

RARE DISEASES, EXTRAORDINARY ASPIRATIONS. HIGHLIGHTS FROM THE SOCIETY FOR MEDICINES RESEARCH SYMPOSIUM, HELD OCTOBER 13, 2016 – COVENT GARDEN, LONDON, UK

P. Jeffrey¹, R. Lock², D. Pryde³ and J. Ritchie⁴

¹Pfizer Ltd, Granta Park, Great Abington, Cambridge; ²Aucuba Sciences Ltd., Canterbury, Kent; ³Curadev Pharma Ltd., Sandwich, Kent;

⁴Centre for Drug Development, Cancer Research UK, London, UK

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SUMMARY

More than 7,000 rare diseases are known that collectively affect some 6-7% of the developed world's population. Individually, any single rare disease may only affect a handful of people making them historically a relatively unattractive prospect for the biopharmaceutical industry to target. However, ground-breaking legislation starting with the Orphan Drug Act that was passed in the U.S. in 1983 now provides financial incentives for companies to develop orphan drugs,

designated to treat rare or “orphan” diseases. Today, rare diseases and the drugs that treat them are sufficiently attractive that many companies and research institutes now have units dedicated to this area of research. A panel of international experts from academia, industry and disease advocacy organizations shared their views on how rare diseases can be effectively tackled, as exemplified using case studies and opinion pieces covering basic science, drug discovery and development, and the impact of regulatory factors. This Society for Medicines Research Symposium was held at The Grand Connaught Rooms, Covent Garden, London, with sponsorship provided by Charles River, The European Federation for Medicinal Chemistry, GlaxoSmithKline, Pharmidex and Vertex.

Key words: Rare diseases – Orphan drugs – Drug discovery – Clinical development – Genetic disorders

PUTTING PATIENTS ON THE TEAM TO IMPROVE THERAPY DEVELOPMENT

The opening talk of the meeting considered the important role that patients and families with life-limiting and chronic conditions can play in influencing the priority-setting agenda for innovative research and how novel therapies are developed against rare diseases.

Genetic Alliance UK incorporates over 180 patient support groups for individuals and families with a rare disease, including those that affect just a handful of people through to those that affect thousands. It advocates strongly that research is the key to progress and that robust translation from in vitro or laboratory studies into humans is essential. The director of Genetic Alliance UK, Alastair Kent OBE, described several powerful examples where the involvement of patients in rare disease research has helped to understand the condition, define the most relevant clinical question to pose, recruit volunteers and validate the outcomes. One example is in the case of Duchenne muscular dystrophy (DMD) wherein a common clinical endpoint is the 6-minute walk test. While a loss of mobility can be distressing for patients and their families, a loss of motor function in the hands is even more devastating for boys with the

condition as it limits their ability to interact, socialize and engage with others despite clinical trials not being powered to address these endpoints. The James Lind Alliance has been established to bring together patients, carers and clinicians to work together to agree which clinical uncertainties about the effects of a new treatment matter most and deserve priority attention.

Groups such as the British Tinnitus Association have influenced research priorities through publishing the top most compelling research areas for the condition, which has been used by researchers as proof of patient engagement when approaching funding agencies. The AKU Society used a crowdfunding approach to raise money to run a clinical trial with the drug nitisinone in patients with alkaptonuria (AKU or black bone disease, a rare genetic condition that leads to a gradual build-up of homogentisic acid leading to significant damage to bone, cartilage and tissue). Social media and the Internet have provided opportunities for people to share knowledge of their conditions, treatment options and symptoms more widely than was ever previously possible. SWAN UK (Syndromes Without A Name) provides an accessible resource for families who would otherwise struggle to obtain a diagnosis for their child. Online registries such as Patients Like Me have become established means to share knowledge and research data quickly and widely and offer peer-to-peer support.

This has also led to improved means of recruiting patients for clinical trials. A good example is Pompe disease, a rare genetic disorder that leads to the accumulation in the body of glycogen due to a deficiency in the lysosomal enzyme α -glucosidase. Through online registration, patients were identified and then recruited for a clinical trial that otherwise would have been very difficult to source.

Patients and families are now becoming more informed about the options that are available to them, and less accepting of poor quality information and data. For example, Eurogentest is an E.U. project to harmonize high-quality genetic testing across all of the E.U., available to all. They provide detailed information leaflets in over 30 languages covering what patients would like to know about their conditions and how testing is performed and interpreted. Armed with more information, patients and carers are more empowered and want to have a say on benefit/risk, study designs and how best to support participation in research projects.

It is essential that new experimental treatments are properly regulated with real world, meaningful evidence and incentives offered for drug repurposing against a new indication when the data support it.

