna-based-medicine>, <https://www.ema.europa.eu/en/medicines/human/EPAR/onpattro>). TTR amyloidosis is a systemic disease with abnormal amyloid protein deposition affecting the heart, peripheral, and autonomic nervous systems. Patisiran acts by inhibiting the TTR protein in the liver by specifically targeting hepatocytes and has shown exceptional efficacy during clinical studies with >80% gene silencing and an excellent safety profile²³. LNPs are the most well-established nano carriers today that are composed of an ionizable lipid,

a diffusible PEG-lipid, and other lipids such as PEG, with each component playing a significant role in the therapeutic efficacy and specific targeting²⁴. The unique characteristics of lipids used in the development of LNPs allow liver targeting through LDL receptor binding for their internalization into hepatocytes, further releasing the RNA machinery in the cytoplasm²⁴. This is achieved by LNPs binding to ApoE serum proteins that facilitate binding to the LDL receptor expressed on the surface of hepatocytes, enabling selective uptake of LNPs into hepatocytes via receptor-mediated endocytosis²⁴. Upon internalization, LNPs are distributed to endosomal compartments. Endosomal release of LNPs is a critical step in the RNA therapeutic delivery process. Although it remains a complex and less clear process, studies have indicated that the acidic environment in the endosomes could lead to the protonation of ionizable lipids. This destabilizes the endosomal membrane, facilitating the release of the RNA machinery into the cytoplasm²⁴. Overall, the FDA and European Commission approval of Patisiran for TTR amyloidosis reflects the remarkable progress of RNA nanomedicine for genetic diseases that have a systemic impact and suggests significant potential in regenerative medicine (<https://www.fda.gov/drugs/spotlight-cder-science/new-class-drugs-fulfills-promise-rna-based-medicine>, <https://www.ema.europa.eu/en/medicines/human/EPAR/onpattro>).

Several types of RNAs, such as small interfering RNA (siRNA), messenger RNA (mRNA), microRNA (miRNA), and antisense oligonucleotide (ASO), are currently being studied for the treatment of regenerative diseases. These types of RNA contain distinctive features that enable/disable their use during specific therapies. Amongst these, siRNAs are currently the most widely used RNA that function by downregulating gene expression through RNA-induced silencing complex (RISC)-mediated mRNA degradation. Similarly, miRNAs also recruit RISC to the complementary mRNA sequences, and miRNA mimics are designed to upregulate the mRNA expression²⁵. Conversely, mRNAs are used when a dysfunctional protein replacement is required, or antigen encoding is needed for a longer-term immunity to pathogens such as SARS-CoV-2. A distinctive disadvantage of

mRNA is that the transcription occurs *in vitro* and therefore they cannot have site-specific chemical modifications²⁵. On the other hand, ASOs act by either inducing the degradation of the mRNA or by inhibiting the progression of splicing or gene translation^{19,26}. The RNA size is also taken into account when designing delivery systems. siRNAs and ASOs are much smaller than mRNAs and can therefore be delivered through small conjugates or antibodies; however, mRNAs are large and need to be encapsulated before any conjugation²⁵.

Additionally, other emerging non-viral technologies are also being used for RNA delivery in regenerative medicine, such as engineered extracellular vesicles (EVs) and tissue nanotransfection²⁷. Extracellular vesicles are lipid-bound vesicles that function as endogenous intercellular cargo transfer systems for RNA moieties due to their natural cell-targeting abilities, pharmacokinetics, and biodistribution²⁷. Overall, EVs can be further classified as exosomes and microvesicles, whereby exosomes are formed from endosomal compartments while microvesicles are produced through budding and pinching from the cell membranes²⁷⁻²⁹. EVs offer the advantage of natural cell-targeting ability as opposed to lipid nanoparticles that are often engulfed and exocytosed by endosomes. Reports have shown successful loading of mRNA, noncoding RNAs, and miRNAs into EVs for regenerative medicine applications, and studies have indicated that, depending on the source of EVs, they may show lesser immunogenic properties due to fewer transmembrane proteins on their surface as compared to the parental cells in regenerative medicine²⁹. On the other hand, tissue nanotransfection has also paved its way through advancements to become the future of regenerative medicine by permitting direct cytosolic delivery of regenerative factors using a non-invasive array of nanochannels supplied with focused electroporation³⁰. Interestingly, researchers have demonstrated that the combination of RNA-based EVs with tissue nanotransfection can trigger cellular or tissue regeneration more effectively³⁰, however, more in-depth studies are still required to generate a proof-of-concept of these technologies in regenerative medicine. Interestingly, researchers have demonstrated that the combination of RNA-based EVs with tissue nanotransfection can trigger cellular or tissue regeneration more effectively³⁰; however, more in-depth studies are still required to generate a proof-of-concept of these technologies in regenerative medicine. Following these advancements, innovative strategies are being explored to enhance regenerative outcomes.

