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Statistical Considerations for Gene Therapy Rare Disease Clinical Trials

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Abstract

On December 18, 2017, the FDA approved Luxturna (voretigene neparvovec-rzyl), a new gene therapy product developed for treating patients born with a kind of retinal disorder due to biallelic *RPE65* gene mutation. Luxturna is a major medical advance since it is the first gene therapy to be granted approval by FDA that targets on a single gene. Approval of Luxturna may provide new prospects for curing various intractable and life-threatening rare diseases. As indicated by the former FDA Commissioner Scott Gottlieb, FDA can see a bright future of gene therapy, through which many dreaded, usually terminal medical problems may be solved, and now it is a critical time for the development of this novel form of treatment. As a result, FDA has been devoted to establishing an integrated, appropriate policy framework to encourage and administrate gene therapy research. FDA will also gradually promulgate a series of disease-specific guidance papers to demonstrate the clinical development and detailed approval process of investigational gene therapy products to discuss some critical and creative thinking that might be helpful to evaluate the efficacy of GT products and accelerate approval process. In this article, FDA guidance on clinical trial design issues for gene therapy development of rare diseases and some current innovative statistical considerations are reviewed. In addition, a case study of Luxturna, the first approved gene therapy for a rare disease is discussed.

Keywords: Rare disease drug development; Demonstrating effectiveness or not in effectiveness; Real-world evidence; Therapeutic index

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Introduction

Gene therapy is a technique to treat diseases by introducing exogenous normal genes into targeted cells to destroy abnormal genes or compensate beneficial protein products. There are three main mechanisms of gene therapies, namely, (i) replacing a defective gene with a normal copy of the gene, (ii) inactivating a defective gene, and (iii) transmitting a functional gene which can effectively treat the disease to the human body [1]. As indicated in the *2020 FDA guidance on Long Term Follow-Up after Administration of Human Gene Therapy Products*, human gene therapy attempts to treat or cure human genetic diseases by gene modification technologies that help certain biological processes return to normal [2,3]. Gene therapy products can be applied in a wide range of illnesses, such as cancer, communicable diseases, and hereditary diseases.

Without a carrier called vector, the modified gene cannot be inserted into body and work successfully. Certain vectors include plasmid DNA, viral vectors and bacterial vectors [1]. A plasmid is often a circular DNA molecule separated from bacteria, which can reproduce in other organisms. Thus, plasmids are a suitable tool to deliver therapeutic genes to specific tissues. Viral vectors are another natural carrier in gene engineering. Before being used as vehicles to transfer genetic materials, infectious viruses have to be modified to become a harmless form. Many gene therapy products are derived from viruses, such as lentiviral vectors used to treat sickle cell disease, AAV virus vectors applied in tuberous sclerosis [4,5]. The mechanism of bacterial vectors is similar to viral vectors, which is firstly removing the infectivity and then inserting the combination of bacteria and the therapeutic gene into human tissues. In addition to vectors, human gene editing technology and patient-derived cellular products are frequently used in gene therapy. For human gene editing technology, the principle is to stop the activity of defective genes or repair mutated genes. For patient-derived cellular gene therapy products, we directly extract pathological cells from the patient and finally returned the genetically modified cells to the patient [1].

As indicated by the National Institutes of Health (NIH) reports, there are about 7,000 rare diseases and over 25 million people in the United States suffer from them. Research shows that approximately 80% of rare diseases are due to single gene mutation, and are commonly diagnosed in children [3]. Moreover, most of the rare diseases are very hard to cure and usually fatal. However, effective therapies are limited. Thus, it becomes necessary for the research of effective treatments for rare diseases. Additionally, many rare diseases exhibit multiple sub-types or variations. As a result, the development of gene therapy products in the area of rare diseases has become very popular.

The remaining of this article is organized as follows. In the next section, FDA's guidance on gene therapy for rare diseases is reviewed. Some statistical considerations are given in Section 3, while Section 4 provides a detailed discussion of a recent FDA approved gene therapy regulatory submission. Some concluding remarks are given in the last section of this article.