Close relationships with rare disease patients and families can create a virtuous circle to attract resources, increase relevance, facilitate partnerships, secure dissemination, increase uptake and demonstrate added value for healthcare systems and all members of the community. Their involvement is increasing and essential, as partners not subjects, in research projects of the future.

Further information on Genetic Alliance UK and the other organizations described herein is available at these websites: www.geneticalliance.org, www.akusociety.org, www.cancerresearchuk.org, www.patientslikeme.com, www.undiagnosed.org.uk, www.jla.nih.ac.uk/about-the-james-lind-alliance.

CYSTIC FIBROSIS DRUG DISCOVERY AND DEVELOPMENT – MORE THAN VENTURE PHILANTHROPY

Dr. Chris Penland, Vice President of BioPharma Programs at the US Cystic Fibrosis Foundation, shared his view of the way that drug discovery and development can be spurred along for an orphan disease like cystic fibrosis (CF) and ways to address challenges that success in a small disease population can bring. CF is a recessive genetic disease and is primarily characterized by enhanced mucus accumulation in the lung with accompanying microbacteria infections that ultimately leads to mortality. The functioning of several other major organs is also disrupted in CF (liver, pancreas, small intestine, skin, reproductive tract and skeletal system) and the average life expectancy, although improving, is approximately 40 years. Mutations in the cystic fibrosis transmembrane regulator (CFTR) are responsible for the disease and over 1,000 variants in the human *CFTR* gene have been described to date. The molecular consequences of *CFTR* mutations can be broadly classified as follows: Class 1 – premature translation termination; Class 2 – impaired folding/trafficking; Class 3 – impaired channel gating; Class 4 – poor channel conductance and Class 5 – deficient membrane residence time.

The most prevalent mutation is deletion of phenylalanine at position 508 (F508del), which interferes with protein folding, trafficking to the apical membrane, membrane residence time and channel gating. In addition to F508del a further mutation of note is G551D, which results in defective gating but with little effect on trafficking and affects ~4% of the CF population.

By the late 1990s, two CF drugs that targeted and helped alleviate some of the symptoms of CF had been approved with the Foundation's support. However, there were no treatments in development to address the underlying mechanistic cause of the disease. In addition, pharmaceutical companies showed little to no interest in rare diseases. As a result, the U.S. CF Foundation pioneered venture philanthropy (whereby a voluntary health organization funds drug development with for-profit companies) and in 1998 began an effort to stimulate CF-specific drug discovery and development.

In 2000, the CF Foundation made its first large investment with Aurora Biosciences (now part of Vertex Pharmaceuticals) to discover compounds that might correct the core genetic defect in people with CF. The joint drug discovery and development efforts led to identification of the CFTR modulators VX-770 and VX-809 (Fig. 1). In recombinant cells VX-770 increased CFTR channel open probability (P_o) in both the F508del processing mutation and the G551D gating mutation. VX-770 also restored chloride anion secretion in G551D/F508del human bronchial epithelia to approximately 50% normal. Furthermore, VX-770 reduced excessive sodium ion and fluid absorption to prevent dehydration of the apical surface and increased cilia beating in epithelial cultures (1). In cultured human bronchial epithelial cells (HBECS) isolated from patients with CF homozygous for F508del, the CFTR corrector VX-809 improved F508del-CFTR processing in the endoplasmic reticulum and enhanced chloride secretion to approximately 14% of non-CF human bronchial epithelial cells, a level associated with mild CF. F508del-CFTR corrected by VX-809 exhibited biochemical and functional characteristics similar to normal CFTR (2). Furthermore, in combination, the positive effect of VX-809 in increasing airway surface liquid height in HBECS was magnified by VX-770 coadministration.

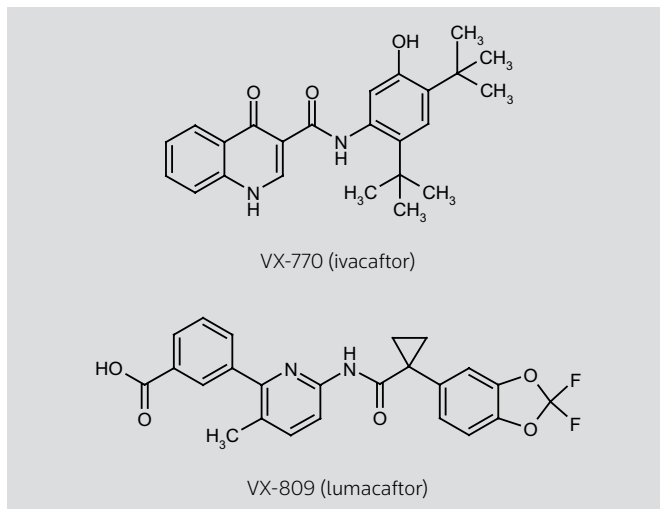


Figure 1. Chemical structures of VX-770 (ivacaftor) and VX-809 (lumacaftor).