Emerging technologies in regenerative medicine

Ocular diseases. The FDA approval of Luxturna®, an adeno-associated virus vector-based gene therapy for the treatment of a form of Leber Congenital Amaurosis (LCA) that causes inherited blindness, has opened new avenues for RNA nanomedicine in the treatment of ocular diseases³¹. Studies have discussed the potential of RNA nanomedicine in eye diseases and disorders. Ocular blindness is caused by the degeneration of PRs, RPEs, and retinal ganglion cells, and he emphasized that over the past decade, retinal regenerative medicine has permitted the generation of specific cells from progenitor cells for cell repair and restoration that can be strategized through RNA nanomedicine³². Also, a surge in RNA nanotherapies for the treatment of ocular diseases has been witnessed over the past few years, owing to their ability to penetrate complex ocular barriers such as the corneal-retinal and blood retinal barriers^{33,34}. This further contributes to minimal to no extra-ocular delivery, thereby limiting off-target toxicities. Recently, Dulla et al. investigated an antisense oligonucleotide (ASO) based treatment of retinitis pigmentosa caused by USH2A exon 13 mutations in a zebrafish model³⁵. The results validated the ASO QR-421a as a candidate for an exon-skipping therapy and are currently being clinically tested in a phase ½ trial (NCT03780257).

Neurodegenerative disorders. Although advances in stem cell therapy have enabled neuron replacement and reconstruction in ischemic stroke patients, most stem cells do not exclusively differentiate into functional neurons, resulting in inefficient clinical translation. In this regard,

nanoparticles offer an unprecedented advantage of co-delivering stem cells with genes that favor neuronal differentiation. In a recent investigation, Lin et al. studied the co-delivery of neuronal stem cells with Pnky siRNA that silenced the gene responsible for the inhibition of neuronal differentiation of neuronal stem cells, and surprisingly, the results indicated that this combination of nanomedicine promoted neuronal differentiation and hence enhanced the therapeutic efficacy of regenerative medicine in stroke³⁶. In addition to this, the convergence of nanomedicine and regenerative medicine also holds immense scope for the treatment of devastating neurodegenerative diseases. The past decade has witnessed stem cell transplantation in these diseases, along with nanotechnology to enable these therapies across the blood-brain barrier (BBB) as the most promising strategy for the treatment of neurodegenerative diseases³⁷. Previously, a clinical study (NCT01640067) demonstrated the therapeutic effect in reducing the progression of Amyotrophic Lateral Sclerosis (ALS) post exogenous transplantation of neural stem cells; however, utilizing nanoparticles in conjunction with this therapy could have also activated endogenous neurons along with the exogenous populations for a more potent pharmacological effect^{38,39}.

Pancreatic diseases. Tremendous advancement has been witnessed in pancreatic regenerative medicine to prevent and treat diabetes. With substantial progress in understanding the disease, several researchers are designing pancreatic stem cells for differentiation into pancreatic β -cells and islet-like organoids that efficiently regulate glucose and secrete insulin^{40,41}. Most recently, Melamed et al. have developed mRNA LNPs that can be specifically targeted to the pancreas for the treatment of pancreatic diseases and disorders. In this study, the intraperitoneal route of delivery was utilized as a strategy to specifically deliver the mRNA to the pancreas located in the peritoneal cavity and reduce systemic toxicity associated with the intravenous route of delivery. The results indicated excellent mRNA-induced protein expression in the insulin-producing β cells with LNPs formulated using three different ionizable lipidoids, and approximately 50% increase in the protein expression was observed in the pancreas when the LNPs were administered intraperitoneally as compared to intravenous administration. Additionally, a proof-of-concept was established to show that the mRNA was delivered by peritoneal macrophage extracellular vesicle transfer, and the mRNA efficacy was not associated with any systemic toxicity⁴².