FDA Guidance on Gene Therapy for Rare Diseases

To assist the sponsors to develop a human Gene Therapy (GT) product for rare diseases, FDA released an industry guidance document on *Human Gene Therapy for Rare Diseases* in 2020 [3]. The guidance is aimed to offer recommendations to researchers who seek to promote human gene therapy medication development targeting a specific rare disease in terms of the possible elements and issues necessary to be considered during each stage of the clinical trial design program. That noted this document does not establish a golden standard. FDA encourages any alternative method to support your product as long as it follows all the statutory requirements determined by FDA.

Although one rare disease affects only a few people, the total number of people suffering from rare diseases is enormous. Moreover, many rare diseases have no approved efficient therapies. Phenotypic heterogeneity and multi-subtypes of disease also add the difficulty of treatment. Therefore, natural history studies are quite important in treatment development for rare diseases since natural history studies can provide comprehensive knowledge of a disease. However, limited information from the natural history of rare diseases is available for clinical trial development [3,6,7].

Many factors should be considered during all phases of clinical trials design. One example is ethical issues since most of the rare diseases happen during childhood which involves enrollment of children and permission from parents. *Human Gene Therapy for Rare Diseases* proposes an outline of suggested elements could be considered into the development of rare diseases treatments (focus on, but not limited to gene therapy). The content is as follows [3]:

Study population

For the selection of study population, two points should be taken into account: First, potential adverse events and benefits should be identified in advance based on previous preclinical or clinical data. Second, the study population should be representative and have the potential to generate instructive safety and efficacy data [3,8]. When conducting gene therapy clinical trials for rare diseases, the following general principles should be considered [3]:

(i) If the disease is caused by a genetic defect, the sponsor should perform genetic tests for the specific defects in all clinical trial subjects. This information is important to ensure correct diagnosis of the disorder of interest. Since many of the disorders may involve different mutations of a specific gene, safety and effectiveness may be linked to specific genotype in unpredictable ways. Thus, early understanding of such associations may help in planning future clinical trials.

(ii) As pre-existing antibody to any component of the GT product may pose a potential risk to patient safety and limit its therapeutic effect, FDA suggested that sponsors may exclude patients with pre-existing antibodies to the product. In such cases, it is strongly suggested that the sponsor should develop a companion diagnostic to detect antibodies to the product.

(iii) At the stage of clinical GT trials design, adverse events, the anticipated risk, and potential benefits to subjects should be considered [3,8]. In addition, subjects with severe or advanced disease who might experience confounding adverse events related to the underlying disease should also be considered.

(iv) Since most rare diseases are pediatric diseases or show symptoms in childhood, pediatric studies are a critical part of drug development and need to be considered seriously. FDA also offers additional safeguards for children in clinical investigation. As indicated in 21 CFR 50.51, a clinical investigation should not show a risk greater than minimal risk unless presenting the prospect of direct benefit to individual subjects (21 CFR 50.52). If neither condition is satisfied, but the study may yield generalizable knowledge about the disorder or condition, may involve children as set forth in 21 CFR 50.53. Note that adequate provisions must be made to obtain the permission of the parents and the assent of the child as per 21 CFR 50.55.

(v) The risks of most GT products include the possibility of permanent unintended effects, along with adverse effects due to invasive procedures that may be necessary for product administration. Because of these risks, it is generally not acceptable to enroll normal, healthy volunteers into GT studies. A well-written informed consent document is also essential.

Study design

For rare diseases drug development, limited number of qualified patients can be enrolled in each phase of clinical studies is the most challenging issue. Insufficient study subjects increases the pressure for more pertinent data (e.g., adverse events, efficacy outcomes, biomarkers) collected from each patient, which is beneficial for further trials design. As a result, it is suggested that the sponsor should refer to the following general principles for selection of an appropriate study design [3]:

(i) A randomized, concurrent-controlled trial is generally considered the ideal standard for establishing effectiveness and providing treatment-related safety data. Randomization in early stages of development is strongly encouraged when feasible.

(ii) Sponsors should consider designing their first-in-human study to be an adequate and well-controlled investigation that has the potential to provide evidence of effectiveness and safety of the test treatment under study in support of regulatory submission and marketing application.