On January 31, 2012, the U.S. Food and Drug Administration (FDA) approved ivacaftor (Kalydeco™; VX-770) for the treatment of CF in patients ages 6 years and older who have the specific G551D mutation in the *CFTR* gene. Two 48-week, placebo-controlled clinical studies involving 213 patients, one in patients ages 12 years and older and another in patients ages 6 to 11 years, were used to evaluate the safety and efficacy of ivacaftor in CF patients with the G551D mutation. In both studies, treatment with ivacaftor resulted in significant and sustained improvement in lung function. The FDA Commissioner Margaret A. Hamburg, M.D. stated that “the unique and mutually beneficial partnership that led to the approval of Kalydeco serves as a great model for what companies and patient groups can achieve if they collaborate on drug development.” The Foundation’s targeted investment with Aurora Biosciences had paid off for thousands of people with CF.

Ivacaftor treats a minority of the CF population, but a wider proportion of the community received a treatment option when the FDA approved the first combination therapy in July 2015. Lumacaftor/ivacaftor (Orkambi™; VX-809/VX-770) addresses the underlying cause of CF in people who have two copies of the F508del mutation (half of the CF population). The safety and efficacy of Orkambi was studied in two double-blind, placebo-controlled clinical trials of 1,108 participants with CF who were 12 years and older with the F508del mutation. In both studies, participants with CF who took Orkambi, two pills taken every 12 hours, demonstrated improved lung function compared to those who took placebo. Clinical trial results also indicated significant increases in BMI and decreases in hospitalizations and the need for intravenous antibiotics.

Dr. Penland went on to share aspects of the CF Foundation Venture Philanthropy Model (tailored financing, multi-year support, performance measurement, engagement) and the additional measures taken by the CF Foundation approach to de-risk pharmaceutical company involvement in rare diseases like CF (nonmonetary assistance such as access to a “CFTR toolbox”, clinical trial infrastructure, patient education). In order to address the operational aspects

of development, the CF Foundation developed “The Cystic Fibrosis Therapeutics Development Network” (TDN), which is the largest CF clinical trials network in the world, bringing together experts from across the U.S. to evaluate the safety and effectiveness of new CF therapies through clinical studies. Over the last 5 years, the total number of TDN trials has approximately doubled. But there are opportunities for improvement in clinical trial participation through patient access to the CF Foundation clinical trial finder page (www.cff.org/Trials/finder) and recruitment into interventional studies. To improve education in CFTR, the Clinical and Functional Translation of CFTR (CFTR2) website (www.cftr2.org) is available to the general public, patients and their family, healthcare professionals and researchers and documents the disease liability of CFTR variants. While it is clear that CF drug discovery and development is more than just “venture philanthropy”, the Foundation’s approach to this has been nationally recognized and praised by leaders in medicine, business and healthcare advocacy. During President Barack Obama’s 2015 State of the Union address, he cited the story of CF as an example of how non-profits, the pharmaceutical industry, researchers, patients and their families can work together to produce more targeted and effective treatments for diseases. The approach has been widely emulated by many other rare disease nonprofits and a National Institutes of Health initiative has adopted CF Foundation strategies to advance drug development for rare and neglected diseases (www.cff.org).

LENTIVIRAL GENE THERAPY FOR MONOGENIC DISEASES OF THE BONE MARROW: CURRENT PROGRESS AND FUTURE PROSPECTS

Professor Bobby Gaspar, Professor of Paediatrics and Immunology, Centre for Immunodeficiency, University College London Institute of Child Health and Great Ormond Street NHS Trust and CSO of Orchard Therapeutics, gave an excellent and inspiring talk on the application of gene therapy for the treatment of immunodeficiencies in children.

Gene therapy approaches to target rare diseases are gathering momentum, with research reported against retinal abnormalities, CF, leukemias, lysosomal storage diseases, hemophilia and skin conditions to name but a few. Primary immunodeficiencies have played a major role in the development of gene therapy for monogenic diseases of the bone marrow. The last decade has seen convincing evidence of long-term disease correction as a result of ex vivo viral vector mediated gene transfer into autologous hematopoietic stem cells, which obviates the need for bone marrow transplants and avoids graft-versus-host disease developing. The success of these early studies has been balanced by the development of vector-related insertional mutagenic events. More recently, the use of alternative vector designs with self-inactivating (SIN) designs which have an improved safety profile has led to the initiation of a new wave of studies which are showing early signs of efficacy.