Liver diseases. Liver, as an organ, presents exceptional ability for regeneration. However, liver diseases are the 11th leading cause of death worldwide, accounting for 4% of all deaths worldwide, with liver transplantation being the mainstay for patients with end-stage liver disease incapable of inherent regeneration^{43,44}. Also, the major anti-fibrotic therapies employed during non-alcoholic steatohepatitis, liver fibrosis, and cirrhosis present low therapeutic efficiency and high side effects. To overcome these challenges, in the past few decades, several researchers have been investigating RNA nanomedicine for the treatment of chronic liver disease and regenerative medicine⁴⁵⁻⁴⁹. Most recently, Mitchell's group has developed a combinatorial library of anisamide ligand-tethered lipidoids (AA-lipidoids)⁵⁰. These lipidoids showed a high potency and selectivity to deliver RNA payloads to activated HSCs. HSP47 siRNA (*si-HSP47*)-loaded AA-T3A-C12 LNP, which are specifically targeted to activated myofibroblasts, performed approximately 65% knockdown and strongly decreased liver fibrosis, with remarkably reduced collagen fibers and pseudobulbes with minimal collagen deposition. Moreover, AA-T3A-C12/*si-HSP47* LNP-treated fibrotic mice demonstrated almost normal histological structure and had a lower number of fibrotic septa and apoptotic hepatocytes, indicating exceptional potential for RNA nanomedicine to target undruggable liver diseases with a potential for regenerative medicine. Overall, RNA nanomedicine represents an exciting field of research for the treatment of end-organ diseases through regenerative medicine.

Strategies to deliver RNA nanomedicine directly into target cells/tissues

Tissue/cell-specific delivery. Since most nanoparticles are systemically administered, an urgent challenge lies in the specific delivery of the circulated mRNA molecules to the site of interest. They are also highly prone to nuclease degradation and, therefore, susceptible to degradation prior to reaching the organ of interest, further affecting therapeutic efficacy. Moreover, the mRNA may require cellular penetration for gene editing; hence, strategies for cytosolic delivery also need to be considered^{51,52}. To overcome these obstacles, different surface ligands and careful design of nanoparticles are necessary for a successful targeted and efficacious therapy. Recently, for lung targeting, Qui et al. reported a novel PEGylated KL4 peptide (PEG₁₂KL4) that acts as a pulmonary surfactant protein B (SP-B) mimic and can be conjugated on nanoparticle surfaces for effective delivery to the lung epithelial cells^{53,54}. Additionally, researchers have also discovered that although the majority of LNPs are made of ionizable lipids, utilizing ionizable lipids with low pKa and unsaturated hydrocarbons and/or 0.5% PEG during LNP formulation elicits greater transfection efficiency in the retinal pigment epithelium (RPE) cells of the eye following subretinal injections⁵⁵.

Similarly, while most lipid nanoparticles accumulate in the liver, it is crucial to identify the liver cells of interest more specifically for therapeutic targeting and to avoid any off-target distribution and toxicity. Considering this, Han et al. have recently developed an anisamide ligand-tethered lipidoid (AA-T3A-C12) as a high-affinity ligand for the overexpressed sigma receptors on activated fibroblasts and HSCs⁵⁰. These novel LNPs showed greater RNA delivery to activated fibroblasts in the liver compared to the current FDA-approved MC3 ionizable lipid. Also, 2-fold fibroblast uptake and decreased liver fibrosis were evident using the AA-T3A-C12 formulated LNPs with heat shock protein 47 siRNA as the therapeutic target for liver fibrosis⁵⁰. Alternatively, researchers have discovered that apolipoprotein E (ApoE) and N-acetylgalactosamine (GalNAc) can direct the nanoparticle uptake specifically to hepatocytes through the lipoprotein (LDLR) and asialoglycoprotein (ASGPR) receptors, respectively^{46,56,57}. Hepatocyte targeting is crucial for several diseases, including hereditary amyloidosis that is currently being treated by GalNAc-mediated hepatocyte targeting LNPs – Onpattro.

Subsequently, RNA nanomedicine has also shown promise in cardiac regeneration for myocardial infarction and gene modulation in inherited cardiac diseases; however, insufficient RNA delivery to the cardiac tissue is often an obstacle. Researchers have been studying different approaches to maximize cardiac tissue delivery by optimizing the various excipients used in LNP formulation development. In a recent study, a positively charged ionizable lipid C12-200, along with higher dioleoylphosphatidylethanolamine (DOPE) and lower cholesterol, was used to formulate LNPs for cardiomyocyte targeted gene therapy, and the results indicated that these novel LNPs were able to target the cardiomyocytes safely and effectively⁵⁸. Collectively, such approaches suggest that specifically targeted nanomedicine holds great potential in cardiac regeneration by targeting the hard-to-transfect cardiomyocytes.

