SPECIAL REPORT

Gene Therapy: The View from NCATS

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INTRODUCTION

The National Center for Advancing Translational Sciences (NCATS) at the National Institutes of Health (NIH) was established in December 2011. NCATS mission is to catalyze the generation of innovative methods and technologies that will enhance the development, testing, and implementation of interventions that improve human health across a wide range of human diseases and conditions. Notably, the "innovative methods and technologies" that are NCATS focus include gene therapy, and the "wide range of human diseases" includes rare diseases, which are a special area of focus for NCATS, and for which gene therapy may have a particularly large impact.

In contrast to other institutes and centers at NIH, NCATS does not focus on individual diseases or organ systems, but on what is common among them, and on the common roadblocks and limitations in the translational science process. NCATS defines translation as the process of turning observations in the laboratory, clinic, and community into interventions that improve the health of individuals and the public—from diagnostics and therapeutics to medical procedures and behavioral changes. We define "translational science" as the field of investigation that seeks to understand the scientific and operational principles underlying each step of the translational process. We study translation on a system-wide level, focusing on creative solutions to both scientific and operational problems.

A hallmark of NCATS approach to translation is collaboration and team science. The enormous range of disciplines—from target qualification through intervention development to demonstration of clinical efficacy to implementation in the community—means that successful translation requires multidisciplinary teams including individuals from diverse scientific backgrounds, as well as patients and patient advocates, working together. Nowhere is the importance of collaboration and team science seen more clearly than in rare diseases. As we will highlight below, patients and patient advocacy groups (PAGs) are an essential part of collaborative teams, and in many cases are driving rare disease intervention development, including gene therapy.

One of the biggest hurdles facing therapeutics development in rare diseases is the sheer number of individual conditions, commonly estimated at around 6000. Well over 90% of these rare conditions have no FDA-approved treatment, and at the current rate of new drug development, hundreds of years will pass before all rare diseases are treatable with a therapy shown to be safe and effective. To address this major challenge, NCATS is particularly interested in "platform"-type approaches that can be readily adapted to multiple diseases. One example is high-throughput screening of all clinically approved drugs[1] to find those that can be "repurposed" expeditiously to treat other diseases. Another strategy is to take advantage of expanding knowledge of disease biology, and focus drug development on shared underlying molecular etiologies rather than clinical phenotype.[2] Gene therapy approaches, including viral vector-mediated *ex vivo* or *in vivo* gene transfer, genome editing, and other nucleic acid therapeutics, are inherent platforms, and of obvious relevance for the treatment of the more than 4000 known rare monogenic disorders.

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In this editorial, we first consider NCATS support for gene therapy across its different programs, including NCATS new initiatives to establish gene therapy-based platforms for rare diseases. We then highlight some areas and aspects of gene therapy we see as particularly promising and how they relate to NCATS mission and priorities.

NCATS SUPPORT OF NUCLEIC ACID THERAPEUTICS

NCATS supports gene therapy development through several programs, some of which are briefly outlined here; more information can be found at https://ncats.nih.gov/programs

The CTSA Program

The Clinical and Translational Science Awards (CTSA) Program, within the NCATS Division of Clinical Innovation, is a national network of over 60 medical research institutions in the United States that are working together to improve translational science. At these institutions, referred to as CTSA hubs, as well as partner institutions affiliated with each hub, NCATS supports gene therapy trials indirectly through the funding of CTSA hub resources. NCATS can directly support translational gene therapy research via CTSA pilot projects, K and T awards.

In addition to the hub awards, NCATS has developed several new funding opportunities for the CTSA program. The CTSA Collaborative Innovation Awards (PAR-15-172 and PAR-15-173) are intended to support teams of investigators from at least three different CTSA hubs to collaborate on projects to overcome important roadblocks in translational science. Projects related to gene therapy could potentially be supported by this mechanism. More broadly, the implementation of other CTSA initiatives designed to accelerate and optimize clinical trials in the United States (RFA-TR-15-004 and RFA-TR-002) could also benefit gene therapy trials. For additional information, see http://ncats.nih.gov/ctsa/funding