X-linked severe combined immunodeficiency (SCID-X1) results from abnormalities of T and NK cell development due to the growth of lymphocytes being blocked. Professor Gaspar described new gammaretroviral SIN vectors for gene therapy of SCID-X1 that are showing startling efficacy in small patient studies with a sustained recovery of CD33⁺, CD8⁺ and CD4⁺ cell populations after a single gene therapy treatment, with extremely low levels of insertional mutational errors.

Another example of a SCID is that due to adenosine deaminase deficiency (ADA-SCID), an autosomal-recessive genetic disorder that leads to a build-up of deoxyadenosine in cells which is toxic to lymphocytes. The majority of patients with ADA-SCID do not have a matched human stem cell donor and survival rates remain low. A lentiviral vector gene therapy study in a murine model of ADA deficiency showed correction of T-cell compartments with a very low mutational frequency. This has stimulated a clinical study in both the U.K. and the U.S. to investigate lentiviral gene therapy for patients with ADA-SCID using autologous CD34⁺ cells to restore T-cell and B-cell function in a lasting and sustained manner, which follows on the heels of the very recent approval of market authorization for Strimvelis™ (3), a gene therapy product for ADA-SCID developed by GlaxoSmithKline. Other SCIDs that have been targeted using lentiviral gene therapy include Artemis deficiency (4), RAG3 deficiency (5, 6) and Wiskott-Aldrich syndrome (7).

A final example discussed by Professor Gaspar was chronic granulomatous disease (CGD), a hereditary disease in which cells of the immune system cannot form the reactive oxygen species required to kill pathogens leading to granulomata in many organs. In a murine model of stem cell gene therapy for CGD, a lentiviral vector targeted to myeloid cells resulted in high levels of gp91 (phox) expression in committed myeloid cells and granulocytes and restored normal NADPH-oxidase activity (8). A multicenter clinical trial with the lentiviral vector is continuing.

Recently, an E.U.-supported consortium with Horizon 2020 funding, SCIDNet (<https://scidnet.eu/>), has been established to convert much of the promising research in this area into genetic therapy of SCIDs through clinical trials and, in the case of ADA, taking an existing candidate through to market authorization of a licensed medicine.

All of the studies described above are multicenter using lentiviral vectors and have the potential to recruit patients rapidly and to show efficacy and safety. We are now seeing the first ever cures with gene therapy that are now available for specific SCID and metabolic conditions. The ongoing development of safer vector platforms and gene editing technology together with improvements in cell transduction techniques and optimized conditioning regimes is likely to make gene therapy amenable to a greater number of pediatric immunodeficiencies (PID). If long-term efficacy and safety are shown, gene therapy will become a standard treatment option for specific forms of PID. These technologies may also be important for other monogenic disorders of the hematopoietic system.

IRCI – SETTING UP AN INTERNATIONAL RARE CANCERS INITIATIVE TO PROMOTE GLOBAL RESEARCH IN A DIFFICULT FIELD

Professor Matt Seymour, Professor of Gastrointestinal Cancer Medicine at the University of Leeds, Director of Clinical Research for the UK National Cancer Research Institute and Specialty Cluster Lead within the NIHR Clinical Research Network, gave his perspective on the unmet needs and challenges associated with the clinical development of new therapies for rare cancers. Rare cancers are generally defined as having an age standardized rate (ASR) of less than 6 cases per 100,000 population. When taken together they account for over 20% of new cancer diagnoses per year, which is more than any single “common” cancer such as breast or lung cancer. Nevertheless,

the average outcomes for patients diagnosed with a rare cancer are generally inferior compared to common cancers with poorer mortality, survival and there has been less improvement over time.

Difficulties in finding sufficient patients at the national level to enable drug development via normal drug development paradigms have led to a continued dearth in effective therapies for rare cancers. Because of their perceived low marked potential, rare cancers are rarely included in pharmaceutical company-sponsored trial programs or supported for investigator-initiated studies, while small patient numbers and uncertain feasibility makes rare cancer trials less likely to secure academic grants. Due to this, the International Rare Cancers Initiative (IRCI) was formed in 2011 with the aim of stimulating and facilitating the development of international clinical trials for patients with rare and particularly very rare cancers (ASR < 2/100,000 new cases per year). The founding international partners of IRCI are government and charity-supported clinical trial networks in the U.K. (National Cancer Research Institute), Europe (European Organisation for Research and Treatment of Cancer) and the U.S. (National Cancer Institute Cancer Therapy Evaluation Program). The IRCI has subsequently grown to include partners from Canada, Australia, Japan and France.

