Biomere

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CELL & GENE THERAPIES FOR RARE DISEASES

INTRODUCTION

NOT SO RARE - 1:10 AMERICANS HAVE A RARE DISEASE[1]

A rare disease typically impacts a small number of patients, so the patient population can range from less than 10 to 200,000. More than 7,000 rare diseases have been identified so far and it is possible that the number will continue to grow with the development of new diagnostic testing. Collectively, rare diseases affect 350 million worldwide and 30 million Americans[1] resulting in an enormous impact on public health. Many patients suffer reduced quality of life and require symptomatic treatment throughout their lives. Sadly, many rare diseases are fatal and, in some cases, fatalities can happen in infancy.

Less than 10% of rare diseases have approved therapies available even though therapies for rare diseases are being approved at an expedited rate in the US and Europe. Several decades ago, the pace of drug discovery for rare diseases lagged far behind that for common diseases. However, this situation has changed due to the Orphan Drug Act (ODA) of 1983, the efforts of the National Organization for Rare Diseases and other advocacy groups, and significant expansion of government funding initiatives in rare disease research. Since the ODA, the FDA has approved over 770 drugs primarily in the oncology (34%) and metabolism & endocrinology (15%) areas with 34 novel drugs approved in 2018 for rare diseases. The R&D pace continues to grow with over 560 assets in development for rare diseases[2].

Most rare diseases are due to changes in single genes (monogenic), and the rapid evolution of sequencing technologies has uncovered disease drivers in various diseases. Additionally, the ability to create improved preclinical models for specific rare diseases using gene editing, phenotypic assays, patient derived cells etc. has accelerated the development of targeted therapies. The toolbox of therapeutic modalities has dramatically expanded thanks to continued advancements in protein-based therapies including monoclonal antibodies, peptides and proteins, RNA therapies including antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), splicing modulators and DNA therapies including viral vectors and gene editing. Cell-based therapies are in earlier stages of development and face a major challenge in that the mechanism of action of cell-based therapies need to be completely characterized to ensure consistency, efficacy and patient safety.

The availability of sufficient patient populations is one of the key challenges to conducting clinical trials. However, patients and their families are an active community and are motivated to participate in clinical trials. The community has partnered with or formed disease foundations that partner with government agencies to build international registries and clinical data repositories. Additionally, there is an increased trend to develop innovative clinical trial designs and strategies. Some of the strategies include n-of-1 trials, adaptive trials, master protocols for umbrella and basket trials and approaches to combine different historical data sources[3]. Another critical enabler of rare disease clinical trials, is the replacement of control groups with of historical controls. Natural history studies are critical to define the target population and identify clinically relevant endpoints. The FDA has recognized this approach by approving therapies based on clinical trials that included historical controls and not a placebo group[3].

THE BROADER IMPACT OF RARE DISEASE RESEARCH

Importantly, scientific interest in rare diseases has increased in the era of personalized medicine. More prevalent diseases are being stratified into smaller and smaller group based on disease drivers or biomarker expression or expected outcomes. Therefore, developing detailed knowledge on rare disease pathophysiology and clinically relevant biomarkers are very helpful to provide insights into biological pathways involved in more prevalent diseases.

TARGETING RARE DISEASES AT THE DNA LEVEL

Gene therapy

Gene therapies uses viral vectors to deliver a payload and can be used to address two types of therapeutic needs. Gene therapy can be used to compensate for a loss of activity of a non-functional gene by delivering the correct gene or reduce the impact of a pathogenic gene by reducing its expression. There are two broad types of delivery methods – *ex vivo* and *in vivo*. In *ex vivo* gene therapy, the patient's cells (typically blood or bone marrow cells) are transfected with the gene outside the body and then transplanted back into the patient. Other cell therapies are actively being developed including chimeric antigen receptor (CAR)-T cells that target rare cancers and iPS derived cells to treat retinal disorders. In *in vivo* gene therapy, the vector is injected directly into an appropriate tissue (e.g., muscle, liver) or intravenously into the intrathecal space for delivery to the central nervous system.

Adeno-associated viruses (AAVs) are currently the most widely used vector type for a few key reasons[4].