Office of Rare Diseases Research

Office of Rare Diseases Research (ORDR) guides and coordinates NIH-wide activities involving research for a broad spectrum of rare diseases. These activities include scientific meetings related to gene therapy for rare diseases.[3] Of the various programs within ORDR, the one that is most relevant to nucleic acid therapeutics is the Rare Diseases Clinical Research Network (RDCRN). RDCRN is a network of 22 consortia, each focused on a group of 3 or more rare diseases. A unique aspect of RDCRN is the requirement for active participation with PAGs. Collectively, there are more than 200 rare diseases under investigation within RDCRN (https://ncats.nih.gov/rdcrn/consortia). At present, gene therapy projects are ongoing in two consortia: urea cycle disorders and primary immune deficiency treatment. In addition, the RDCRN program requires longitudinal natural history studies, which can identify outcome measures that are an essential requirement for clinical trials, including gene therapy. Like all NCATS programs, RDCRN is catalytic and collaborative, and we would encourage investigators developing gene therapy for rare diseases under study by RDCRN to consider collaborative opportunities.

The Therapeutics for Rare and Neglected Diseases Program

The NCATS Therapeutics for Rare and Neglected Diseases (TRND) program (http://ncats.nih.gov/trnd) collaboratively develops treatments for rare and neglected diseases with an emphasis on developing technology platforms and operational models that aim to increase the efficiency and effectiveness of translating candidate molecules into safe and efficacious human therapies, across a multitude of diseases. TRND develops a full range of therapeutic modalities, including small-molecule, biologics, and nucleic acid- or cell-based therapies from lead optimization to first-in-human studies, with the aim of derisking therapeutics development projects to the point of licensing to biopharmaceutical or other organizations for completion of clinical development. TRND operates via a unique operational model whereby chosen partners form a joint project team with TRND scientists; support is provided via in-kind drug development both internally at TRND and via contract resources. For example, TRND supported the development of an RNA-based antisense oligonucleotide therapeutic for exon skipping in Duchenne muscular dystrophy (https://ncats.nih.gov/trnd/projects/complete/avi-4038-duchenne-muscular-dystrophy). Currently, TRND program accepts gene therapy projects for collaboration.

The Bridging Interventional Development Gaps Program

The Bridging Interventional Development Gaps (BrIDGs) program assists researchers in advancing promising therapeutic agents in any indication to achieve successful investigational new drug (IND) applications. Through the BrIDGs program, NCATS makes its scientific drug development expertise and government contract resources available to eligible and competitive extramural research projects through collaboration. In the past, the BrIDGs

program has supported multiple nucleic acid therapeutics projects, including successful production of clinical-grade adeno-associated virus (AAV) vectors carrying gene products for treating Parkinson's disease, aromatic acid decarboxylase deficiency, and osteoarthritis. BrIDGs program also completed a successful manufacturing campaign of an antisense oligonucleotide drug candidate for testing in Alzheimer's disease (https://ncats.nih.gov/bridgs/projects).

Small business opportunities

Like all other institutes and centers, NCATS participates in the NIH Omnibus Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) programs (http://ncats.nih.gov/smallbusiness). In addition, we have a specific program announcement entitled "Platform Delivery Technologies for Nucleic Acid Therapeutics" (PA-14-307 and PA-14-308). This funding opportunity was motivated by the knowledge that, for many disease targets, the limiting factor is the ability to deliver nucleic acid therapeutics to specific cells and tissues.

Extracellular RNA communication

An emerging area of interest to NCATS is the role of extracellular RNA in communication between cells and tissues. NCATS participates in the NIH Common Fund program on extracellular RNA communication (https://commonfund.nih.gov/Exrna/index)[4] and directs the components of this program focused on the clinical utility of extracellular RNAs as therapeutics (https://ncats.nih.gov/exrna). The recent publications[5–7] from that effort highlight the therapeutic potential of novel RNA delivery vehicles.

NCATS VIEWPOINT

In this section, we highlight some recent developments in gene therapy that are particularly relevant to NCATS mission and priorities.