Rare cancers are selected for clinical trials by the IRCI based on several criteria that include an incidence < 2/100,000 per year but sufficient patients for a trial to be feasible, not usually being included in ‘common’ trials, no existing international trial group(s), no (or inadequate) existing trials, a potential for an interventional trial (randomized if possible) and enthusiastic champions in at least two of the partner organizations. Once an indication has been identified, a study group is then launched to drive the set-up and delivery of the clinical trial. Eleven IRCI study groups have been set up to date covering a diverse set of indications.

The initial aim of the IRCI was confined to developing randomized interventional studies, but this has subsequently been relaxed due to associated design feasibility challenges and a lack of baseline standard of care therapy(ies) for many rare cancers. Consequently, some groups are now able to consider nonrandomized designs or observational cohorts to complement their interventional and randomized clinical trials. An important part of the IRCI program has been to develop and apply novel trial methodologies to overcome the difficulties of research with fewer patients, and the group has published recommendations on clinical study design for rare cancers (9).

Certain aspects such as clinical researcher enthusiasm, research funder and consumer engagement are working well, whereas other areas still remain a challenge. These include the following:

- Difficulties in funding rare cancer research across the international stage. Principally due to lack of understanding of the disease(s) and associated methodologies, as well as the risk of funding not being approved by different national review bodies (i.e., “double” or “triple jeopardy”).
- Lack of industry engagement produced by a perceived low return on investment.
- Complexity of setting up legal agreements across international jurisdictions, leading to slow and costly trial set up procedures.

In order to tackle some of these issues, several different approaches to conducting clinical trials are being tested. These include “piggy

backing” lower-powered, rare cancer arms onto single protocols alongside more common cancers, incorporating multiple different rare cancer types under a single protocol that can be continually amended to drop/add arms, and the use of population level genotyping of common and rare cancers together to enable joint molecular bucket trials.

Further information on the IRCI can be found at their website: www.irci.info.

OVERCOMING THE “RARE DISEASE” HURDLE IN DEVELOPING DRUGS FOR CHILDHOOD CANCER

Professor Pamela Kearns (Professor of Clinical Paediatric Oncology and Director of the Cancer Research UK Clinical Trials Unit Institute of Cancer and Genomic Sciences, University of Birmingham) gave an excellent presentation on drug development for pediatric cancers, its unique challenges compared to that of adult cancers and how they are being addressed at the international level.

Pediatric cancers are relatively rare, affecting around 1 in 500 children with many indications being specific to children. Despite the fact that over 75% of children with cancer can be cured of their disease (considered disease free after 10 years), it remains the most common cause of death in childhood. Current standard of care often involves surgery, radiotherapy and multiagent chemotherapies. This is associated with long-term side effects and morbidities in 20-40% of survivors such as cardiovascular impairment thought to be triggered by effects of therapy on the developing immune system. Given this, there is an urgent need for development of new drugs for children with cancer that are better targeted to combine greater efficacy with reduced toxicity.

Drug development for childhood cancers is challenging due to the small patient groups involved, requiring novel trial designs such as adaptive and Bayesian approaches. Due to this situation, there is a lack of commercial incentive to invest in developing innovative cancer therapies for childhood cancer. Establishment of the Paediatric Medicines Regulation in 2007 and the requirement for organizations such as pharma to implement pediatric investigation plans (an agreed development plan to ensure that the data required to support approval of medicines for children is in place) was developed to overcome this lack of commercial interest. Even so, cancer drug development for pediatric populations is still reliant on and driven by the needs of more common adult cancers. In the majority of instances, a class waiver that exempts development in children (e.g., crizotinib in the E.U.), deferred development until just prior to market authorization (e.g., immune checkpoint inhibitors) or halting pediatric development if an agent fails in adult cancer trials are the norm. This is despite there often being a strong scientific rationale for development of modern targeted therapies specifically in pediatric cancer indications and the many pitfalls of directly extrapolating adult trial data to children. For example, some pediatric cancers have distinctive underlying molecular pathologies that may produce different responses to therapy, children handle drugs differently due to altered pharmacokinetics, and often have higher tolerability thresholds when compared to equivalent adult doses. Rather disappointingly, only three new oncology products have been approved for treatment of childhood cancers under the Paediatric Medicines Regulations (imatinib, Abraxane® and bevacizumab).

In order to tackle the challenges of developing innovative new therapies for childhood cancers, a European academic consortium (Innovative Therapies for Children with Cancer [ITCC]) was set up in 2003. The ITCC offers a comprehensive program of early clinical evaluation of anticancer drugs for children and adolescents with treatment-resistant cancers across 9 countries and comprises 50 academic institutions. Achievements of the consortium to date include delivery of more than 35 phase I and II trials, recruitment of over 1,100 children and adolescents to new drug trials using 37 new anticancer drugs and contributing to 26 pediatric investigation plans across commercial and academic studies in all tumor types. Specific challenges of delivering multi-country, academic and academic-industry collaborative clinical trials within the current European regulatory framework have also been addressed by the ITCC.

