PharmaVOICE

THERAPEUTIC DIGEST

RARE DISEASES

PROGRESS AND CHALLENGES AHEAD

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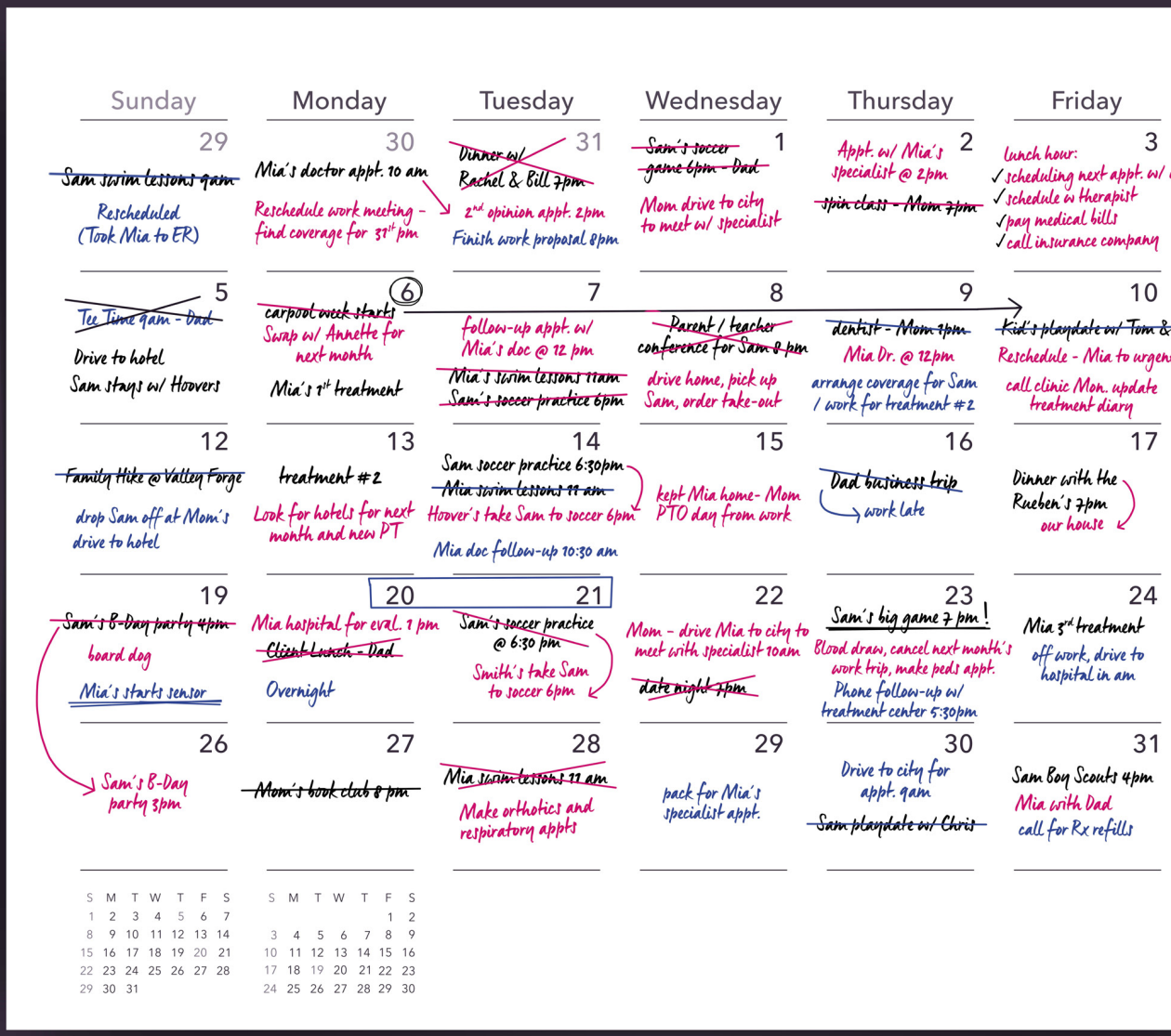
DECEMBER 2020

THE CENTER FOR RARE DISEASES



Rare changes everything.

Every day can be delayed, postponed, or canceled when you or a loved one has a rare disease. At the Center for Rare Diseases, we never forget that rare alters entire lives. We're committed to changing that.

To find out more, visit us at rarechangeseverything.com.

PRAHealthSciences

Table of Contents

thinkgen Editorial content provided by ThinkGen (www.think-gen.com)
Written by Kristina Erfe Pines

Q&A with PRA's Juliane Mills

How are rare disease clinical trials different from traditional clinical trials?

Every patient is a precious resource in clinical research. When conducting a clinical trial in a rare disease, there are by definition only a few eligible patients who can participate in a clinical trial. If someone drops out, there may not be anyone else to invite. The way we engage with patients needs to be reflective of the value of their participation. This means doing all we can to make it easy to volunteer such as with plain-language communication, reimbursement for time and travel, and carefully planning visit procedures.

Rare disease trials are also different due to the statistical challenges when researching a low number of patients. Just as every patient is precious, every data point is critically important. Because there are fewer patients, there are less data overall, and we may not have the statistical power in rare disease trials to discriminate noise from a true signal. To reduce 'noise' in the data set, the methods used to collect data need to be standardized. We need to make sure every trial site in a study is consistently using the same staff and the same methods to assess each patient. Every person involved, from the doctors administering the assessment to the study coordinators and site staff, must follow the same procedures.

Many families search for a diagnosis for years. Historically, it's been incredibly difficult to obtain an accurate diagnosis. Some studies have estimated that up to 40% of rare disease patients are misdiagnosed at least once. What can be done to change how rare diseases are diagnosed?

When patients are misdiagnosed or bounced around from doctor to doctor, it can certainly upend the doctor-patient relationship. Sometimes a diagnosis of a rare disease is a process of ruling out other diseases, which means a lot of tests, painful procedures, and time. The lack of answers can completely erode a patient's faith in the medical system.

Honesty on both sides of the patient-doctor relationship is a great way to move forward. Doctors need to be willing to hear and listen to what patients are telling them. Conversely, patients need to understand that doctors may not have all the answers. Doctors and patients are partners in the journey. Patients should be empowered to do their own reading, talk to other people who have the same symptoms, and share their ideas about treatment with their doctor. It's never a one-way street.

Juliane Mills
Director,
PRA Center for Rare Diseases
PRA Health Sciences

PRA Health Sciences

90% of all rare disorders do not have an FDA-approved treatment. How can the R&D process address the smaller patient populations inherent with orphan or rare disease trials?

Registries are often set up by R&D companies, but more and more we see advocacy groups setting them up and encouraged to do so by regulatory authorities. When advocacy groups set up registries on their own, they can democratize ownership of all this data, which puts ownership in the hands of patients. By building registries populated with shareable observational data, we can establish consensus in rare disease research and form a better singular view into these patient populations.

As we share what we know about rare diseases, we find that some rare diseases operate in the same biological pathway but may manifest with very different symptoms. Even though these patients have the same disease pathway, different types of doctors may treat them depending on where the symptoms occur. Advocacy groups have been bringing together doctors from all different specialties as well as academic researchers to build consensus on biomarkers, disease terminology, and treatments. ■

By partnering with patients in your rare disease research, you can develop more meaningful endpoints. **Learn how** in our latest white paper.