While LNPs mostly tend to accumulate in the liver, which facilitates liver targeting as opposed to targeting other organs, liver localization is not an inherent property of all nano carriers, and chemical modifications in the nanoparticle design can allow minimal liver involvement. This has evidently been demonstrated by the discovery of a selective organ targeting (SORT) strategy to rationally develop nanoparticles for extrahepatic RNA delivery. Dilliard et al. added a SORT molecule as a fifth component to the conventional LNPs that are comprised of PEG, cholesterol, amphipathic phospholipids, and ionizable cationic lipids to alter the biodistribution profile of the nanoparticles⁵⁹. Most importantly, the SORT molecule is selected based on the cells or organ of interest and which proteins would most abundantly absorb to the LNPs for extrahepatic delivery, for example, increased pulmonary delivery has been observed with LNPs that contain a quaternary ammonium headgroup as a SORT molecule⁶⁰.

Local delivery. All speakers and discussants agreed to the fact that the route of delivery is extremely crucial for specifically targeting organs and tissues. Studies have indicated that different routes of administration, such as intranasal, intravitreal, and topical, have shown enhanced delivery to the lungs, brain, eye, and skin, respectively. Pulmonary delivery has been considered challenging due to its specialized cell types, mucus barrier, and mucociliary clearance. Researchers are aiming for inhalational and intratracheal delivery formulations for lung diseases and lung tissue regeneration. For the treatment of cystic fibrosis, MRT5005 is reportedly the first-in-human inhaled mRNA candidate currently in Phase I/II clinical trials that delivers a codon-optimized CFTR mRNA via a nebulizer⁶¹. The ongoing study results indicate that this aerosol delivery was safe and well tolerated during the 28-day follow-up post-treatment completion, and no severe adverse effects or off-target effects were reported except minor adverse events such as cough and headache⁶². Although improvements on nanoparticle stability for nebulization are still required, *in vivo* studies have shown highly efficient pulmonary mRNA delivery and gene editing in the lung with excellent cellular uptake⁶³. Also, minimal to no off-target distribution was evident by selecting this route of administration for lung delivery.

Furthermore, intranasal delivery has been the preferred route of drug administration for neuron regeneration in neurodegenerative diseases. Although the current standard of care for most CNS diseases is oral medications, it has been proven that oral therapy has a limited therapeutic effect due to its poor absorption and inability to cross the BBB to reach the brain and cells of interest. For this reason, researchers are taking advantage of the nerve pathways, blood vessels, lymphatic system, and cerebrospinal fluid located within the nasal system that enable routing nanoparticles to the brain⁶⁴. Also, nasal instillation allows the drug to immediately enter the systemic circulation, thereby avoiding any drug degradation that occurs in the gastrointestinal tract during oral therapy. Most essentially, nanomedicine enables efficient drug delivery of low molecular lipophilic molecules past the BBB, which is equipped with abundant tight junctions and undergoes limited pinocytosis for the maintenance of CNS homeostasis⁶⁵.

Similarly, for targeting the degenerative diseases of the eye, researchers have been focusing on subretinal and intravitreal injections of RNA nanoparticles. Studies have shown how RNA nanoparticles can be designed to not only specifically deliver the gene therapy to the eye, but also to specifically deliver target genes in different cells of the eye by designing specific target peptides for specific cells. Studies have indicated that lipid nanoparticles (LNPs) are able to predominantly deliver gene therapy to retinal pigment epithelium RPE cells and Muller glia cells through subretinal or intravitreal routes; however, LNPs have been unable to penetrate the neural retina for photoreceptor (PR) targeting. To overcome this challenge, researchers have successfully developed and evaluated novel peptides for mRNA delivery to the neural retina in mouse models. Moreover, these chemically decorated LNPs showed excellent delivery in a retinal degenerative nonhuman primate model with potent protein expression in RPE, Muller glia, and PRs, opening the scope for eye regenerative medicine^{66–68}.