More recently, a multi-stakeholder pediatric oncology platform called ACCELERATE (www.accelerate-platform.eu) has been set up in a partnership between the Cancer Drug Development Forum (CDDF), the European Society for Paediatric Oncology (SIOP-E), parents and patient representatives (Unite2cure), industry and regulatory bodies (10) with the aim of further accelerating new oncology drug development for the front-line treatment of pediatric cancers. Several proposals in order to achieve this goal have been put forward by ACCELERATE:

- Mandatory pediatric investigation of drugs based on ‘mechanism of action’ rather than adult disease (11).
- Prioritization of drugs in order to match the best available therapies to children with rare or refractory malignancies.
- Reduction in the delays in starting pediatric development of potentially life-saving innovative drugs.
- More effective and flexible rewards to better incentivize the development of new and specific pediatric medicines.

TREATMENTS FOR SANFILIPPO SYNDROME A: APPROACHES TO ENZYME REPLACEMENT THERAPY

Dr. Stephen James, Vice President for Research & Translational Science and a member of the executive leadership team at SOBI, described the company portfolio of drug discovery and development projects that span a range of therapy areas from genetics and metabolism through inflammation and hemophilia. Dr. James then expanded on the MPS IIIA project as an example of the SOBI approach to a rare disease.

Mucopolysaccharidosis type IIIA (MPS IIIA, Sanfilippo syndrome A) is a severe degenerative disease (12). It is an autosomal-recessive lysosomal storage disease caused by a deficiency in the enzyme sulfamidase, a sulfatase involved in the step-wise degradation of the glycosaminoglycan (GAG) heparan sulfate (HS). HS accumulates in the lysosomes of cells, causing cell dysfunction and, eventually, cell death and in particular affects the central nervous system (CNS) (13). The incidence of MPS IIIA is 0.28-4.1/100,000 live births. Babies can at first appear normal, but through their toddler years develop more slowly than their peers and can present difficult behaviors such as wakefulness at night and hyperactivity. By the second decade of life it is common for complete motor disability and cognitive decline. Survival into the third decade of life is rare and there is currently no effective treatment for MPS IIIA beyond palliative care.

Experimental therapies generally fall into one of three approaches: substrate reduction, enzyme replacement and gene therapy. The latter approach is gaining more attention in the biomedical community, including intracerebral injections of a gene encoding a sulfhydrolyase (14, 15) and a single delivery of a normal copy of the defective gene into the CNS of the patient (16). Results from trials using these approaches are eagerly anticipated. Clinical trials are continuing with enzyme replacement therapy (ERT) approaches. For example, Shire has been using ERT of the deficient enzyme, sulfamidase, via intracerebrospinal fluid injection to access the brain, reducing neuropathological changes and improving symptoms in a congenic mouse model of the disease (17). Many of these approaches are invasive in nature and SOBI had set out to develop an intravenous agent that would be suitable for lifelong treatment of MPS IIIA.

SOBI-003 is a chemically modified variant of recombinant human sulfamidase with a prolonged plasma half-life, which facilitates uptake of the molecule across the blood-brain barrier (BBB). Modifications to the glycan units of sulfamidase extend the plasma half-life of SOBI-003 to several hours, in contrast to the several-minute half-life of the wild-type enzyme, and stabilize SOBI-003 in the targeted lysosomal compartment. This results in enrichment of SOBI-003 at the BBB and in brain parenchyma, which was shown to be a non-saturable slow process to reach the CNS compartment and therefore unlikely to be via a pinocytosis mechanism, although the mechanism of uptake at this stage is not clear.

SOBI-003 reduces brain levels of HS in MPS IIIA mice in a dose-dependent manner, dampens inflammatory signals in the cerebral cortex and thalamus, and improves neuromuscular capability in a wire suspension mouse model to restore a close to normal phenotype in treated mice.

These data have underpinned the progression of SOBI-003 with clinical trials planned in the near future.