Rare Diseases

Progress and Challenges Ahead

Rare diseases, also known as orphan disease, are a group of disorders that affects a small percentage of the population. Commonly presenting in early life, rare disease can also be seen in adulthood with a chronic phase. These diseases are frequently progressive, disabling, and life threatening. Approximately 30% of children suffering will die at 5 years of age[1].

Although rare diseases affect small numbers of patients by definition, they are estimated to collectively affect ~350 million patients globally[2], more than double the number of patients affected by AIDS and cancer combined. While there have been substantial efforts to promote the development of therapies for rare diseases in the past few decades, supported by regulatory and economic incentives[3], most of the estimated ~7,000 rare diseases still lack specific treatments. Moreover, recent analysis indicates that the number of rare diseases could be substantially higher than 7,000.

The vast majority of rare diseases are characterized by Mendelian inheritance, and the recent evolution and broader application of sequencing technologies have revealed the causes of novel rare diseases and have identified new mutations responsible for previously defined disorders[4]. In addition, emerging applications of advanced analytics such as facial recognition have the potential to improve the screening and diagnosis of some disorders[5]. Nevertheless, the rate of translation of knowledge of rare diseases into therapies lag far behind the rate at which knowledge is being generated.

Industry has traditionally focused on small-molecule drugs, but advances in molecular biology and understanding of the human genome have enlarged the drug discovery toolbox, first to protein-based therapeutics (proteins, peptides, and antibodies) and more recently to antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs) and gene and cell therapies. These therapeutic modalities differ in their ability to target molecular disease mechanisms and/or to effectively reach certain cellular compartments. Protein-based therapeutics have enabled the modulation of extracellular targets and the replacement of dysfunctional circulating proteins, whereas ASOs, siRNAs, and gene and cell therapy have widened the druggable target space to include targets and mechanisms that are difficult to address with small molecules and proteins, such as transcription factor targets and compensation for dysfunctional intracellular proteins. Together, these therapeutic modalities allow a broad coverage of targets and mechanisms, which can be expanded by combining modalities, such as small-molecule conjugation with an antibody.

DEFINING RARE DISEASES

There is no universal definition of a rare disease, although such definition on a global basis would be valuable. Rather, there are definitions used in legislation in different parts of the world to incentivize drug development for diseases that have a prevalence below a given threshold in that region. Such products are known as orphan drugs in the United States and orphan medicinal products in the European Union (EU).

Orphan drug designation in the US may be obtained through the Office of Orphan Products Development of the FDA if a sponsor can provide a rationale for the use in the proposed rare conditions and show by documented prevalence that the affected population is less than 200,000 in the United States. For a designation as an orphan medicinal product in the EU, the sponsor must justify the use of products for an approved rare condition, as evaluated by the Committee for Orphan Medicinal Products of the European Medicines Agency. The prevalence threshold is defined as less than 5 in 10,000 in the European Union (corresponding to a population of ~254,000 in the current EU of 28 countries). In the EU, an orphan condition is additionally defined as life-threatening and/or serious, a requirement that is less strictly applied in the US. Furthermore, if there are other satisfactory methods to treat, diagnose, or prevent the condition, the EU legislation mandates that it be demonstrated that the product is of significant benefit to patients.

SMALL MOLECULES

Small molecules are the most well-established drug platform for diseases in general and continue to be attractive as therapeutic agents because of their multiple routes of administration, controlled dosing, stability, scale of synthesis, and generally low cost of goods. Although concerns have been raised for many years that the rate at which small-molecule drugs reach the clinic is slowing[6], new screening technologies and advances in synthetic chemistry, computational screening and structural biology are enabling the discovery and design of novel bioactive molecules. There is also huge potential to expand the knowledge of previously understudied gene s as drug targets, given that less than 700 of an estimated 3,000 disease-associated proteins encoded in the human genome are targeted by currently approved drugs[7,8]. Furthermore, even if a mutated-gene product may not be a druggable target, analysis of the associated pathway may identify a suitable target for small-molecule intervention.

Progress

The identification of small-molecule drug candidates generally depends on the screening of cell lines with libraries that typically range ins ice from ~10^3 to ~10^6 compounds. This approach has been boosted in the past two decades by the introduction of more efficient screening technologies and developments with chemical libraries to increase hit rates and quality[9]. An example is the filtering of chemical libraries to remove structures that may be more likely to have poor pharmaceutical properties (e.g. based on Lipinski's rule of five)[10] and structures that are likely to be false positives owing to assay interference, although this approach is not without controversy[11,12]. Medicinal chemists then investigate derivatives of promising hits to optimize the effects in disease models, as well as their absorption, distribution, metabolism, excretion, and toxicology (ADMET) characteristics, before selecting a candidate to carry forward into clinical testing.

The ability to set up a high-throughput screen where the readout related directly to human physiology has been one of the limitations in translating small-molecule candidates from such screens into therapies for use in the clinic[13]. Importantly, however, for rare diseases, the molecular cause is often well characterized, in contrast to more common diseases. Furthermore, several recent developments, including induced pluripotent stem (iPS) cells, technologies for gene editing such as Crispr-Cas systems[14] and organoids[15] have made possible the development of cellular disease models that are anticipated to have a strong translational relevance, as well as provide much higher throughout than possible previously.

Theoretically, iPS cells can be established from a patient's skin biopsy sample and differentiated into the cell type of interest expressing the phenotypic characteristic of the disorder[16]. One of the first high-throughput screens using iPS cells derived them from the fibroblasts of a patient with spinal muscular atrophy (SMA) and differentiated them into motor neurons[17]. These cells demonstrated the characteristic disease features: notably, a decreased ability to differentiate into neurons. Screens using such cells have led to drug candidates, including the phase II SMA therapy LMI70, a small molecule that boosts production of a protein known as survival motor neuron protein (SMN) by binding to a complex of the SMN2 pre-mRNA and the cellular splicing machinery.[18] Three-dimensional organoid cultures provide an even closer mimic of tissue organization and functionality, making them an excellent model for screening for small-molecule drugs, particularly when drugs targeted at particular mutations are required[19].

Screens in model organisms including Saccharomyces cerevisiae (yeast), Caenorhabditis elegans (nematode), Drosophila melanogaster (fruit fly) and Danio rerio (zebrafish) are also emerging as important genetic and chemical discovery platforms, particularly for small-molecule drugs that

may modify a disease phenotype[20]. These screens also take into account drug uptake into cells and toxicity considerations. The genomes of all these organisms have been sequenced, and they can be used in modifier screens for the identification of drug targets. The advent of CRISPR–Cas9-based genome editing and transgenic technology allows the introduction of specific human mutations. Simultaneous validation across several model organisms could accelerate the movement of potential therapies towards the clinic.

Clinical Success and Approvals

The success of small molecules in therapy for rare diseases has been driven by targeted screens and better disease modelling. For example, for cystic fibrosis, therapeutic small molecules have been derived from cell screens defined by knowledge of the underlying mutations in the CFTR gene, which lead to defects in protein production, trafficking, function, misfolding or premature degradation. In vitro screens led to the identification of the CFTR potentiator ivacaftor,[21,22]which was initially approved to treat 10 different mutations in patients with cystic fibrosis, with approval subsequently expanded to an additional 23 mutations[23]; Further studies have indicated that it could be applicable to many more[24]. An assay for the correction of the folding and processing of CFTR allowed the development of lumacaftor, a compound that promotes CFTR trafficking, for use in combination with ivacaftor for patients with cystic fibrosis with the most common F508del mutation[25].