Design considerations for regenerative medicine: mRNA vs small RNA for RNA vaccines

Amongst others, one crucial hurdle is the immune response as a viral defense mechanism experienced during RNA therapy that has previously led to the termination of clinical studies. Although this could favor while designing RNA vaccines, immunogenicity might not be the ideal response during RNA therapy, and therefore it is extremely important to understand this phenomenon and take into consideration while designing RNA vaccines for regenerative medicine⁶⁹. Most importantly, both single and double-stranded RNA moieties are recognized by the immune system via toll-like receptor (TLR) signaling that further activates several pathways resulting in a type I interferon response, production of inflammatory cytokines, and activation of nuclear factor- κ B (NF- κ B)⁷⁰. As a proof-of-concept, Kariko et al. demonstrated that mRNAs with naturally modified nucleotides had a

higher translational capacity than unmodified mRNAs. Also, only the unmodified mRNAs depicted immunogenic behavior with elevated levels of interferon α as opposed to the modified mRNAs⁷¹.

To understand this, researchers have extensively studied self-amplifying RNA (saRNA) versus mRNA for the development of vaccines and how different cargos are sensed intracellularly to produce the immune response. Researchers have been studying whether different RNAs have shown variations in the optimal parameters for cellular activation, and the studies indicated that most parameters remained the same irrespective of the RNA; however, the size of RNA had an impact on the encapsulation efficacy and eventually the mechanism of action. Also, researchers are employing second and third-generation chemical modifications, such as 2'-ribose modifications on siRNAs and phosphoramidate morpholino oligomers (PMOs), to reduce the associated immunogenicity by neutralizing charge and restricting protein interactions that could trigger immune system activation⁷²⁻⁷⁵.

Most importantly, the stability and rapid degradation of RNAs critically determine the fate of the nanoparticle in the body. Many researchers have been studying ways to improve the RNA half-life for optimal efficacy. Reports have indicated the importance of the Cap, untranslated regions (UTRs), and poly(A) tail located at the 5'- and 3'- ends of the mRNA structure for stable and effective mRNA. Some of the ways to improve mRNA stability include (a) increasing G-C fractions in mRNA, and (b) fine-tuning the poly(A) tail, 5' Cap, 5'-UTR/3'-UTR, and rare codon⁷⁶. Similarly, chemical modifications on siRNAs and ASOs also enhance RNA stability and protect them from rapid nuclease degradation. The most common ASO and siRNA modification is a phosphorothioate modification that enhances DNase resistance and improves cellular uptake due to an increase in hydrophobicity⁷⁷⁻⁷⁹.

Additionally, several other potential solutions are being investigated, such as performing more in-depth immune-related adverse event screening in human studies with TLR interaction methods, using short 7-8 nucleotide RNAs that show successful low immunogenicity in preclinical studies, administering metronomic (regular and frequent) doses of RNA therapy, and combination treatments of RNA and immune-based therapies.

Overall, the lessons learnt from the COVID-19 mRNA vaccine are important to drive RNA nanotherapeutics in autoimmune diseases, inflammatory conditions, and end-organ diseases as regenerative medicine.

Barriers to clinical translation: understanding the factors contributing to the translational gap in chronic and end-organ diseases for regenerative medicine

Most importantly, a better understanding of the underlying disease mechanisms is needed for the successful clinical translation of regenerative medicine. Currently, most of the regenerative medicine research is based on genetically engineered animal models that do not offer a precise model of the complex traits in the human disease^{80,81}. Subsequently, researchers have also been working to utilize human cells; however, the hurdles in specific cell isolation from whole tissues cannot be overlooked. Most recently, technological advances have enabled researchers to generate human models of different diseases by developing and expanding different types of differentiated cells from both human induced pluripotent stem cells (iPSC) and human embryonic stem cells (hESC) from patients with the specific disease⁸². However, the biggest challenge still lies in the perfect isolation and expansion of certain rare cell populations in order to completely differentiate them, and the high variability in the cell lines.

Furthermore, when particularly studying cardiovascular regenerative medicine, the heterogeneity of the cardiac progenitor cells that convert to cardiomyocytes in the heart, as opposed to their conversion to arterial and venous smooth muscles, endothelial cells, atrial and ventricular cardiomyocytes, and cells of the conduction system *in vivo*, presents a critical challenge that is extremely difficult to overcome^{83,84}. Therefore, for an optimal model development for studying cardiac regenerative medicine, it is extremely important to understand these progenitor cells and their corresponding pathways that dictate their fate in the heart.