FAMILIAL AMYLOID POLYNEUROPATHY: HOW A GENETIC DISEASE INFORMS DRUG DISCOVERY

Dr. Chris Bulawa's (Pfizer Rare Disease Research Unit) presentation entitled, "Familial amyloid polyneuropathy: How a genetic disease informs drug discovery," began with a history lesson. In 1945 Dr. William B. Castle (the famous American physician and physiologist who is credited for transforming hematology "from an art... to an interdisciplinary science") and Dr. Linus Pauling (the Nobel Prize winning chemist) met by chance and talked about some of the work Castle had been doing on sickle cell. Castle mentioned that when red cells sickled, they changed shape showing birefringence in polarized light. He believed that some kind of molecular alignment or orientation was occurring and Castle suggested that this might be something in which Pauling might be interested. It turned out that it was and the following year, Pauling and his colleagues at the California Institute of Technology began the studies that eventually showed that the hemoglobin in sickle cell disease was abnormal. In November 1949, Pauling and coworkers published their work demonstrating that individuals with sickle cell disease had a modified form of hemoglobin in their red blood cells, and that individuals with sickle cell trait had both the normal and abnormal forms of hemoglobin. This was the first demonstration causally linking an abnormal protein to a disease, and also the first demonstration

that Mendelian inheritance determined the specific physical properties of proteins, not simply their presence or absence and heralded the dawn of molecular genetics. However, despite this early promise, treatment options for the 13 million estimated sickle cell disease (SCD) patients worldwide remain limited. Currently there is only one approved drug for SCD, hydroxyurea, and despite continued research the exact molecular mechanism is not fully understood. The challenge for this disease and for many other genetic diseases is that while the cause may be known, the feasibility of pharmacological repair is uncertain.

The transthyretin amyloidoses (ATTR) are often fatal diseases characterized by progressive neuropathy and/or cardiomyopathy. ATTR are caused by aggregation of the circulating protein transthyretin (TTR), a natively tetrameric protein involved in the transport of thyroxine and the vitamin A-retinol binding protein complex. Mutations within TTR promote its misfolding, aggregation and deposition as amyloid in peripheral nerves. Tafamidis is used in the treatment of transthyretin-type familial amyloid polyneuropathy (TTR-FAP) and slows disease progression in those patients with the V30M TTR mutation. Early extensive studies in 1952 in Portuguese patients identified the phenotype in fisherman who in later life would become thin and weary and begin walking slowly and bending their knees to compensate for an abnormal foot drop. Subsequent genetic studies showed that in populations of Portuguese descent, inheritance of one copy of V30 TTR typically led to TTR-FAP with severe clinical manifestations. However, a few V30M carriers developed either much milder symptoms and in some cases none at all. It transpired that these carriers have a different mutation, T119M, resulting in the formation T119M/V30M, a more stable tetramer comprising of the V30M and wild-type subunit. It was therefore postulated that mimicking the action of T119M with a ligand that stabilizes the tetramer, so-called TTR kinetic stabilizers, could yield disease-modifying agents. Using a structure-guided screening approach, researchers at FoldRx identified a series of potent benzoxazole stabilizers that were shown to dose-dependently inhibit fibril formation *in vitro*. However, demonstrating *in vivo* activity proved challenging in that transgenic mice expressing human mutant TTR (huTTR) while showing a conserved sequence (80% identity vs. human) do not deposit amyloid. Transgenic mice overexpressing huTTR by 30-fold were shown to produce amyloid but the doses required to achieve suppression of the high nonphysiological levels of circulating TTR were extremely high suggesting that overexpression of the target to achieve pharmacological proof of principle is not necessarily always the best approach.

In phase II and III clinical trials, tafamidis demonstrated clinical efficacy through kinetic stabilization in V30M TTR-FAP patients. Tafamidis was initially approved for commercial use in Europe in 2011 and has since been approved for use in Japan, Mexico and Argentina where it is used as a first-line treatment option for patients with early-stage TTR-FAP. In concluding and reflecting on the SCD introduction, Dr. Bulawa remarked that the important insights gleaned from human genetic studies combined with biophysical studies of TTR variants all contributed to the therapeutic strategy around native state stabilization which led to the discovery and development of tafamidis, the first and currently only small-molecule, disease-modifying oral treatment for an amyloid disease.

MODULATORS OF UTROPHIN PRODUCTION FOR THE TREATMENT OF DUCHENNE MUSCULAR DYSTROPHY

Duchenne muscular dystrophy (DMD) is a severe progressive neuromuscular condition characterized by a generalized weakness and progressive loss of muscle strength leading to debilitating cardiac and respiratory difficulties. DMD is one of the most common fatal genetic disorders diagnosed in children with ca. 50,000 patients in the developed world and a life expectancy of only 20 years. The disease is an X-linked recessive disorder with mutations, mostly deletions, causing altered expression of dystrophin. In DMD, no dystrophin is produced, whereas in Becker muscular dystrophy (BMD) truncated and semi-functional dystrophin is produced with the result of a less severe disease phenotype. Dystrophin is a rod-shaped, cytoplasmic protein that forms a crucial part of the dystrophin protein complex (or costamere) that connects the cytoskeleton of a muscle fiber to the surrounding extracellular matrix, enabling stabilization of the membrane during muscle contraction and relaxation. The lack of functional dystrophin causes repeated cycles of muscle necrosis and regeneration resulting in the eventual replacement of functional muscle fibers by adipose and connective tissue. There is currently no effective treatment for DMD.