Recently, a three-drug combination for patients with one or two F508del alleles (representing ~90% of patients) demonstrated efficacy in phase III trials[26] and has received FDA approval[27]. None of these combination treatments is a cure, but by targeting different phenotypic outcomes of a given mutation, it is possible to achieve significant clinical benefit. It is also noteworthy that in vitro assays, such as those based on cystic fibrosis intestinal organoids,[28] can indicate whether particular combinations of drugs that address different defects in CFTR function are likely to be effective in particular groups of patients. On the basis of the results of such assays and the drug safety profile, the FDA staff have worked with patients, advocacy groups, industry and academia to allow the use of approved drugs in additional patient populations with CFTR mutations that are too small for traditional clinical trials,[29] an approach that may also be relevant for other drugs targeting specific mutations in the future.

Small molecules can potentially target all tissues, although tissue exposure depends on the chemical structure. Lysosomal storage disorders (LSDs), many of which are caused by defects in lysosomal enzymes, are a good example of a set of rare diseases where this could be an advantage[30]. Enzyme replacement therapy (ERT) is a well-established effective platform for

some groups of patients with LSDs, but is costly to manufacture, requires injection and can be limited by the lack of penetrance of the enzyme to key pathological sites, such as the central nervous system (CNS). Two small-molecule LSD therapies that inhibit the biosynthesis of the substrates of defective enzymes (miglustat and eliglustat for Gaucher disease) and one that acts as a chaperone to stabilize and restore function to a mutant enzyme (migalastat for Fabry disease) are already approved, with further candidates in clinical trials, including the CNS-penetrant compound ibiglustat for Fabry disease.

Small molecules that promote stop codon readthrough (SCR) are promising for drug discovery for rare diseases caused by such mutations in a particular gene[31]. For example, ~13% of patients with the muscle-wasting disease Duchenne muscular dystrophy (DMD) have stop codon mutations in the gene coding for dystrophin,[32] and a small molecule, ataluren, that promotes SCR demonstrated efficacy in the mdx mouse model of DMD.[33] However, translation into patients has been difficult because of the low levels of readthrough, and ataluren is currently approved in the European Union but not in the United States.

Small-molecule drugs can also be used to increase the levels of proteins that can compensate for a lack of a protein product. For example, increased expression of the dystrophin-related protein utrophin has been shown to prevent pathology in the mdx model of DMD,[34] although small molecules which increase utrophin levels have not yet been successful in clinical trials. In DMD, several small molecules that address downstream effects such as inflammation and fibrosis are also showing efficacy in the clinic and can be used in combination[35]. Finally, small-molecule proteostasis modifiers, which increase the endogenous cellular response to stress and upregulate the chaperone heat shock protein 70 to promote protein folding, are being developed for LSDs[36].

Strengths and Limitations

Small molecules remain at the forefront of drug discovery because they can target many tissues, they can be produced at reasonable costs and their manufacturing is scalable. For rare diseases, if the causative molecular target is in a class with established tractability for small molecules, such as G-protein-coupled receptors or kinases, the vast scientific, clinical and regulatory experience with this platform can also be an advantage compared with emerging platforms discussed elsewhere in this article. Furthermore, the potential for phenotypic screening to identify molecules that have the desired therapeutic effect through unknown novel mechanisms could also be an advantage for rare diseases for which the molecular cause is unclear or multifactorial.

The major challenge is to find the right molecule that displays an excellent pharmacological effect and excellent pharmacokinetics but with few off-target effects, which sometimes requires

extensive optimization of a lead candidate. The other main hurdles are access to sufficient numbers of chemical entities (outside biopharma companies) and the development of screens which are relevant to the disease state in vivo. There has been substantial progress in tackling both of these challenges in recent years; for example, through initiatives such as the European Lead Factory to enable academic researchers and small and medium-sized enterprises to screen novel targets with large pharma-quality compound libraries (see Related links). Furthermore, as screening for disease phenotypes improves, many of the drugs already shown to be safe and well tolerated in one condition may be repurposed to treat a (different) rare disease where there might be a common pathway for intervention, as discussed later in this article. Finally, as understanding of rare disease mechanisms improves, combination therapy targeting different aspects of disease pathogenesis, a common scenario in cancer, may become possible.

ANTIBODY THERAPIES

The first therapeutic monoclonal antibody (mAb), muronomab-CD3, was approved in 1986 for the treatment of organ allograft rejection.[37] Since then, this class of products has steadily grown such that therapeutic mAbs (and antibody-related products such as Fc-fusion proteins, antibody fragments and antibody–drug conjugates (ADCs))[38] have become a dominant product class for the treatment of a variety of diseases, particularly cancers and immune disorders[39]. Antibodies exert their effect by modulation signaling pathways, recruiting cells or proteins to specific sites, delivering cytotoxins or neutralizing or modulating circulating factors.

Progress

mAbs are naturally produced by B lymphocytes, recognizing foreign antigens during the humoral immune response. The two key characteristics of a mAb are its specificity for a particular antigen and that this specificity is continuous. Efforts to exploit these features therapeutically date back to the 1970s[40,41]. However, the first murine mAbs had immunogenicity and a short half-life, and scientists realized that mouse/human chimeric mAbs, humanized mouse mAbs or human Abs would be necessary for the development of effective mAb therapeutics. Four main approaches have since been developed to identify and produce such mAbs: phage display,[42] transgenic animals,[43] B cell immortalization and single B cell sorting[44].

Antibody engineering is now well established, and antibodies can be produced as full-length naked mAbs or as smaller engineered antigen-binding fragments (Fab),[45] providing desirable characteristics for specialized applications (for example, reaching higher concentration in

confided settings such as the back of the eye) and characterized by a faster clearance, resulting in reduced systemic bioavailability and consequent reduced toxicity. Engineering techniques can allow the production of bispecific antibodies (BsAbs),[46] which may have advantages over monospecific antibodies, such as the ability to direct effectors of the immune system to target tumor cells or to block two different targets simultaneously[47,48]. BsAb development is less straightforward than mAb development, however, with challenges including stability of the molecules, manufacturing and more complex toxicology assessments. Although multiple BsAb formats have been developed and more than 50 BsAbs have entered clinical trials, so far only two BsAbs have reached the market.

The antibody constant region (Fc) can also be fused to another non-antibody-related protein domain and used as a standalone therapeutic or the full-length antibody can be fused to a small molecule to create ADCs. Fc-fusion proteins confer the advantages of IgG, including binding to the neonatal Fc receptor (FcRn) to facilitate in vivo stability, and the therapeutic benefit of the specific effector function[49]. Today, there are eight approved Fc-fusion proteins. ADCs harness the specificity of mAbs to selectively deliver highly potent cytotoxic drugs to tumor cells that express a particular antigen on their surface, thereby reducing damage to healthy tissues[50]. As with BsAbs, there have been challenges in realizing the potential of the ADC strategy, with only a few ADCs for blood cancers approved so far, but the field is active, with more than 50 ADCs in clinical trials[51].

Clinical Successes and Approvals

The number of mAb-based therapies approved for rare diseases outside the oncology field is limited at present, but the potential of the platform for highly specific targeting of disease linked proteins is beginning to be realized. This therapeutic modality has been primarily developed for large indications and then repurposed, initially off-label, for some rare disease indications.