(iii) Placebo controls are suggested to be adopted for interpreting both safety and efficacy results. In a study that has multiple dose-level cohorts, each cohort should consider establishing a placebo group.

(iv) In case where the performance of the test treatment under study depends upon subjects with different disease stages or severities, sponsors are encouraged to consider stratified randomization based on disease stage/severity.

(v) For some gene therapies with different indications (e.g., a genetic skin disease), the use of an intra-subject control design (e.g., a cross-over design such as n-of-1 trial design) may be useful [9]. Comparisons of local therapeutic effects can be facilitated by the elimination of variability among subjects in inter-subject designs.

(vi) A single-arm trial using historical controls, sometimes including an initial observation period, may be considered if there are feasibility issues with conducting a randomized, controlled trial. If this design pattern is used, then knowledge of the natural history of disease is critical, which may provide the basis of a historical control, but only if the control and treatment populations are adequately matched, in terms of demographics, concurrent treatment, disease state, and other relevant factors.

(vii) As the study's power to detect treatment-effects diminishes due to small sample size and potential high inter-subject variability, alternative trial designs and statistical techniques that maximize data from a small and potentially heterogeneous group of subjects should be considered. Ideally, an endpoint based on a treatment outcome that virtually never occurs in the natural course of the disease would facilitate the interpretability of small trials.

(viii) Adequate measures are suggested to minimize bias. The preferred approach is to use a study design that includes blinding.

(ix) Sponsors are also encouraged to identify relevant biomarkers early and to leverage all available information from published investigations for the disease of interested (or related diseases). Some biomarkers or endpoints are very closely linked to the underlying pathophysiology of the disease. In this case, changes in such biomarkers could be used during drug development for dose-selection, or even as an early demonstration of drug activity.

Dose selection

Dose selection is crucial in GT drug development for rare diseases. The following principles are from FDA guidance [3]:

(i) Dose selection should be informed by all available sources of clinical information (e.g., publications, experience with similar products and experience in related patient populations).

(ii) Leveraging non-human data obtained in animal models of disease and *in vitro* data may be, in some cases, the only way to estimate a starting effective human dose. Additional dosing information can be obtained from predictive models based on current understanding of *in vitro* enzyme kinetics, and allometric scaling.

(iii) For early-phase studies in subjects under serious conditions, it is better to start with a potentially therapeutic dose.

(iv) In practice, some GT products may have an extended duration of activity, so that repeated dosing may not be an acceptable risk until there is a preliminary understanding of the product's toxicity and duration of activity, and a comprehensive assessment of the product's immunogenicity.

Safety considerations

FDA indicates that the first necessity of designing a gene therapy clinical trial is to ensure the safety of patient subjects, and previous human experience of identical or similar gene therapy products can provide valuable reference. However, if few such experiences exist, it is dangerous to conduct large-scale first-in-human trials. To reduce the potential risk, FDA suggests sponsors stagger administration to consecutively enrolled subjects, followed by staggering between dose cohorts [3,8].

Immunogenicity is another factor that can affect safety. Thus, FDA implies that we should develop an effective test for specific immune reactions and keep paying attention to any immune responses against the proposed gene therapy drug during the whole process of the clinical trial [10]. Moreover, possible long-term side effects resulted from gene engineering technology has always been contested. One gene therapy product might not expose safety issues until years later. Therefore, a long term follow-up survey for those patients who received the gene therapy is necessary [2,3].

FDA also notes that a complete protocol of gene therapy research should cover stopping rules, which require a forceful pause once any adverse events happen. These stopping criteria are significant to protect subjects from any unanticipated injury or safety threats [3].

Efficacy endpoints

An endpoint in a clinical trial is a measurable parameter (usually displays a patient's physiological state) that could provide substantial evidence showing a clinical meaningful benefit of the drug/product. Selection of the primary efficacy endpoint is relevant to whether a drug can be approved. FDA proposes the following guidelines for appropriate endpoint selection for gene therapy rare diseases clinical trials [3]:

(i) Sponsors should understand the pathophysiology and natural history of a disease as fully as possible at the outset of product development. Full understanding of disease pathophysiology is important in selection of endpoints or identifying potential surrogate endpoints which are especially helpful for sponsors who are considering seeking accelerated approval of a GT product.

