Healthspan Extension through Innovative Genetic Medicines

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Summary: Genetic medicine has emerged as an innovative class of therapeutics, allowing the development of new and powerful approaches to address a myriad of diseases that were previously untreatable. At the same time, our improved understanding of the mechanisms underlying aging has created novel opportunities to intervene therapeutically in the aging process itself through the targeting of key pathways driving this process. As individuals age, the onset of a multitude of age-related diseases can significantly impact lifespan. The ultimate goal of their treatment is the maximization of healthy, disease-free years, or healthspan. Here, we discuss a number of promising genetic medicine approaches to target both general and specific mechanisms of age-related disease, and their potential impact on healthspan extension. Essential to this topic is the challenge of nucleic acid delivery, and we discuss the technologies that have been developed to address this challenge in highly promising preclinical and clinical development efforts. In particular, we describe a next-generation delivery technology for healthspan applications called proteo-lipid vehicles. (Plast. Reconstr. Surg. 150: 49S, 2022.)

From a regulatory perspective, genetic medicine covers a broad area, comprising the manipulation of both genetic code and gene expression via the exogenous introduction of nucleic acids into a biological system. The first gene therapy clinical trial occurred in 1989, and it took 14 years for one to be approved. However, in the time since, several cell and gene therapies have been approved for clinical use, and at a much faster pace. The first gene therapy approved in the United States entailed the ex vivo manipulation of patient cells, but recent improvements to delivery methods have enabled the use of genetic therapies that are administered in vivo, directly to patients. Notable examples are the latest genetic vaccines designed to immunize against COVID-19. These have set new benchmarks for speed and efficacy with respect to the design and deployment of commercial genetic medicines. These recent successes have ushered in a new era of treatments that can sense and modify genetic pathways with a precision never before possible using small molecules and biologics, which rely on chemistry for their targeting and mechanism of action. The question has now become how to best utilize these new tools, especially for something as complex and multifactorial as healthspan.

Healthspan is commonly defined as the period of time in which an organism is relatively healthy and free from disease. Although this is not a strictly defined phenotype, maximizing healthspan remains a general goal to achieve for the longevity field. From practical and regulatory standpoints, single age-related disease indications and quantifiable metrics are the most practical ways to assess the efficacy of interventions, with potential consequences for healthspan. An effective therapy targets the core aspects of aging that contribute to age-related disease to varying degrees. For example, senolytics, a class of therapies that selectively eliminate senescent cells, has the potential to be beneficial in multiple age-related diseases. In this mini-review, we will expand upon this class...
of therapies, provide an overview of the genetic medicine payloads that are being developed for healthspan extension, and discuss a major challenge of genetic medicines: payload delivery.

**SENOLYTICS**

Senescence is a cell fate characterized by stable cell cycle arrest and secretion of proinflammatory cytokines and molecules known as the senescence-associated secretory phenotype (SASP). The SASP is comprised of cytokines, chemokines, proteases, and growth factors, which impact surrounding healthy cells and alter their function. Seneesnt cells accumulate over time and contribute to a decline in organ function, implicating them in most age-related diseases. The seminal proof-of-concept that showed the removal of senescent cells in vivo can rejuvenate an organism was first shown in 2011. Since then, a rapid expansion of research in senolytic therapies has led to a number of clinical trials. Many small molecule and biologic approaches have been used academically and clinically with varying degrees of success. Despite their broad effect, senescent cells only constitute a small percentage of cells in the body. Senescent cells themselves are also heterogeneous with features that closely resemble surrounding cells. These challenges limit the utility of small molecule drugs due to their indiscriminate nature and resultant systemic toxicity, since many are based on drug repurposing. This is especially true in sensitive tissues like skin, where senescent cells contribute to health and resultant appearance. Senescent cells accumulate over time and contribute to a decline in organ function, implicating them in most age-related diseases. The seminal proof-of-concept that showed the removal of senescent cells in vivo can rejuvenate an organism was first shown in 2011. Since then, a rapid expansion of research in senolytic therapies has led to a number of clinical trials. Many small molecule and biologic approaches have been used academically and clinically with varying degrees of success. Despite their broad effect, senescent cells only constitute a small percentage of cells in the body. Senescent cells themselves are also heterogeneous with features that closely resemble surrounding cells. These challenges limit the utility of small molecule drugs due to their indiscriminate nature and resultant systemic toxicity, since many are based on drug repurposing. This is especially true in sensitive tissues like skin, where senescent cells contribute to health and resultant appearance. 