- **Good safety profile**
- **High transduction efficiency**
- **Broad tissue tropism**
- **Amenable to genomic engineering**

AAVs have a good safety profile as they do not cause human diseases. The primary adverse effect observed with AAVs is a transient rise in liver transaminase that is a readout for liver damage. However, this effect can be easily managed with steroid treatment. AAVs do not integrate into the human genome. They can infect both replicating and non-replicating cells but are lost quickly from rapidly replicating cells. Thirteen different AAV serotypes have been identified that have different sequences in the viral capsid proteins resulting in broad tropism for various tissues.

For example, AAV serotype 8 has been used in clinical trials targeting the liver while AAV-9 has been used in clinical trials targeting the CNS and have been shown to cross the blood-brain barrier via retrograde transport. AAVs have been shown to have varying transduction efficiencies in various cell types so it is essential to screen AAV serotypes for transduction efficiency in the cell type of interest[5]. For example, AAV-1 and AAV-6 were shown to have relatively high efficiency to transduce some stem cells but not others. The viral genome is small and highly amenable to engineering where endogenous genes are replaced with expression cassettes containing the payload of interest.

Retroviruses/lentiviruses have been widely studied as viral vectors. Retroviral vectors from murine leukemia virus were tested in X-linked SCID and Wiscott-Aldrich syndrome trials but were found to induce leukemias due to viral integration into the genome. Lentiviral vetors were found to be a better alternative as they have a better safety profile and can infect both dividing and non-dividing cells[4].

In *ex vivo* gene therapy, cells are modified outside the patient's body before being reintroduced. Cell therapies can be either autologous (patient derived) or allogeneic (off the shelf) or stem cell derived (induced pluripotent stem cells or iPSCs). Genetic modification of cells is typically done through genome editing methods or viral transduction and the genetically modified cells are injected into the patient.

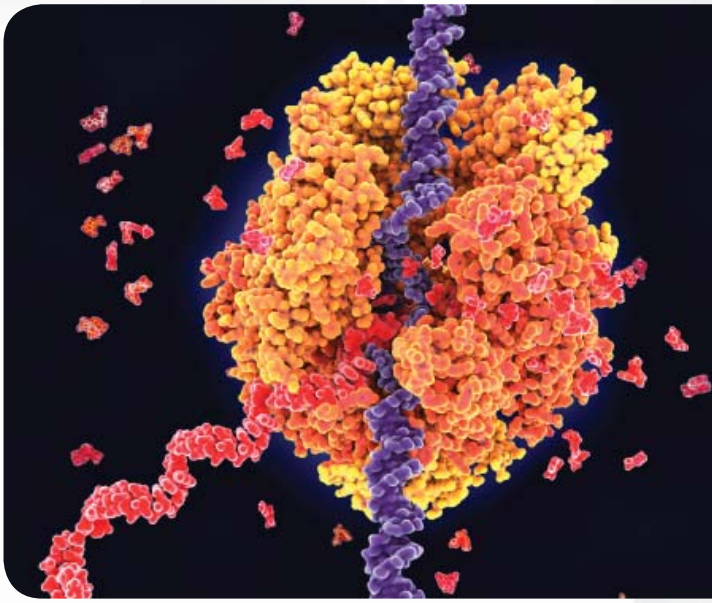
Genome editing

Genome editing has the potential to expand therapy development for a broad range of diseases while overcoming challenges associated with gene replacement therapy. Genome editing approaches enable precise, targeted repair of the patient's own DNA and causes permanent changes to the target gene, so theoretically only one therapy administration is needed, assuming every cell in the target organ is reached. While the majority of genome editing efforts are being applied to monogenic diseases, there is potential to expand the range of diseases.

Genome editing could be particularly useful for dominant disorders involving expanded repeat mutations, such as Huntington's disease and certain forms of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), if the editing technology would be able to access every neuron in the adult brain. The normal functions of the target genes are not fully defined, so there is an inherent risk in the knock-out approach. Correcting the gene to restore normal function is a lower risk alternative. Finally, genome editing offers the possibility of correcting several different mutations simultaneously. The most accessible organ systems for clinical application of genome engineering are liver and circulating blood cells as in both cases, either a high percentage of cells can be edited or edited cells can re-populate the patient's adult cell population.

The techniques currently used for genome editing are ZFNs (zinc finger nucleases), TALENS (transcription activator-like effector-based nucleases), meganucleases and the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats) system[6]. The first clinical trials of gene editing therapies are already underway for Hunter's syndrome, sickle-cell anemia and β-thalassemia but it is too early to draw conclusions.