(ii) Sponsors should identify specific aspects of the disease that are meaningful to the patient and might also be affected by the GT product's activity.

(iii) Considerable information can be gained by collecting clinical measurements repeatedly over time. Such a longitudinal profile allows the assessments of effect, largely based on within-patient changes, that otherwise could not be studied.

Patient experience

Since patient experience data may provide important additional information about the clinical benefit of a GT product, FDA encourages sponsors to collect patient experience data during product development, and to submit such data in the marketing application.

Statistical/Scientific Considerations

In addition to the above important elements to be considered, the following statistical considerations are necessarily implemented during the conduct of gene therapy rare diseases clinical trials.

Small sample size

In clinical research, power analysis is often performed for sample size calculation. The purpose is to achieve a desired power of correctly detecting a clinically meaningful difference at a pre-specified level of significance if such a difference truly exists. However, this method

may not be appropriate for rare disease clinical trials due to limited available patients can be enrolled. To solve this problem, Huang and Chow proposed an innovative method based on a probability monitoring procedure to calculate the sample size in 2019 [11]. The concept is to select an appropriate sample size for controlling the probability of crossing safety and/or efficacy boundaries at a pre-specified level of significance. For rare disease clinical development, an adaptive probability monitoring procedure may be applied if a multiple-stage adaptive trial design is used. More details regarding the probability monitoring procedure for sample size determination of rare diseases can be found in Huang and Chow [11].

Endpoint selection

In experimental treatment trials, the determination of a proper clinical endpoint is essential to project success since the endpoint or outcome provides evidence of whether a clinical benefit exists. A good endpoint should demonstrate both the safety and effectiveness of a proposed treatment with statistical significance and the treatment benefit must be clinically meaningful. In real cases, we sometimes find that there are multiple choices of clinically meaningful endpoints, all of which well measure or characterize the therapeutic effect of interest. Since the study endpoint is important for further decision of the sample size under a desired power, it is important to choose the primary endpoint which can best inform the disease status and measure the treatment effect. To better understand how to choose a primary study endpoint, the concept of therapeutic index was created by Chow and Huang. Each candidate endpoint is assigned a pre-specified weight based on previous observed *p*-values and then apply a linear model to develop the therapeutic index function. More details regarding the development of therapeutic index can be found in Chow and Huang [12].

The use of biomarkers

A biomarker is a measurable characteristic which can reflect your physiological changes to a medical treatment (e.g. blood pressure). Since we do not have a full understanding of most rare diseases, using a biomarker as the endpoint might be more appropriate than a standard endpoint such as survival rate. There are several advantages of using biomarkers: (i) it is easily monitored and can be measured frequently during the whole trial, (ii) it is sensitive to minor treatment effect under a small sample size, (iii) it can predict/explore the promise of an investigational drug in early stage, and (iv) it may help accelerate the process of study and approval for the drug. In addition, biomarkers are able to guide adaptive trial designs [13]. In some cases, the study treatment does not show significant improvement in general population but might be effective in a fraction of people who manifest some similar characteristics. Biomarkers can be utilized to identify the target population. Thus, under the assumption that there is a well-established relationship between a biomarker and clinical outcomes, the use of biomarker in rare disease clinical trials can not only allow screening for possible responders at enrichment phase, but also provides the opportunity to detect signal of potential safety concerns early and provides supportive evidence of efficacy with a small number of patients available [14].

The Use of RWE for regulatory approval

FDA recommends proper use of Real-World Data (RWD) and Real-World Evidence (RWE) as support for regulatory decision making in clinical research. The 21st Century Cures Act, which was enacted back in 2016, turns the attention to the utilization of such data for the purpose of promoting approval of new therapy [15]. For example, advanced electronic tools, mobile devices and other healthcare applications allow people to generate massive amounts of valuable health data, which is an excellent complement to data generated from Randomized Clinical Trials (RCTs). Although the real-world evidence is considered as a powerful support for regulatory decision making, the assessment of treatment benefit based on RWE could be biased due to lack of randomization and heterogeneity of RWD. Thus, in real clinical trials, where there are inconsistencies between RWE and substantial evidence, measures are needed to filter those qualified RWD and diminish the variability of RWD from standard substantial data for an accurate evaluation of the therapeutic benefit [14].