Major advances in viral vectors for the treatment of rare diseases

Emergence of AAV as a clinical gene therapy platform. AAV offers significant advantages over adenovirus and retroviruses as a platform for therapeutic gene transfer, particularly with regard to safety considerations.[8] Within the past several years, evidence of clinical efficacy for AAV vectors in the treatment of monogenic disorders has emerged. Notable examples include the use of AAV for the treatment of hemophilia resulting from mutations in factor IX,[9] and treatment of a retinal disorder, Leber's congenital amaurosis.[10,11] A very recent report of encouraging results from a pivotal phase 3 trial of LCA (www.fiercebiotech.com/press-releases/spark-therapeutics-announces-positive-topline-results-pivotal-phase-3-tria) notes plans to file a Biologics License Application (BLA) in 2016, raising the possibility of the first approval of a viral vector-based gene therapy in the United States. Notably, an AAV vector expressing lipoprotein lipase, Glybera (alipogene tiparvovec), was approved by the European Medicines Agency (EMA) in 2012 for the treatment of lipoprotein lipase deficiency, a rare monogenic disease.[12]

Many rare genetic diseases affect the nervous system, and the blood–brain barrier presents a significant obstacle for gene delivery. The discovery that a specific serotype of AAV, AAV-9, could cross the blood–brain barrier[13] represented a major breakthrough in the field. Compelling evidence of efficacy in a mouse model of spinal muscular atrophy (SMA)[14] led to a clinical trial (https://clinicaltrials.gov/ct2/show/NCT02122952) in which an AAV-9 vector containing the survival of motor neuron (*SMN*) gene was given intravenously to SMA type 1 patients, with the goal of transducing motor neurons in the spinal cord. Although it is too early to assess clinical benefit, publicly available information on the safety profile of the protocol is notable,[15] given the relatively large doses of viral vector administered to young children, much of which would be expected to impact the liver.

Another trial focuses on giant axonal neuropathy (GAN), a progressive neurodegenerative disease affecting spinal motor neurons (www.clinicaltrials.gov/ct2/show/NCT02362438?term=GAN&rank=1). This trial utilized intrathecal injections of AAV-9 containing the gigaxonin *GAN* gene, based on preclinical studies demonstrating transduction of spinal motor neurons (as well as many other cell types) in nonhuman primates.[16] According to publicly available information, one patient has been injected with the vector (http://news.unchealthcare.org/news/2015/june/gan-treatment).

We note the important role of PAGs in these two trials, in providing funding and driving the clinical programs forward, including participation at meetings of the Recombinant DNA Advisory Committee. Although these programs are being supported by patient groups focused on individual diseases, both efforts represent potential platforms that could be adapted for the treatment of other similar diseases by changing the transgene. These patient groups are following in the footsteps of other rare disease patient advocacy organizations, in efforts that began decades ago.[17]

We also note that the patient support group that funded the GAN gene therapy trial is also

supporting a small-molecule drug discovery effort with the NCATS Division of Preclinical Innovation (https://ncats.nih.gov/pubs/features/adst-fellows). Nucleic acid-based and small-molecule therapeutics are not mutually exclusive, and could well be complementary. Because the ultimate clinical impact of either cannot be predicted in advance, pursuing multiple therapeutic strategies where possible is prudent.

Retroviruses for *ex vivo* gene therapy. One striking example of successful *ex vivo* gene therapy was in the adenosine deaminase (ADA)-deficient severe combined immunodeficiency (ADA-SCID), also known as "bubble boy disease." In this approach, hematopoietic stem cells (HSCs) were isolated from patients and transduced with a retrovirus encoding ADA, and then infused back into the patients following a bone marrow conditioning regimen to improve engraftment.[18,19] The conditioning step is important to achieve a therapeutic benefit.[20] Based upon the success of this approach, an application for approval has been submitted to EMA, in a partnership between a large pharmaceutical company and private research foundation (www.pmlive.com/pharma_news/gsk_files_immune_deficiency_gene_therapy_in_europe_730965).

However, use of γ-retroviruses in gene therapy for other diseases has been plagued by serious adverse events, including leukemia, resulting from oncogene activation secondary to insertional mutagenesis (see ref.[21]). Subsequent work employed lentiviral vectors for gene transduction, which are less prone to oncogenic activation. Two studies of clinical efficacy using this approach were published in 2013, for Wiskott–Aldrich syndrome[22] (a primary immunodeficiency), and metachromatic leukodystrophy (MLD), a lysosomal storage disorder affecting the brain.[23] For MLD, the transduced hematopoietic cells can enter the brain, where they are believed to differentiate into microglial cells that secrete active enzyme that is taken up by other cells by cross correction.[24]

Although the results in MLD are impressive,[23] the complexity of the protocol may limit the platform potential for this approach compared with AAV-based strategies for neurologic diseases. Recently, Katz et al.[25] demonstrated therapeutic benefit of an AAV vector delivered to brain ventricular cells in an animal model of Batten disease. Batten disease and MLD are two examples of a group of "cross-correctable" neurodegenerative disorders, which could benefit from a platform delivery approach.