In presenting "Modulators of utrophin production for treatment of DMD," Dr. Jon Tinsley (Summit Therapeutics) described three key areas of current research efforts and treatment strategies for DMD. Dystrophin restoration (exon skipping, nonsense mutations and gene therapy), which is unlikely to have broad utility as it will only be suitable in small and fragmented patient populations; symptomatic treatments (targeting different aspects of muscle biology including inflammation, fibrosis and muscle mass), and finally functional replacement, which based on a truly disease-modifying approach that has the potential to target 100% of DMD patients, irrespective of the gene mutation. The potential of utrophin to functionally replace dystrophin is not new and the discovery and development of utrophin modulators is not as an exciting opportunity for a transformational therapy. Utrophin (so named as a contraction of ubiquitous dystrophin) shares both structural and functional similarities with dystrophin; both dystrophin and utrophin provide the essential link between the actin cytoskeleton and cell membrane thus providing stability and elasticity in muscle fibers and both are expressed in the same location in the developing muscle. In studies using the mdx mouse model, Dr. Tinsley and coworkers observed that the loss of dystrophin could be compensated for by increasing the levels of the dystrophin-related protein, utrophin. Novel orally available small molecules capable of transcriptional upregulation of the utrophin gene that target both skeletal and cardiac muscle would therefore have the potential to treat all DMD patients.

Ezutromid (SMTC-1100) was discovered through a pharmacological-based approach using a novel phenotypic cell-based screen for early in vitro compound triage and subsequent evaluation in the mdx mouse for more in-depth in vivo phenotypic screening evaluating muscle biology. Murine H2K cells were transfected with a human utrophin promoter linked to a human reporter gene and stable mdx myoblasts generated (H2K-mdx utrA-luc) to support high-throughput screening (HTS). Using this HTS screen, researchers identified seven structurally distinct series based on screening a

3,000 diversity compound set with a hit rate (based on > 200% luc activity) of ca. 0.5-1%. From these seven series, a further 700 compounds were then synthesized to screen for potency, in vitro ADME and safety prior to in vivo evaluation in the mdx mouse. In total, 38 compounds were tested in vivo using a 4-week dosing regimen with both physiological and pathological endpoints. Of the four best compounds discovered with this iterative approach, ezutromid was the most potent (EC_{50} 0.47 μ M vs. luc) with an encouraging in vivo preclinical disease-modifying profile. Both utrophin protein and RNA levels were modulated combined with a reduction in membrane damage, muscle degeneration, fibrosis, chronic inflammation and myodystrophy. This overall improvement of whole muscle function plus the positive disease modification profile underpinned the decision for further clinical evaluation.

Ezutromid entered phase I clinical development in 2012. Single-dose (up to 400 mg/kg) and multiple-dose (up to 200 mg/kg b.i.d. for 10 days) studies in healthy volunteers were completed and although the drug was considered safe and well tolerated, the pharmacokinetics were variable, nonlinear and subject to a significant food effect (ca. 4-fold increase in both AUC and C_{max} following a fat meal). After further evaluation in both DMD patients and healthy volunteers investigating diet modifications and different formulations, a phase II proof of concept study has now commenced. In this multi-site U.S. and U.K. open-label trial of 48 weeks dosing, performed in ca. 40 DMD boys aged between 5 and 9 years old, the primary endpoints will be a change in baseline leg muscle parameters assessed by MRI examination on weeks 12, 24, 36 and 48. Secondary endpoints will be based on baseline changes in muscle biopsy samples measuring utrophin expression and muscle regeneration biomarkers while the 6-minute walk test, time to stand and other blood biomarkers at weeks 12, 24, 36 and 48 are exploratory only. Utrophin expression is anticipated to reduce the numbers of regenerating fibers, and novel staining and imaging techniques to quantify the number of regenerating fibers within the whole biopsy section are currently under development.

The clinical development of ezutromid is an exciting opportunity in the field of DMD therapy, but it is not the final chapter, Dr. Tinsley concluded. In collaboration with Oxford University, Muscular Dystrophy UK, the Muscular Dystrophy Association and the MRC Functional Genomics Unit, Summit Therapeutics is committed to delivering a pipeline of future generation utrophin modulators. A new series of small molecules, including those with a potentially differentiated mechanism from ezutromid, are currently undergoing lead optimization and it is hoped that these research efforts will deliver soon.

DISCLOSURES

P. Jeffrey, R. Lock, D. Pryde and J. Ritchie are in paid employment of their respective organizations. All authors are SMR Committee members for which no remuneration is paid.

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