APPROVED GENE THERAPIES



The first clinically approved use of gene therapy was in 1990, for a young girl with adenosine deaminase deficiency-severe combined immunodeficiency (ADA-SCID). The treatment used *ex vivo* transfection of her white blood cells with a functional copy of ADA and a later follow up showed long term expression of ADA[7]. However, the gene therapy field faltered for many years after that, because of issues with inefficiency of gene transfer, loss of therapeutic effect over time, insertional mutagenesis leading to cancer, and severe immune reactions (including one death).

It was not until 2012, after over 1800 trials that the first gene therapy drug received approval. The following gene therapies have been approved for clinical use.

- Glybera was approved in Europe for lipoprotein lipase deficiency, an ultra-rare disease. The drug's marketing approval in Europe was allowed to expire in 2017 and it was not approved in the US, so the first approved gene therapy is no longer available.
- Strimvelis®, a lentiviral *ex vivo* gene therapy, approved in Europe for use in ADA-SCID in 2016.
- Kymriah®, a chimeric antigen receptor (CAR)–T cell therapy for acute lymphoblastic leukemia was approved in the United States in 2017.
- Yescarta®, another CAR-T cell therapy was approved in the United States for relapsed or refractory large B-cell lymphomas in 2017.
- Luxturna® was the first *in vivo* gene therapy approved in the United States and subsequently Europe to treat *RPE65* mutation-associated retinal dystrophy.
- Zolgensma®, an AAV-based gene therapy was approved in the United States for Spinal muscular atrophy.

The number of gene therapy clinical trials continues to increase and 370 phase I, II and III trials have been reported in Q3 2019[8].

TARGETING DISEASES AT THE RNA LEVEL

Several therapeutic approaches targeting gene transcripts have been developed including antisense oligonucleotides (ASO), small interfering RNAs (siRNAs) and splicing modulators. One of the key features of targeting RNA is a good safety profile as the RNA modulators do not integrate into the genome and cause long-term effects.

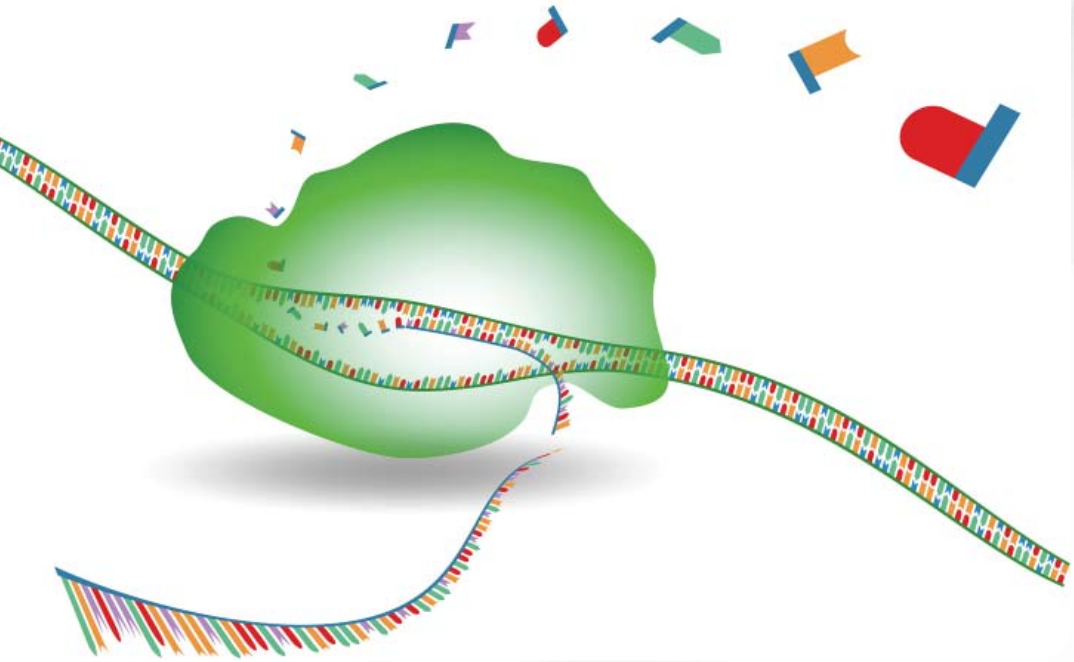
Antisense Oligos (ASOs)

Oligonucleotides are synthetic nucleic acid sequences that bind to specific complementary RNA sequences. The paired sequences are then degraded by ribonuclease H or RNase H leading to lower expression of the target protein. To date, oligos have been used primarily for gene silencing in diseases caused by toxic gain-of-function mutations Oligos can potentially be applied to any gene transcript, and have very high specificity for their RNA targets compared to traditional small molecule drugs. ASOs can be injected directly into the target tissue or administered subcutaneously without the need for a viral vector. The evolution of next-generation sequencing technologies has revolutionized the diagnosis and subsequent design of oligo-based therapies.