Demonstrate not-ineffectiveness

A standard statistical test to support the efficacy of the drug is to reject the null hypothesis of *ineffectiveness*, in turn, we can accept the alternative hypothesis of *effectiveness*. However, Chow and Huang challenged this interpretation, proposing that rejecting *ineffectiveness* implies *not-ineffectiveness* (or *non-inferiority*) instead of *effectiveness* (or *superiority*) [16]. The concept of "not-ineffectiveness" should include "inconclusiveness" and "effectiveness". For this reason, Chow and Huang pointed out that effectiveness of the test treatment could be concluded if two conditions are met: (i) ineffectiveness is rejected; (ii) the probability of inconclusiveness is negligible. Chow and Huang have therefore developed a groundbreaking two-stage drug discovery trial method for rare diseases. At the first stage, employ a small number (n_1) of patients from targeted population, then test for not-ineffectiveness at a pre-specified significance level. Note that n_1 may be determined by the probability monitoring procedure as mentioned in small sample size section. If the null hypothesis of ineffectiveness is rejected, move to the second stage. To achieve the statistical power (like 80% power), N subjects are needed. It is suggested to recruit n_2 patients and borrow N-n_2 data from Real-World Data (RWD) to eliminate inconclusiveness. If the test result indicates that the probability of inconclusiveness can be neglected (like <5%), effectiveness of the studied therapeutic intervention under investigation is claimed [16].

Innovative trial design

As we mentioned before, small available sample is the main difficulty to conduct clinical study for rare diseases. Thus, it is impossible to use traditional clinical trial design methods to meet FDA's standards for approval of products for rare diseases. In some cases, we need to adopt some innovative trial designs which could help us obtain substantial evidence of effectiveness and safety of the product with small sample size. These innovative trial designs include, but are not limited to, the n-of-1 trial design, an adaptive trial design, master protocols, and a Bayesian design. The flexible use of combination of these innovative trial designs with the innovative thinking discussed earlier is not only to fix the dilemma of sample available (e.g., n-of-1 trial design and master protocol) but also to ensure effectively, accurately, and reliably assess the treatment effect and increase the probability of success of the intended clinical trials (e.g., adaptive trial design in conjunction with Bayesian approach for borrowing real-world data) [14].

Case Study-Approval of Luxturna

In this section, we will discuss a case study regarding gene therapy product Luxturna (voretigene neparvovec-rzyl) approved by the FDA on December 18th, 2017. Luxturna is a novel gene therapy that was specifically developed for patients with an inherited retinal

Table 1: Efficacy results of the phase 3 study at year 1, compared to baseline.

Efficacy Outcomes	LUXTURNA n=21	Control n=10	Difference (LUXTURNA minus Control)	P- value
MLMT score change using both eyes, median (min, max)	2 (0, 4)	0 (-1, 2)	2	0.001
MLMT score change using the first treated eye, median (min, max)	2 (0, 4)	0 (-1, 1)	2	0.003

Source: FDA Statistical and Clinical Reviews

disorder resulted from biallelic *RPE65* gene mutation. Luxturna is a major medical advance since it is the first gene therapy to be granted approval by FDA that targets on a single gene [17].

Background

Biallelic *RPE65* mutation-associated retinal dystrophy is an autosomal recessive genetic disorder often diagnosed in young children. Patients inherit one copy of mutated *RPE65* gene from both parents (mutation site may be different). The function of *RPE65* gene is to make *RPE65* protein, an enzyme involving in the visual cycle (converts light into electrical signals) which is important for normal vision. If two copies of *RPE65* genes are mutated, the number of *RPE65* proteins will rapidly decrease or disappear, resulting in the block of the visual cycle and causing visual impairment. The condition of vision loss will become worse with age, and the patients may even be totally blind in young adulthood. In the United States, almost 1,000 to 2,000 people are affected by eye diseases due to biallelic *RPE65* mutation.