Eculizumab, a mAb that targets the terminal complement protein C5, was first approved for paroxysmal nocturnal hemoglobinuria more than a decade ago, and has since been approved for two other rare diseases in which the complement system has an important role: atypical hemolytic uremic syndrome and myasthenia gravis. Canakinumab, a mAb targeting the pivotal inflammatory cytokine IL-1β that was originally developed for rheumatoid arthritis,[52] was repurposed and approved for cryopyrin-associated periodic syndromes in 2009. It has since been tested in clinical trials for other diseases, including an ‘umbrella trial’ that provided the basis for its approval for three rare other periodic fever syndromes linked to IL-1β[53]. IL-1β is also the target of rilonacept, a fusion protein consisting of the ligand-binding domains of the extracellular portions of the human IL-1 receptor component and IL-1 receptor accessory protein linked to the Fc portion of human IgG1, which was approved for cryopyrin-associated periodic syndromes in 2008.

One of the two BsAbs approved so far is for a rare disease: the hemophilia therapy emicizumab acts by binding to factor IX and factor X, bringing these proteins close to each other and initiating a coagulation cascade[54]. The pioneering nanobody therapeutic caplacizumab, which targets von Willebrand factor, has recently been approved in the European Union and in the United States for acquired thrombotic thrombocytopenic purpura[55].

Strengths and Limitations

A key strength of mAb-based therapies as a platform in general is their high specificity. This limits the risk of off-target toxicity, which is frequently observed with small molecules. This is particularly relevant in the treatment of rare diseases, which often involves long-term drug administration. Related to this, another advantage of mAbs is that their stability in vivo allows infrequent (for example, once-monthly) dosing regimens in such contexts. For rare diseases caused by 'gain of function' of a particular protein that is present in the circulation and/or on the surface of cells, approaches to identify suitable mAb therapies are well established. Importantly, some approaches such as phage display are becoming increasingly accessible not just to major biopharma companies but also to small and medium-sized enterprises and universities[56]. Furthermore, other functionalities are also possible for mAb-based therapies, demonstrated earlier by the example of the BsAb emicizumab, although such approaches are less straightforward to pursue.

However, the large size of mAbs limits their tissue and cell penetration, preventing the pursuit of some theoretically desirable targets such as intracellular proteins, although this is one area in which novel fragment formats such as nanobodies hold promise. The manufacturing costs for mAbs may also be prohibitive owing to the need for large cultures of mammalian cells followed by extensive purification steps, under good manufacturing practice conditions. In addition, mAbs need to be injected (with the consequent need for very high standards of sterility in the formulation phase), and may initiate injection-site adverse reactions — a feature they share with other large molecules such as those used in protein replacement therapies.

Although the mAb platform is currently only marginally deployed in rare conditions, its future will probably look different owing to two major developments. First, the capability to identify and manufacture mAbs efficiently and safely is 'democratizing'. That, together with the much higher mAb titres that can be achieved today in batch, fed-batch or continuous perfusion cell culture, is greatly reducing the cost of goods and the flexibility of use of moderately sized good manufacturing practice upstream and downstream manufacturing suites[57].

Second, the entry into the market of cheaper biosimilar versions of pioneer mAbs may facilitate repurposing efforts. For example, the use of artificial intelligence to match the mechanisms of action

of well-characterized mAbs with pathway information derived from large sequencing efforts (such as Genomics England) implicated in rare diseases might benefit patients with rare diseases that currently lack therapeutic options.

PROTEIN REPLACEMENT THERAPIES

While the mAb platform discussed in the previous section is well suited to the development of therapies for rare diseases linked to gain of function of a particular protein, another biologic platform — protein replacement therapies — has long been a cornerstone in the treatment of rare diseases linked to the loss of function of a particular protein. One prominent example is the administration of factor VIII or factor IX to treat patients with hemophilia A or hemophilia B, respectively. This area has seen substantial innovation in the past few decades, progressing from plasma-derived products to recombinant proteins, to recombinant engineered proteins that have superior therapeutic characteristics, including modifications such as pegylation, to the latest advances such as emicizumab. These developments have been comprehensively reviewed recently,[58] so we focus here on a broad strategy — ERT — to illustrate the platform.

Diseases caused by missing or defective enzymes can be treated by replacement with exogenously supplied enzymes, either purified from human or animal tissue or produced by recombinant techniques[59]. The concept of systemic delivery of a deficient enzyme to rescue cellular function in patients with LSDs goes back to the 1960s[60,61,62], but the first ERT to be developed successfully was human α1-antitrypsin (A1AT) to treat emphysema associated with severe A1AT deficiency, which was approved by the FDA in 1987.

The focus of most ERT development so far though has been various LSDs, which are genetic diseases caused by missing, insufficient or malfunctioning enzymes in the lysosomes, leading to a pathological build-up of their substrates[64,65,66]. LSDs are progressive, and often ultimately fatal, although characterized by a spectrum of clinical manifestations, with variable disease progression rates, and beginning in fetal life. In the 1980s, Brady and colleagues at the US National Institutes of Health provided the proof of principle for ERT to treat LSDs by showing that glucocerebrosidase purified from placentae could be used to treat Gaucher disease[67]. Purified human placental glucocerebrosidase was further developed by Genzyme and first approved as a commercial ERT by the FDA in 1991. For safety and supply reasons, Genzyme developed a recombinant form of glucocerebrosidase, which was first approved by the FDA in 1994.

Progress

Enzymes used for ERT are either natural forms or recombinant proteins showing a high degree of homology with the human enzymes. The emergence of HIV/AIDS as well as potential supply limits made the use of natural enzymes less desirable. Therefore, most enzymes used in ERT are recombinant, which also allows modifications to provide a longer half-life, more potent activity, resistance to degradation or targeting to a specific organ, tissue or cell type[68]. ERTs are typically produced using mammalian cell lines, most commonly Chinese hamster ovary (CHO) cells, although modified human cells are also used. Prokaryotic systems are not useful for the expression of lysosomal enzymes because they cannot perform the post-translational modifications (such as N-linked glycosylation and mannose phosphorylation) needed for lysosomal enzyme stability, synthesis and/or activity[69,70]. An exception to the main use of CHO cells is the production of taliglucerase alfa in plant (carrot) cells in suspension, which does not require additional processing for glycosidic modifications[71]. As with all recombinant protein therapeutics, purification of the manufactured enzymes from the bioreactor broth is complex and needs to be highly controlled to preserve the biological activity of the final product and to ensure sufficient yield. Also, changes in manufacturing parameters such as the scale of the bioreactor can cause differences in the characteristics of the final product that may be considered clinically meaningful by the regulators, as exemplified by alglucosidase alfa, an ERT for Pompe disease. The product derived from two sizes of bioreactor has been approved by the FDA under two different trade names, whereas products from both sizes of bioreactor were considered sufficiently identical from a clinical perspective by the European Medicines Agency (EMA) to be approved under the same name.

Clinical Successes and Approvals

The emergence of ERTs for some LSDs has made it possible to treat patients and save their lives[72,73]. Recombinant ERTs have been developed and approved to date for 11 different LSDs worldwide (10 approved by the FDA, 9 approved by the EMA), including Gaucher disease, Fabry disease, Hurler–Scheie disease (also known as mucopolysaccharidosis type I (MPS I)), Hunter disease (MPS II), Pompe disease, Maroteaux–Lamy disease (MPS VI), lysosomal acid lipase deficiency (Wolman disease), Batten disease (neural ceroid lipofuscinosis type 2), Morquio A syndrome (MPS IVA) and recently Sly disease (MPS VII) and α-mannosidosis. For some LSDs, more than one commercial ERT is available. Several ERTs are in development for additional LSDs, including Sanfilippo A syndrome (MPS IIIA) and Sanfilippo B syndrome (MPS IIIB)[74,75,76]. Outside the field of LSDs, a few ERTs have been approved for A1AT augmentation therapy[77] and for adenosine deaminase (ADA) deficiency-associated severe combined immunodeficiency disease (SCID),[78] using natural (human and animal) enzymes, and recombinant ERTs were approved for hypophosphatasia (in the United States and the European Union), and phenylketonuria (in the United States)[79].