Other nucleic acid therapeutics

Oligonucleotides. In addition to viral vector-based therapies, other nucleic acid therapeutics are advancing rapidly (see ref.[26] for a very recent review). One antisense oligonucleotide drug, Kynamro (mipomersen sodium), has been approved by the FDA for the treatment of familial hypercholesterolemia (www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm372598.htm). Further advances in this area are being driven by improvements in nucleic acid chemistry, as well as better delivery methods. Conjugation of siRNAs with N-acetyl-galactosamine[27] is a particularly promising approach for targeting hepatocytes, and is now in clinical trials (www.clinicaltrials.gov/ct2/show/NCT02035605). Similar strategies to target other tissues and cell types would be very valuable. As many rare diseases affect the CNS, finding ways of getting nucleic acids across the blood–brain barrier without using viral vectors is an important goal. One strategy is direct injection into the intrathecal space, which is being utilized in an ongoing phase 3 trial of antisense oligonucleotides in SMA patients (https://clinicaltrials.gov/ct2/show/NCT02193074?term=isis+smnrx&rank=6). Conceivably, pumps or ports could be utilized to allow continuous infusion into the CSF, avoiding the need for repeated intrathecal injections.

Another potential platform therapeutic approach for genetic disease is the use of oligonucleotides to alter RNA splicing. Much of the work using the exon-skipping approach has focused on Duchenne muscular dystrophy, as a result of the scientific and clinical observations that various truncated forms of the dystrophin protein can partially restore its biological function. Although a recent FDA evaluation of a phase 3 clinical trial of an exon-skipping oligonucleotide was negative (www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/PeripheralandCentralNervousSystemDrugsAdvisoryCommittee/ucm475950.htmtrial), a final decision on approval has not been made. Results from a phase 3 trial of another exon-skipping oligonucleotide, made with a different backbone chemistry, are expected in early 2016 (www.clinicaltrials.gov/ct2/show/NCT02255552?term=NCT02255552&rank=1).

Messenger RNA. For molecular biologists who learned early on about the fragility of RNA, the concept of using messenger RNA as a drug[28] is surprising. As with synthetic oligonucleotides, progress in this area has depended upon chemistry, including novel chemically modified ribonucleotides that can be incorporated into RNA during

transcription. These modified bases both increase the stability of messenger RNA, and also avoid TLR-mediated activation of the innate immune system,[29] which is a major potential source of toxicity. When used to deliver messenger RNA encoding a protein that is absent in a genetic disorder, an mRNA would have to be administered repeatedly. However, mRNAs could be used to deliver nucleases that would carry out gene editing (see below). This approach could allow somatic gene editing and permanent modification of the genome without viral vectors. Moreover, the transient nature of mRNA delivery would be a benefit, because continued expression of the nuclease could result in additional genetic damage. As with other nucleic acid therapeutics, the limiting factor appears to be effective delivery methods.

CELiD: beyond plasmid DNA. Viral vectors are derived from viruses that have evolved as highly effective gene delivery vehicles. However, even with AAV, immune responses against capsid proteins can occur.[30] Therefore, the development of nonviral delivery methods for nucleic acids remains of interest. An emerging issue here is the ability of the nucleic acid to generate an immune response. The vast majority of early studies of nonviral delivery agents used reporter genes carried on circular plasmid DNAs produced in bacteria. Although convenient to use and produce, plasmid DNAs contain base modifications that are unique to prokaryotes, and have a higher representation of unmethylated CpG dinucleotides that can generate an innate immune response.[31] Minicircle DNA[32] is devoid of most plasmid DNA backbone sequences, but is still produced in bacteria. In contrast, the recently described[33] closed-ended, linear duplex (CELiD) DNA is generated as an intermediate in AAV replication in eukaryotic cells, and thus does not contain any prokaryotic DNA. After hydrodynamic injection into mouse liver, CELiD DNA was shown to have longer persistence than plasmid DNA, as well as a longer duration of transgene expression.[33] Importantly, the use of CELiD DNA avoids the transgene size limitation imposed by the requirement for packaging into the AAV viral capsid, which is a significant limitation for AAV-mediated viral vectors. Thus, when combined with suitable delivery vehicles, the use of CELiD has significant potential advantages as a gene therapy platform.