**DURABLE GENE REPLACEMENT THERAPIES FOR IMPROVING HEALTHSPAN**

Age-related diseases are typically chronic, and it is reasonable to assume that their treatment will require prolonged or repeat dosing. Although the half-lives of small molecules and many biologics are relatively short, gene therapies are unique in that they can be engineered to persist indefinitely. The first approved gene therapy in the United States, Kymriah, introduced a gene for a chimeric antigen receptor on a patients T cells (CAR-T) ex vivo before reinfluencing them to treat their acute lymphoblastic leukemia. Since then, CAR-T research has expanded to multiple hematological malignancies, solid tumors, and more recently to target senescent cells based on a surface cell marker. CAR-T cells are designed to persist indefinitely within the patient to exert their protective effects, so great caution must be taken with a persistent senescent cell clearance therapy. Treatment of monogenic diseases via gene replacement therapies is an excellent example of the durable effect afforded by genetic medicines. In addition to the approved gene replacement therapies, efforts are being made to understand the genes that decrease with either age or age-related diseases, and several have been identified that demonstrate protective antiaging effects and healthspan benefits. For example, a gene therapy is being developed to increase the expression of the DNA-repair enzyme, SIRT6. There are multiple groups developing therapies to extend telomeres via the expression of telomerase. A triple gene therapy approach to treat mitral valve disease is being developed for canines, with an aim to eventually progress to humans. A therapy based on work from the University of South
Alabama is introducing a gene in macrophages ex vivo that can metabolize oxidized cholesterol. Efforts to treat epidermolysis bullosa have had dramatic results from two groups using either transgenic ex vivo keratinocytes or intradermal injection of collagen VII. Using their genetic medicine platform, the authors are developing multiple gene replacement therapies to treat sarcopenia, mitochondrial diseases, collagen defects, and skin disease. Attempting to recapitulate the effects of gene replacement therapies via recombinant protein therapy is prohibitively expensive and often precluded by delivery challenges and complicated manufacturing.

The discovery of the CRISPR/Cas9 gene editing system to fix single genetic mutations rather than replace the gene outright has dramatically broadened the potential applications of genetic medicines. There are numerous age-related diseases driven by point mutations that could be treated via CRISPR/Cas9-based gene therapies, such as ALS, Alzheimer disease, Parkinson disease, and rheumatoid arthritis, and more as CRISPR-based research progresses. However, although these tools hold great promise to correct genetic diseases, in vivo delivery of these tools to the cells of interest remains a significant challenge.

**GENETIC APPROACHES CAN BE TUNED FOR SPATIOTEMPORAL SPECIFICITY**

Given the highly regulated spatial and temporal nature of gene expression, genetic medicines must be designed to express exogenous genes in the right cell population at the right time. Engineering highly selective DNA promoters and
enhancer/repressor combinations allows for a gene therapy to deliver throughout the body and still achieve precise cell and tissue.

Temporal specificity is also important for gene therapies affecting healthspan. The type of payload can also affect temporal specificity, such as mRNA, which provides rapid and robust yet transient cargo expression. However, in some indications, this approach is desirable. For example, there are multiple efforts to utilize Yamanaka factors to partially reprogram cells into a more youthful state.\(^{46-50}\) With their senolytic approach, the authors are eliminating senescent cells in a similar “hit-and-run” approach that can be delivered body-wide repeatedly at regular intervals, further reducing toxicity and effectively re-creating what has been shown by other studies involving transgenic animals (Fig. 2).\(^{10}\)

THE CHALLENGE OF NUCLEIC ACID DELIVERY

There are some significant hurdles that genetic medicines must overcome to gain more broad applicability. The plasma membrane is a highly effective physical barrier that actively repels or sequesters exogenous macromolecules such as negatively charged nucleic acids. This necessitates the use of a carrier vehicle to facilitate entry into the cytosol to exert the intended therapeutic effect (Fig. 3). Viral vectors have dominated gene therapy efforts due to their high efficiency of gene expression, with adenoviral, retroviral/lentiviral, and adeno-associated viral (AAV) vectors leading the field.\(^{1,51}\) The two subtypes of retroviruses, \(\gamma\)-retroviruses, and lentiviruses contain an RNA genome that undergoes reverse transcription into DNA upon entry into a transduced cell. The new DNA genome is then integrated into the host cell therefore enabling durable gene expression.\(^{52}\) Retroviruses have demonstrated their clinical success via ex vivo gene transfer into hematopoietic stem cells (HSCs)\(^{53,54}\) and T cells.\(^{55,56}\) Genetically altered cells can be reintroduced into patients and reverse deficiencies in HSCs, or target cancer cells. The risk of insertional mutagenesis has hindered the systemic use of retroviruses and further testing and validation will be required before this platform can be adopted to improve health span.

Adenoviral and AAV vectors have predominately been the chosen viral vectors for systemic gene therapy. These vectors have a DNA genome and possesses an ability to transduce quiescent cells, making them an ideal candidate for the treatment of monogenic diseases and potentially aging.\(^{57}\) However, initial studies examining the use of adenoviruses as gene therapy vectors were

![Fig. 2. Oisin Biotechnologies clinical approach. Schematic detailing the mechanism of action of a senolytic PLV infusion.](image-url)
met with resistance after it was reported that serious and life-threatening side effects could occur,\(^5^8\) causing a shift to the use of safer AAV vectors.\(^5^9^,^6^0\) Sustained gene expression from a single dose is often required for therapeutic effect, as adaptive immune responses prevent AAV vectors from being administered repeatedly.\(^6^0^,^6^1\) Additionally, preexisting neutralizing AAV antibodies can exist even without prior vector exposure further hindering gene expression.\(^6^1^–^6^4\) Although immune stimulation be advantageous in the field of vaccines, where robust T-cell responses induced by the gene delivery vector can help facilitate long-lasting immunity to the antigen, this immunogenicity hinders repeat dosing.\(^6^5\)