Strengths and Limitations

On the basis in part of 20 years of experience using ERT to treat more than 5,500 patients with type 1 Gaucher disease, the following general points can be made about ERT as a platform[80]. First, ERT can be very effective if the replacement enzyme can be delivered at the right dose into the right tissue and cells early enough in the course of the disease (that is, before major irreversible organ damage has occurred)[81,82,83]. Enzyme delivery is receptor mediated and dose dependent, and in Gaucher disease, mannose molecules on the enzyme surface help the enzyme enter the relevant cell type, macrophages[84,85]. However, for other LSDs, such as mucopolysaccharide disorders, Fabry disease and Pompe disease, ERT proved more difficult to develop because pathological substrate build-up occurs in other cell types that lack or express low levels of mannose receptors[86,87]. In addition, when the enzyme needs to be delivered into organs or tissues less served by the vascular system, much higher amounts may be needed. For example, in Pompe disease, skeletal muscle cells have low levels of mannose receptors, so very high amounts of enzyme (20–40 mg kg^{-1}) are necessary to achieve the right therapeutic effect[88,89,90]. In addition, intravenous ERT is not effective for neurological manifestations of the neuropathic subtypes[91,92,93], as enzymes are too large to cross the blood–brain barrier. ERT with intrathecal injections into the CNS is therefore being tested in some LSDs[94,95].

Second, the safety record of ERT is excellent. Very few patients experience significant infusion-related reactions. Hypersensitivity can be a difficult problem however, not just causing allergic reactions but potentially also limiting the efficacy of therapy owing to the formation of antibodies to the recombinant enzyme, and in severely affected patients with irreversible organ damage, ERT may not have any therapeutic effect[96,97]. Overall, the relevance of antidrug antibodies specific to ERTs for LSDs remains a mixed picture that will require time and continued clinical follow-up to resolve for each specific condition and treatment[98,99].

Third, technologies for ERT are well developed, but will continue to have limitations, including the cost of manufacturing and of purification of recombinant enzymes and the time to build manufacturing capabilities for new products. ERT dosing (~1 mg per kilogram body weight for Fabry disease, MPS and Gaucher disease, versus 20–40 mg kg^{-1} in Pompe disease therapy) will remain an important factor in determining the size of the manufacturing facility required.

Looking to the future for ERT, the establishment of relevant study end points in clinical trials for ERT is of growing importance, together with the understanding of what comprises a minimal clinically important difference in these end points for the patients. It may be insufficient to use subclinical parameters rather than clinical outcomes or to evaluate end points for which relevance to patients' outcomes remains unclear[100,101,102]. LSDs are likely to remain a strong focus in the

near future, as of the 70 or more of these rare monogenic diseases (which collectively affect 1 in 5,000 live births)[103], 'only' 11 of them have an approved ERT. Nevertheless, the monogenic nature of LSDs and the detailed knowledge of the function of many of the proteins defective in these disorders provide multiple therapeutic intervention points[104,105], and so several alternatives to ERT are being developed or investigated. These include small-molecule strategies mentioned earlier, stem cell transplants and gene therapy mentioned later, which would potentially allow the 70% of LSDs with neurological involvement to be addressed[106,107,108,109,110]. Combination therapies are being tested as well[111]. Therapies that target converging elements of the pathogenic cascade and thus may be applicable to more than one LSD may also be considered but may be less effective[112,113]. The challenges and successes of therapy development for LSDs may inform the treatment for other rare diseases[114].

OLIGONUCLEOTIDE THERAPIES

Another broad strategy to specifically target disease-associated genes is to intervene at the level of RNA. Several approaches to targeting RNA have been developed, with the most extensively investigated of these being ASOs and siRNAs, which can both reduce the production of a specific disease-associated protein by promoting degradation of its mRNA. Like antibodies, ASOs and siRNAs can be highly specific interventions for rare diseases with a well-defined molecular cause, with the additional advantage that in principle any gene product can be targeted, rather than just cell-surface or circulating proteins. The development of ASOs and siRNAs has been a long and challenging process, particularly with regard to delivery, but recent approvals and an extensive clinical pipeline indicate that these platforms are now poised to fulfil their potential.

Progress

Oligonucleotide therapies are synthetic nucleic acid sequences that bind to RNA targets through sequence-specific base pairing and thereby affect gene expression in various ways. The first oligonucleotide therapies to be investigated were ASOs, for which research began in the late 1970s. ASOs are single-stranded molecules that bind to complementary mRNA by Watson–Crick base pairing, and initiate its selective degradation by ribonuclease H, leading to knockdown of the expression of the corresponding protein. The identification of various chemical modifications, such as phosphorothioate backbones to increase the resistance of ASOs to degradation by nucleases in vivo, has also been crucial in developing ASOs as a robust platform for gene knockdown[115].

The second class of oligonucleotide therapies that degrade target mRNAs are synthetic siRNAs,

which are based on the discovery of RNA interference as an endogenous mechanism for gene regulation in 1998. While these are double-stranded rather than single-stranded like ASOs, they also incorporate modified chemical backbones to enhance their pharmaceutical properties. Delivery has been a greater challenge for siRNAs than ASOs, and to address this, they are also typically conjugated to carriers such as lipid nanoparticles or N-acetylgalactosamine, with most siRNA drug candidates so far exploiting the ability of such carriers to achieve delivery to the liver[116].

A third class of oligonucleotide therapies act in a different way, hybridizing to pre-mRNAs or mRNAs without leading to their degradation[117]. Depending on the nature of the interaction, these single-stranded RNA- blocking agents (which are also chemically modified as with ASOs and siRNAs) can have various effects that may be useful in the treatment of rare disorders linked to gene dysfunction, such as exon skipping, cryptic splicing restoration or even changing levels of alternate gene splicing[118]. Importantly, gene function can be restored by such therapies, in contrast to ASOs and siRNAs, which only inhibit gene function.

Clinical Successes and Approvals

Oligonucleotide therapies have demonstrated clinical efficacy for the treatment of multiple human diseases, with the first FDA approval of an ASO in 1998, fomivirsen for cytomegalovirus retinitis in immunocompromised patients, including those with AIDS, an orphan condition. Although fomiversen has since been withdrawn, other ASOs have followed, such as the ASO mipomersen, which was approved in 2013 for familial homozygous hypercholesterolaemia; this ASO targets mRNA for apolipoprotein B-100, the principal apolipoprotein of LDL and its metabolic precursor, VLDL[120].

However, the application of oligonucleotide therapies is perhaps most promising in rare neurological conditions, which has led to several pioneering approvals in recent years. Two of the approved products harness different platforms for the treatment of hereditary transthyretin (TTR)-mediated amyloidosis. Patisiran, a lipid-conjugated siRNA, became the first siRNA therapy to be approved by the FDA (in 2018)[122], and this was followed shortly after by the FDA approval of the ASO inotersen[123]. Both agents act by degrading the mRNA encoding TTR, resulting in a reduction in serum TTR and TTR deposits in tissues[124] and clinically relevant improvements in the neurological manifestations of hereditary TTR-mediated amyloidosis.