Tissue chips for toxicity testing?

A bottleneck for all nucleic acid therapeutics is toxicity testing, which is a key component of the regulatory approval process. A recent publication[34] outlined many of the important issues in oligonucleotide toxicity testing, including the limitations of the current animal-based assays. In this context, the NCATS-DARPA-FDA Tissue Chip program (https://ncats.nih.gov/tissuechip), which is working to develop three-dimensional organoids that represent the structure, function, and drug responsiveness of human organs, may offer an attractive option. In addition to reducing animal usage, toxicity testing in these human organs-on-chips should be much faster and less expensive than animal testing, and the modular nature of the system could be adapted for relatively high-throughput testing. Most importantly, such a system holds the potential to allow assessment of the human innate immune response to oligonucleotide drugs in different target cell types.

Gene editing nucleases. The clinical findings described above are certainly encouraging, and technological advances continue to drive the field forward. In particular, the use of "gene editing," technologies, including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases, and most recently clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9, is a rapidly developing therapeutic strategy (see refs.[35–37] for review). These tools allow the possibility of inactivating target genes, or inserting therapeutic genes into the genome, without integrating viral vectors. Even though potential off-target effects of engineered nucleases are a concern,[38] retroviral vectors not only carry the risk of insertional mutagenesis, but also contain viral transcriptional enhancers that have been linked to oncogenesis.[21]

Tebas et al.[39] used ZFNs to edit the *CCR5* gene (encoding an HIV co-receptor) in autologous T-cells, which were then infused into patients with HIV. Within the limits of this small study, the authors found the procedure to be safe. For *ex vivo* gene therapy in rare diseases, a recent proof-of-principle study demonstrated efficient gene editing using ZFNs in human HSCs, which were then engrafted into mice.[40]

Importantly, nuclease-based approaches are not limited to *ex vivo* applications. Yin et al.[41] used Crispr-Cas9 to correct a point mutation in the *Fah* gene in a mouse model of hereditary tyrosinemia, and also correct a clinical phenotype (body weight). This study used hydrodynamic injection of plasmids expressing Cas9 and guide RNAs, as well as a single-stranded DNA donor, to target the liver. Though useful for proof-of-principle studies in animals,

hydrodynamic injection is a relatively inefficient method that is not suitable for clinical use. However, a smaller Cas9 enzyme from *Staphylococcus aureus* that can fit into an AAV vector enables a more efficient and clinically applicable delivery method.[42] Three very recent articles demonstrated the feasibility of this approach in animal models of Duchenne muscular dystrophy.[43–45]

Sharma et al.[46] used AAV vectors to deliver ZFNs targeting the albumin locus for site-specific integration of human factors VIII and IX to the liver of knockout mice. This strategy resulted in clinically relevant clotting factor levels and improved blood clotting in the mouse models. To further emphasize the platform aspect for rare diseases, they also demonstrated site-specific integration of four other genes that are defective in different lysosomal storage disorders. Notably, clinical trials using this approach for the treatment of hemophilia and a lysosomal storage disorder were presented to the Recombinant DNA Advisory Committee in 2015 (http://videocast.nih.gov/summary.asp?Live=16926&bhcp&bhcp=1, http://videocast.nih.gov/summary.asp?Live=17731&bhcp=1).

One potential limiting factor for all nuclease-based gene editing strategies relates to double-strand-break processing. There are two alternative pathways for repairing double-strand breaks in DNA: nonhomologous end-joining, which often generates insertion or deletion mutations, and homologous recombination (HR), which is considered to be error-free. HR requires a donor DNA that contains the correct sequences. In nondividing cells, HR is generally down regulated, which means that the error-prone NHEJ pathway will be favored, which can be problematic for some therapeutic applications. However, a very recent publication[47] demonstrated that the suppression of HR in nondividing cells is reversible, and provided several insights into the control mechanism. Further studies along this line may ultimately facilitate HR in quiescent cells, further extending the therapeutic utility of *in vivo* nuclease-based gene editing.

CONCLUSIONS

NCATS mission is to catalyze the development, demonstration, and dissemination of methods and technologies that will get more treatments to more patients more quickly. Gene therapy, in all its myriad and expanding manifestations, represents just such a transformational translational technology, and NCATS looks forward to helping drive realization of the field's enormous potential for science and health in the years to come.

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