To overcome limitations surrounding viral vectors, the focus in recent years has largely shifted to the development and optimization of the safer nonviral delivery vectors.\(^5^1\) Nonviral delivery vectors are easier and cheaper to manufacture than viral vectors, allowing for rational design and large-scale testing of novel materials and formulations with nucleic acid delivery potential.\(^5^6^,^6^7\) Of the various nonviral delivery vectors, lipid nanoparticles (LNPs) have demonstrated their success with the FDA approval of patisiran (Onpattro), an LNP-based RNA interference agent that treats amyloidosis caused by abnormal transthyretin production.\(^5^8^,^6^9\) LNPs are formulated with cationic or ionizable lipids that by neutralizing the anionic charge of nucleic acids enable transport of nucleic acids across the charge-restrictive plasma membrane via endocytosis.\(^7^0\) Despite the immunogenicity benefits LNP vectors have over viral vectors, LNPs containing cationic or ionizable lipids can stimulate toxic and inflammatory responses, limiting their usefulness clinically. At a cellular level, LNPs facilitate apoptotic cell death that translates to liver toxicity following systemic delivery.\(^7^1^,^7^2\) Interactions between LNPs and the immune system can have systemic effects and lead to secretion of proinflammatory cytokines like tumor necrosis factor alpha (TNF-\(\alpha\)), interferon-gamma (IFN-\(\gamma\)), and interleukin-6 (IL-6).\(^7^3^,^7^4\) Furthermore, LNPs can stimulate complement activation-related pseudoallergy (CARPA), a hypersensitivity reaction resulting in death in severe circumstances.\(^7^5^–^7^7\)

The authors have taken an approach that combines the strengths and mitigates the weaknesses
of viral and nonviral delivery vectors by developing proteo-lipid vehicles (PLVs), a platform that incorporates a fusion-associated small transmembrane (FAST) protein into a lipid-based formulation.\textsuperscript{78–101} FAST proteins are the smallest viral fusogens that have been described (≈100–200 residues), with a single transmembrane domain fixing the FAST protein in a \textsuperscript{exo}plasmic/\textsuperscript{cyt}oplasmic type I membrane topology that exposes an even smaller ectodomain (≈20–40 residues).\textsuperscript{86} FAST proteins function as fusion machines, where incorporation into a lipid-based nanoparticle is sufficient to facilitate nanoparticle-cell fusion and delivery of encapsulated cargo directly into the cytosol of target cells.\textsuperscript{87}

This enables FAST protein-containing PLVs to be formulated with reduced ionizable and cationic lipids, substantially improving safety while retaining efficacy, which allows for genetic medicines that require high systemic doses. Furthermore, FAST proteins are nonimmunogenic enabling repeat dosing. Notably unlike other LNP platforms, FAST-PLVs have demonstrated the ability to successfully deliver plasmid DNA systemically after intravenous administration, similar to viral vectors.\textsuperscript{85}

**DISCUSSION**

Genetic medicines are rapidly becoming a distinct and revolutionary class of therapeutics. The durability, specificity, and ease of manufacturing allows for a plethora of creative and effective strategies. The COVID-19 pandemic has shown the world that a gene therapy can be rapidly developed and deployed globally. This has already begun to catalyze the interest and capital that will be leveraged toward gene therapies going forward. Much like LNPs, PLVs are relatively inexpensive to manufacture at scale compared to viral-based delivery methods, which enables the development of cost-effective therapeutics that can be more broadly applied.

Although there are still considerable efforts involved in designing genetic medicines, the ability to progress from target to preclinical testing in a matter of months is typically shorter than the development that many small molecules go through before preclinical in vivo testing. As a result, researchers developing genetic medicines can take a more iterative approach and de-risk their clinical efforts.

Next-generation gene delivery platforms such as PLVs enables the development of more sophisticated genetic medicine approaches utilizing DNA or self-amplifying RNA. This allows for innovative gene therapies that can be exquisitely regulated. For example, a genetic program that expresses a gene of interest X exclusively in keratinocytes but is repressed when that keratinocyte expresses another gene Y is a kind of logic gate that is possible utilizing DNA. There is enormous potential in the gene therapy space, which can synergize with and supplement surgical efforts. Platform technologies like PLV are also likely essential for the treatment of chronic diseases of aging as individuals will require systemic treatments over the course of many years. We are focusing our initial efforts on the development of a first-in-class senolytic gene therapy while engineering innovative solutions to ameliorate age-related diseases and promote healthspan extension using multiple complementary approaches. The future of gene therapy in the context of healthspan could be seen as an accumulation of genetic programs that prevent or correct the normal trajectory of age-related disease and could be taken acutely and/or repeatedly as the demand arises.

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