Two further RNA-blocking oligonucleotide therapies have also been approved for rare neurological conditions. Nusinersen was designed to treat SMA caused by mutations in chromosome arm 5q that lead to SMN deficiency. It acts by increasing exon 7 inclusion in SMN2 mRNA transcripts, resulting in the production of full-length SMN[125,126] and has been approved in the United States and

the European Union. Eteplirsen was designed to hybridize to exon 51 of dystrophin pre-mRNA, leading it to being skipped during splicing, and thereby correcting the translational reading frame and resulting in the production of shortened, but functional, dystrophin proteins in patients with DMD[127],[128]. However, there has been controversy over the extent to which dystrophin function is restored by treatment with eteplirsen in trials conducted so far, and while the FDA granted approval for eteplirsen in 2016, a marketing authorization application to the EMA received a negative opinion in 2018.

Strengths and Limitations

The mechanistic characteristics of oligonucleotide therapies result in high specificity, ability to address targets that are otherwise inaccessible with traditional therapies and reduced toxicity owing to limited systemic exposure[129]. This greatly expands the numbers and types of selectable targets[130]. With the majority of the known rare diseases being of genetic origin, RNA targeting by oligonucleotide therapies provides a key opportunity to reduce the vast morbidity and mortality associated with rare conditions[131]. However, the fact that oligonucleotides do not readily cross the blood– brain barrier, and therefore require invasive delivery methods such as intrathecal or intraventricular routes, remains one of the most substantial obstacles for their clinical applications in CNS disorders. Despite this, the number of recent successes that resulted in regulatory approval are likely to result in greater research and development for other rare conditions. For example, an ASO that targets the mRNA for huntingtin (HTT), known as RG6042, has recently entered phase III trials for Huntington disease in the hope that this may represent the first disease-modifying therapy for this neurodegenerative disorder[132].

GENE AND CELL THERAPY

Gene therapy harnessing viral vectors can be used in two general contexts for rare diseases. For diseases in which the therapeutic goal is to compensate for a loss of function of a particular protein, such as in SMA, the vector is used to express a transgene (with the endogenous sequence or codon optimized) that encodes the desired protein, under the control of an appropriate promoter. Conversely, for diseases such as Huntington disease, where the aim is to suppress the impact of a pathogenic gene, a transgene that encodes an RNA (such as a short hairpin RNA) that can harness RNA interference mechanisms to inhibit gene expression can be introduced.

There are also two broad approaches for delivery of gene therapy, depending in part on the cells in which the gene needs to be expressed to treat disease. In some cases, viral vectors containing the therapeutic gene can reach the desired cells following injection of the vector, often directly into

the tissue or organ, such as the eye, which can promote uptake and minimize off-target effects. In other cases, cells are genetically modified out- side the body to produce therapeutic factors and subsequently transplanted back into patients. This ex vivo gene therapy approach, which can also be considered as a type of cell therapy, is particularly useful for rare inherited blood disorders, for which hematopoietic stem cells (HSCs) can be collected from patients, genetically modified and then transplanted back into patients.

Other types of cell therapy are also being developed for rare diseases, including transplants of cells derived from iPS cells, such as retinal cells for eye disorders, and chimeric antigen receptor (CAR) T cells that target specific tumor antigens for rare cancers. Cells can be injected or incorporated onto scaffolds for placement in the appropriate tissue, such as the eye.

Progress

Two gene therapy platforms based on viral vectors have emerged as the most useful for clinical studies in rare diseases: adeno-associated virus (AAV) vectors and retroviral/lentiviral vectors[133].

With recombinant AAV vectors, a therapeutic trans- gene, including the promoter and other regulatory elements, of up to five kilobases in size can be inserted into the AAV for gene therapy applications. Thirteen different AAV serotypes have been identified, reflecting different amino acid sequences of the capsid proteins. These differences result in differing tropism for different organs, tissues and cell types[134]. On the basis of clinical studies, certain AAV serotypes are emerging as tissue-specific platforms. For example, AAV-8 has been used in three clinical trials for gene delivery to the liver, while AAV-9 has been used in four trials in neurological diseases. On the basis of available crystal structures of capsid proteins, it is now possible to rationally engineer novel capsids in an effort to develop vectors to target specific cells and tissues[135,136]. AAVs can infect both dividing and non-dividing cells. Although wild-type AAV can integrate into the human genome, sequences encoding the viral proteins necessary for integration are deleted in AAV vectors used for gene therapy. Therefore, recombinant AAV vectors are generally considered to be non-integrating — a very important feature with regard to potential safety issues from a regulatory perspective. However, the lack of integration means that AAV vectors will be lost from infected cells as they replicate. For this reason, AAVs are primarily used for gene therapy in non-dividing (or very slowly dividing) cell types.

With regard to safety, it is worth emphasizing that, despite the similarity in name, AAVs are fundamentally different from adenoviruses (the viral vector used in a gene therapy trial in the 1990s that resulted in the death of a patient with a rare disease). Whereas adenoviruses are human pathogens, AAVs are not known to cause any human disease. AAV vectors have an excellent safety

record, having been used in more than 200 human clinical trials[137] without any deaths or without causing cancer. Some recent studies have linked AAV vectors to an increased risk of liver cancer in mice[138], although the relevance of these findings for human cancer is the subject of debate[139]. The most common serious adverse effect resulting from AAV vectors in humans is a transient elevation of the level of liver transaminase (indicative of liver damage), which is related to an immune response to the AAV capsid proteins. However, this can generally be controlled by a course of steroid treatment[140].

Retroviruses contain a single-stranded RNA genome and have the capacity to integrate into the human genome via a mechanism involving reverse transcription. The ability to integrate allows the possibility of permanent modification of the genome, which will persist over time and following cell replication. Lentiviruses are a genus of the retrovirus family. However, in contrast to other types of retroviruses, the entry of lentiviruses into the cell nucleus does not depend on mitosis. As a result, lentiviruses can be used to deliver genes into both dividing and non-dividing cells.

Initial investigations of retroviruses for gene therapy focused on disorders of blood cells, using an ex vivo approach. Retrovirus vectors based on murine leukemia virus were used in some early trials, but these were plagued by poor efficacy. Subsequent work using other gammaretroviruses demonstrated substantial clinical efficacy in ADA deficiency-associated SCID, such that the patients were able to stop ERT. However, patients administered gammaretroviral vectors in X-linked SCID and Wiscott–Aldrich syndrome trials and in an X-linked chronic granulomatous disease trial developed leukemias due to integration of viral vectors adjacent to oncogenes, resulting in transcriptional activation by powerful enhancer elements present in the long terminal repeats of the viral genome. To avoid these problems, the field turned to lentiviral vectors due to their greater efficiency for infecting human HSCs[141]. Specifically, self-inactivating lentiviral vectors, in which critical transcriptional enhancer sequences in the long terminal repeats are deleted in the course of vector production, have emerged as a platform for ex vivo gene therapy using HSCs[143],[144].

Depending on the initial cell source, cell therapies are categorized as patient specific (most often autologous but also allogeneic) or off the shelf (allogeneic). ‘Active ingredients’ differ widely, including T cells, dendritic cells, HSCs, mesenchymal stromal cells, CD34-selected cells, islet cells, fibroblasts, natural killer cells, neural stem cells, embryonic stem cells and iPS cells. Source cells are modified through processes that include some combination of target cell isolation (selection, sorting), culture (expansion and activation), washing, volume reduction and formulation. Cryopreservation may or may not be used to extend the shelf life of incoming cells, intermediate products or finished product. iPS cells have generated a lot of interest due to their ability to differentiate into many different cell types that can target many disease indications[145]. These

cells can be reprogrammed from an autologous sample (for example, dermal tissue) before being differentiated into the target cell type, such as retinal cells for eye disorders.