Clinical evidence of the rapid development of n-of-1 ASO therapy (milasen) has opened up a new frontier for personalized therapies in rare diseases9. ASOs are also being used to treat patient cohorts and several examples zare listed below –

- The first ASO approved in 1998 for cytomegalovirus retinitis but due to the development of superior high-activity anti-retroviral therapy, the need for the drug dropped and it was discontinued.
- Kynamro® or mipomersen was approved in the United States but not Europe for the treatment of homozygous familial hypercholesterolemia (HoFH). The ASO that knocks down expression of apolipoprotein B and reduce LDL cholesterol levels.
- Exondys 51® or eteplirsen was approved in 2016, for DMD that promotes exon skipping during RNA splicing. The approval process is considered controversial due to the small trial size.
- Defitelio® or defibrotide was approved in 2016 and is indicated for severe hepatic veno-occulsive disease that occurs after high dose chemotherapy and autologous bone marrow transplants. The mechanism of action and composition of the drug are very complex. The drug is a natural product derived from porcine intestinal mucosa DNA.
- Spinraza® or nusinersen was approved in 2016 for the treatment of SMA by promoting inclusion of an exon during RNA splicing. The drug is indicated for infants born with SMA types 1 2 or 3.
- Waylivra® or volanesorsen was approved in 2019 and is an ASO targeting apolipoprotein C_3 to treat familial chylomicronemia syndrome (FCS). It is a triglyceride reducing drug.

Tominersen is an ASO developed by Ionis Pharmaceuticals/Roche has shown that delivery of an ASO targeting the huntingtin gene in the CNS resulted in a sustained and significant reduction of the mutant huntingtin protein in CSF. Many more oligonucleotide drugs are in the clinical pipeline, including drugs targeting Alzheimer's disease (AD), Parkinson's disease, ALS, FTD, spinocerebellar ataxia, and hemophilia.



Small Interfering RNAs (siRNAs)

This approach most commonly uses short interfering RNAs or (siRNAs) that are double-stranded nucleic acid sequences, which bind to mature, spliced mRNA targeting it for removal by the RNA-induced silencing complex (RISC). These RNAs are introduced using viral vectors as shRNAs (short hairpin RNAs) or as synthetic oligonucleotides. RNA inhibition (RNAi) therapeutics are considered to be a paradigm shift in the development of novel therapies. Disease targets that are considered "undruggable" by small molecules or other therapies can be targeted by siRNA therapeutics. Additionally, the time-frame to develop a lead asset is short compared to chemical modulators or biologics. However, it is important to note that the therapeutic effect is transient and repeated dosing may be necessary. The first siRNA therapy Patisiran was approved in 2018 for the treatment of hereditary ATTR (hATTR) amyloidosis with symptomatic polyneuropathy (previously known as familial amyloidotic polyneuropathy or FAP)[10].

Modulation of RNA splicing and editing

Mutations that disrupt normal splicing are estimated to account for about 30% of all disease-causing mutations[11]. These mutations result in exon skipping and/or the use of alternative splice sites, resulting in mRNAs that are either degraded or encode defective proteins. Examples of diseases due to mutations affecting RNA splice sites include β+-thalassemia, muscular dystrophies including Duchenne muscular dystrophy (DMD), familial dysautonomia, frontotemporal dementia (FTD) with parkinsonism linked the chromosome 17 (FTDP-17), and some forms of cystic fibrosis.

Antisense oligonucleotide (ASO) therapy can be used to redirect splicing of mutation-bearing pre-mRNA to either prevent splicing (e.g., at an abnormal splice site) or promote splicing (e.g., to induce exon-skipping). An innovative approach is to use ASOs to repair the sequence of the mRNA encoding the disease-associated protein and one example of this therapeutic approach is Eluforsen for Cystic Fibrosis patients with the F508 del mutation. Small molecules that modify the activity of splicing factors are an option but one major drawback is the lack of specificity leading to off target effects.

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