Additionally, overcoming the immunological barriers in regenerative medicine limits its translation from bench to bedside. The immunological responses are usually minimal when the cells are of autologous origin; however, any allogenic or genetically modified cells generate host immune responses, further inducing rejection⁸⁵. Some possible strategies to overcome this hurdle include: administering immunosuppressant drugs or monoclonal antibodies that prevent or treat allograft rejection, adoptive transfer of APCs, NKT cells, T cells, or mesenchymal stromal cells to induce tolerance, hematopoietic stem cell transplantation to induce tolerance, and developing biomaterials that restrict contact between donor and host immune systems, such as nanomedicine⁸⁵.

Most importantly, even after careful consideration and design of pre-clinical studies with RNA nanotherapies, several factors affect their clinical translation. First, species variability highly affects the pharmacokinetics and pharmacodynamics of RNA therapies due to differences in serum lipids, basal metabolic rates, and organ/body ratios^{25,86,87}. These variations have led to difficulties in correlating small animal biodistribution, cellular interactions, and toxicities to those in human studies⁸⁸. To solve this problem, researchers are studying the use of species-agnostic nanoparticle delivery screening (SANDS) that enables determining a species-dependent correlation factor between the delivery of RNA therapies in murine, human cells *in vivo*, and nonhuman primates⁸⁸.

Another crucial factor determining the translational gap between preclinical and clinical studies is the unavailability of well-characterized models that can reproduce preclinical results during clinical research. Although several diseases can be reproduced in small animals, there are likely to be certain differences that result in failure of clinical trials despite promising preclinical data. This is due to several factors, including diverse anatomy, pathophysiology, disease phenotype, and transcriptomic profiles in human versus research animals. Nevertheless, several diseases are still in their preliminary research stages, which contributes to an insufficient understanding of the disease. There has been an emerging advancement in 3D organ reconstitution by re-cellularizing scaffolds from animal or non-transplantable human organs for studying various diseases. To date, the greatest progress has been achieved in developing epithelial models of the lungs and skin^{89,90}. Subsequently, researchers working in the field of dermatology have also experienced a lack of ideal animal models due to the diversity in the skin morphology and disease pathology in humans versus small animals. Studies have indicated that there is 70% genetic variation between the skin of mice and humans. Consequently, researchers have revolutionized preclinical testing by developing microfluidic organ-on-a-chip tissue cultures that act as 3D organ models to evaluate topical RNA nanomedicine in skin regeneration^{89,91-93}. These *in situ* and *ex vivo* gene editing systems using transfected skin and transgenic skin sheets mimic the human setting of the skin diseases, as opposed to animal skin, further offering an advantage since it is possible to combine different cells and extracellular matrices to study a combined pharmacological response. This discussion implies that there is an urgent need for further studies utilizing interdisciplinary approaches for a successful RNA-based nanotherapy to address the issue of immune activation.

Conclusion

Call to action: short-term and long-term goals

Currently, organ transplantation remains the standard of care for most end-stage organ diseases, and this mostly varies patient to patient depending on various factors, including disease state, presence of other diseases, disease heterogeneity, and the ability to metabolize drugs. Unfortunately, certain patients are also ineligible for organ transplantation, and therefore, a dire need for alternate therapeutic options arises. Over the past decades, regenerative medicine has offered novel insights into end-stage organ disease management and could emerge as an excellent therapeutic option for several diseases that are currently unmanageable. Also, owing to the challenges in the development of safe and efficacious regenerative medicine, RNA nanotechnologies present with exceptional features that could accelerate the development of safe, effective, and targeted regenerative therapies

in patients with chronic and end-organ disease. Furthermore, combination therapies of RNA therapeutics with cell therapy or tissue engineering approaches have also shown exceptional progress in the field of cardiac regeneration through pre-treatment of hiPSC-derived cardiomyocytes with modified RNAs encoding for salutary factors that can improve engraftment and/or functional outcomes in preclinical models. This combined approach shows tremendous hope in the advancement of regenerative medicine. Through this paper, we envision the unprecedented promise of RNA nanotechnologies for the development of novel regenerative medicine.

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A.B. and A.L. participated in the literature search, writing and revising the manuscript; R.V., M.L., E.J., H.H., L.R., S.H., G.S., A.B., M.S., B.L., and G.Z. participated in the workshop as speakers and discussants. They also participated in reviewing, providing critical feedback, and editing the manuscript. M.B. and G.Z. led the workshop and reviewed the manuscript at each stage. All authors have read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

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