Clinical Successes and Approvals

AAV-based therapies have demonstrated clinical efficacy for the treatment of multiple human diseases, including SMA, hemophilia A and hemophilia B[148], aromatic l-amino acid decarboxylase deficiency and retinal pigment epithelium-specific 65-kDa protein (RPE65)-mediated retinal degeneration. Three AAV-based gene therapies have been approved for the treatment of rare diseases so far, all due to loss of gene function. Alipogene tiparvovec is an AAV-1 vector that expresses the gene for lipoprotein lipase that is administered by intramuscular injection. It became the first gene therapy to be introduced in a major market, with its approval by the EMA in 2013 for the treatment of lipoprotein lipase deficiency[149], but was withdrawn in 2017 owing to commercial issues. More recently, voretigene neparvovec, an AAV-2 vector that expresses the RPE65 gene, received FDA approval for the treatment of RPE65 mutation-associated inherited retinal dystrophy[150] in 2017 — the first gene therapy to be approved in the United States — and shortly after also received approval in the European Union[151]. In 2019, it was joined by onasemnogene abeparvovec, an AAV-9 vector that expresses the gene encoding SMN (SMN1), which has received approval for the treatment of SMA in the United States and is under regulatory review in the European Union.

Clear evidence of clinical efficacy without malignancy has been observed in studies using a gamma- retroviral vector for ADA deficiency-associated SCID, and with self-inactivating lentiviral vectors for other hematological disorders, including X-linked SCID[152], Wiscott-Aldrich syndrome[153], and β-thalassaemia[154]. Strimvelis, a retrovirus that expresses ADA, became the first ex vivo gene therapy to be approved for a rare disease when the EMA granted its approval for the treatment of ADA deficiency-associated SCID in 2016. A lentiviral platform has shown clinical success in treating three neurological diseases: X-linked adrenoleukodystrophy[155]. Metachromatic adrenoleukodystrophy and cerebral adrenoleukodystrophy[156].

n 2017, two CAR T cell therapies were approved in the United States for rare cancers: tisagenlecleucel[157] for acute lymphoblastic leukemia and axicabtagene ciloleucel for large B cell lymphoma[158]. Clinical data for these approaches have demonstrated transformative efficacy: patients receiving axicabtagene ciloleucel in the phase II ZUMA-1 pivotal trial achieved an overall response rate of 72%, while patients receiving tisagenlecleucel in the phase II ELIANA pivotal trial achieved an overall response rate of 83% — responses previously unheard of in hematological cancers. While the rapidly growing pipeline of CAR T cell therapies[159] are not being developed only for specific

rare cancers (indeed, the hope is that they will be much more broadly applicable), their development has helped establish the processes and regulatory requirements for cell therapies more broadly.

Finally, in June 2019, a product for the treatment of β-thalassaemia based on autologous $CD34^+$ cells encoding the βA-T87Q-globin gene was conditionally approved by EMA.

Strengths and Limitations

On the basis of the clinical experience to date, AAV vectors appear to be an excellent platform for treating rare monogenic disorders[160]. AAV vectors have shown clear evidence of clinical efficacy in multiple diseases of the nervous system, the retina and the liver. These results, as well as studies in animals, indicate that AAV vectors can support transgene expression that persists for years in non-dividing cells. A key limitation is the complexity and cost of manufacturing and production, which are vastly greater than for small molecules. Other limitations of AAVs include the potential loss of AAV-transduced cells due to immune responses, lack of effective vector serotypes for other relevant tissues and cell types and the limited capacity of the genome.

AAVs can also be used to deliver genome editing enzymes such as Cas9 or zinc-finger nucleases to treat rare diseases[161]. Indeed, AAV vectors were used to deliver zinc-finger nucleases in the first clinical trial of genome editing in a rare genetic disease[162]. However, while gene therapy requires long-term expression of a therapeutic gene, which is supported by AAV vectors, long-term expression of genome editors after genome editing has been completed may have negative consequences. Therefore, non-viral delivery vehicles such as lipid nanoparticles are of increasing interest for delivering genome editors[163].

Retroviral vectors have been shown to be effective clinically for ex vivo gene therapy for hematological diseases[164]. A major limitation to the use of retroviruses has been carcinogenesis resulting from integration of the vector into the genome. However, no malignancies have been observed in clinical studies using self-inactivating lentiviral vectors to date, suggesting that this problem has been addressed, although careful observation of treated patients for signs of clonal expansion will be required. Another clinical consideration is the requirement for myeloablative conditioning before infusion of the gene-modified HSCs, which is associated with significant toxic effects.

A key limitation for the use of cells as therapies is the current incomplete ability to characterize such products to ensure consistency, and the precise mechanism of action for these products may also not be clear. This limitation places extreme pressure on making improvements to the chemistry, manufacturing and controls (CMC) for cell therapy candidates, which, due to the early stage of development of the industry, often includes reliance on open and manual technologies. The pressure

to maintain quality, as is necessary to achieve patient safety, comes at the expense of scalability and sustainability. These factors increase the cost of goods for these therapies, especially for patient-specific cell therapies, to a level that is unsustainable in the long term[165]. There is therefore a need for biologists and engineers to collaborate on further developments in both clinical and CMC aspects to solve these challenges, as has been achieved with other biologic platforms discussed above.

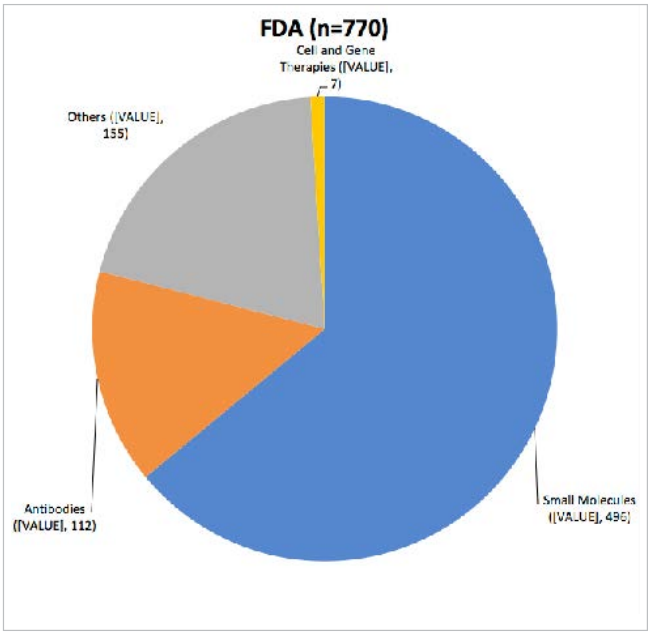
Overall, gene and cell therapies as a platform are at an early stage of development compared with small molecules, antibodies and protein replacement therapies. In addition, the complexity and cost of manufacturing viral vectors and cell therapies are much higher than for small molecules. Nevertheless, the possibility that such therapies could be a one-time treatment or even a cure for a disease has profound implications for the development of rare disease therapeutics.

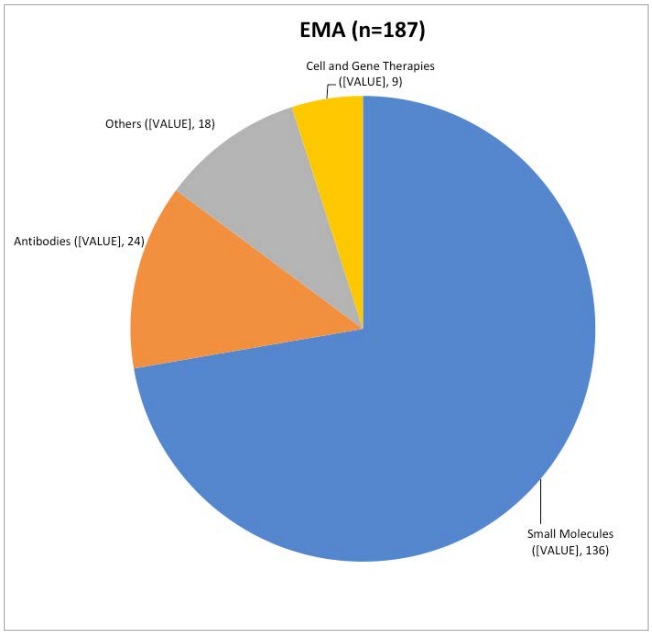
Characteristics of Therapeutic Modalities

Modality	Cause of disease at the protein level		Molecular target			Protein target localization			Delivery		
	Reduction or loss of function	Excessive or detrimental function	DNA	RNA	Protein	Extracellular	Plasma Membrane	Intracellular	Oral	Injection	Inhaled
Small molecule	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Protein replacement	✓				✓	✓		✓		✓	
Antibody		✓			✓	✓	✓			✓	
Oligonucleotide therapy	✓	✓		✓		✓	✓	✓		✓	
Cell and gene therapy	✓		✓			✓	✓	✓		✓	

Sources: ClinicalTrials.Gov; FDA

APPROVALS BY THERAPEUTIC MODALITY





Sources: EMA; FDA

Glossary of Terms

Accelerated approval	This allows a product for a serious condition to be approved on the basis of a surrogate end point or an intermediate clinical end point. Confirmatory post-marketing trials will be needed to verify this benefit.
Accelerated assessment	The evaluation of a marketing authorization application under the centralized procedure in the European Union can take up to 210 days. on request, the time frame can be reduced to 150 days if the applicant provides sufficient justification that the medicinal product is expected to be of major public health interest, particularly in cases of therapeutic innovation.
Adeno-associated Virus vectors (AAV)	AAV vectors are based on wild-type AAV, which has a single-stranded circular genome of roughly 4.7 kilobases. The AAV genome contains two open reading frames bounded by inverted terminal repeats into which a transgene of up to approximately five kilobases can be inserted.
Approval under exceptional circumstances	in exceptional cases, a reduced data set is acceptable by the European Medicines Agency for candidate drugs for a rare indication with a high medical need if it is difficult to obtain sufficient data to fulfil the requirements of a full dossier for marketing authorization in a reasonable time frame. Annual review of clinical data obtained after such approval is required, with the potential to maintain or withdraw the authorization.
Breakthrough therapy designation	This FDA designation can expedite development of drugs for which preliminary clinical evidence indicates that they may offer substantial advantages over existing treatment options for patients with serious or life-threatening diseases. Designated drugs are eligible for the expedited processing that fast-track designation offers, as well as intensive guidance on efficient development from the FDA.
Capsid	A protein shell that originally encloses the viral genome.

CFTR	Gene encoding the cystic fibrosis transmembrane conductance regulator protein, an ion channel in the membrane of cells that produce mucus, sweat, saliva, tears and digestive enzymes. Mutations in CFTR that affect the production, processing or function of the protein underlie cystic fibrosis.
Conditional marketing authorization	This European Medicines Agency pathway is similar to the accelerated approval process in the United States. Applicants may be granted a conditional marketing authorization for medicines for which the benefit of immediate availability outweighs the risk of less comprehensive clinical data than normally required.
Fast track pathway	This can expedite the review of products to treat serious conditions. The process allows sponsors to have more frequent meetings and communications with the FDA to address appropriate data collection and design of clinical trials. it also allows a sponsor to be eligible for priority review and a rolling review of the application.
Good manufacturing practice	A system for ensuring that products are consistently produced and controlled according to defined quality standards.
Hematopoietic stem cells (HSCs)	Cells that can replenish all blood cell types. HSCs derived from bone marrow have been used for many years to treat cancer; patients receive a myeloablative conditioning regimen to remove diseased cells before transplantation, with the transplanted HSCs then reconstituting the hematopoietic system. A similar strategy can also be used to treat inherited blood disorders.
Intrathecal injection	Delivery of a substance directly to the spinal fluid (intrathecal space) through a drug delivery system comprising a pump and a catheter.
Lipinski's Rule of Five	These guidelines identify several physicochemical properties to be considered for small molecules that are intended for oral delivery: molecular mass 500 Da or less; five or fewer hydrogen- bond donors; fewer than 10 hydrogen-bond acceptors; and calculated octanol–water partition coefficient (a surrogate for the ability of a molecule to cross biological membranes) of 5 or less.
Nanobody	A type of single-domain antibody fragment.
Pegylation	Attaching polyethylene glycol chains to therapeutics, particularly proteins, can improve characteristics such as immunogenicity, and pharmacokinetics. For example, pegylation has been used to extend the half-life of factor VIII replacement therapies for hemophilia.
Priority Medicines (PRIME) scheme	A scheme in the European Union that provides early and enhanced scientific and regulatory support for medicines that may offer a major therapeutic advantage over existing treatments, or benefit patients without treatment options.
Priority review	This is a designation that allows the FDA to act on a marketing authorization application in 6 months (compared with 10 months for standard reviews). To be eligible for priority review, the intended medicine should offer significant advancements in safety and efficacy of treatment, diagnosis or prevention of a serious condition.
Regenerative medicine advanced therapy designation	This FDA designation is similar to the breakthrough therapy designation and is available for cell therapies, therapeutic tissue engineering products, human cell and tissue products and combination products if the product is intended to treat serious or life-threatening diseases.
"Umbrella Trial"	A clinical trial design in which a single drug is evaluated in more than one disease simultaneously.

CONCLUSION

The achievements in the last two decades in the diagnosis, management and research on rare diseases have been unprecedented. The advancing studies of rare diseases toward genetic causes and effective therapies have been progressing rapidly. Policies and guidelines concerning rare diseases have been issued in different regions and countries. Life quality of patients with rare diseases has been improving with such advances internationally. Increases in research funding support have been providing more and more coordination in different disciplines/areas of management to provide the forming of best practice.

Awareness of rare disease has been raising quality of life, and the ensuing impact on patients has been improving, although we are still facing challenges in medical and nonmedical issues. These challenges have been reducing with more knowledge and awareness of rare diseases in the community/society in the healthcare strategies and established in vitro diagnostic and bioinformatics systems.

Further and deep studies on rare diseases across different levels and aspects, including the cell types, tissues, and organs, are associated with rare diseases and the interactions between different cell types to explore mysteries.

Rare disease is named historically with the limitation of technologies in the symptomatic era under the condition from clinical data to distinguish from the common diseases, such as nutritional and infectious diseases which were relatively and predominantly higher.

However, it is believed that more rare diseases will be identified and reclassified in the future in the genomic era since we know that “rare disease” is probably not a proper terminology to be used currently to classify such a disease genetic base affecting a large population worldwide.

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THERAPEUTIC DIGESTS

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Rare Disease