There was limited effective drug or treatment to cure the biallelic *RPE65* mutation-associated retinal dystrophy prior to Luxturna. A common applied treatment is using the Argus II Retinal Prosthesis System, an artificial device that can help patients with blindness caused by outer retinopathy restore a basic vision. This device is first approved in the United States under a Humanitarian Device Exemption (HDE) and is now commercially allowed in many countries. The Argus II System includes external components (a pair of glasses with camera and a portable computer) and internal components (a specific microelectrode implanted into eyes by surgery). The camera on the glasses can capture external images and the computer translates the light perception into electrical stimulation which is then received by implanted electrode to activate retinal cells. The Argus II Retinal Prosthesis System is proved to be relatively safe and effective; however, the recovery of vision acuity varies in different patients [18].

The mechanism of Luxturna is to transmit a normal copy of the *RPE65* gene directly to living retinal cells through a transformed adeno-associated virus as a vector. Thus, *RPE65* proteins can be complemented and the visual cycle can continue again.

FDA review of clinical safety and efficacy

This section discusses the clinical trial program of Luxturna, including the study design, important results and FDA's recommendations. The Luxturna regulatory approval is based on a clinical phase 1 analysis and a clinical phase 3 studies that established the key safety and efficacy proof for the BLA submission [19].

Phase 1 and phase 3 studies: The phase 1 study was an open-label, dose-escalation protection study in a total of 12 patients with reported retinal dystrophy associated with biallelic *RPE65* mutations. Eleven of 12 patients with an injection time of 1.7 to 4.6 years received subretinal Luxturna injection to either eye. One patient got just one eye with a subretinal injection. Additionally, the Phase 1 analysis evaluated three doses and results indicated that no apparent dose that might affect safety, bioactivity, or preliminary efficacy. The high dose (1.5×10^{11} vector genome in an injection volume of 0.3 mL) was chosen for the phase 3 study.

Table 2: Magnitude of MLMT score change using both eyes at year 1.

MLMT Score Change	LUXTURNA n=21	Control n=10
1	-0	3 (30%)
0	2 (10%)	3 (30%)
1	8 (38%)	3 (30%)
2	5 (24%)	1 (10%)
3	5 (24%)	0
4	1 (4%)	0

Source: FDA Statistical and Clinical Reviews

The Phase 3 study was an open-label, randomized, controlled, cross-over trial. It was developed to test the effectiveness and efficacy of Luxturna sequential subretinal injection into each eye. Patients were randomized to either the Luxturna therapy group or the observational control group in a 2:1 ratio, and were monitored for the primary efficacy evaluation for duration of one year. After one year of study, patients in the control group were crossed over to receive Luxturna. The phase 3 study recruited 31 patients in the United States from two locations. At the stage of baseline evaluation, 21 of the 31 participants were randomized to the therapy group with one drop. Ten patients were randomized to the control group with withdrawal of consent of one patient at the screening visit. A crossover study was conducted in the nine patients with one-year observation of Luxturna injection. The mean age was 15 years (4 to 44 years), including 20 (64%) pediatric patients (4 to 17 years) and 11 adult patients. The subretinal injection period ranged from 6 to 18 days for each patient's two eyes.

Efficacy evaluation: The primary efficacy endpoint in the phase 3 study was change in Multi-Luminance Mobility Testing (MLMT) performance from baseline to one year after Luxturna administration. MLMT assessed the ability of a patient to traverse a course correctly and at a fair speed at various degrees of ambient light. The MLMT was measured at one or more of seven light levels, ranging from 400 lux (corresponding to a brightly lit office) to 1 lux (corresponding to a moonless summer night), using both eyes and each eye independently. A score code ranging from 0 to 6 was assigned to each light level. A higher score implied that a patient was able to pass the test at a lower light level. Patients who did not pass MLMT in the brightest environment (400 lux) were given a score of -1. Each patient's MLMT was recorded and tested by independent, blinded graders. The MLMT score was the lowest light level at which the patient successfully passed the MLMT. Another parameter defined in this study is the MLMT score change from baseline to the end of study. An increase of the MLMT score suggested an improvement of eyesight. Additional efficacy endpoints included Full-Field Light Sensitivity Threshold (FST) and Visual Acuity (VA). The phase 1 and phase 3 regulatory review analyses are summarized below.

The results of the primary endpoint (MLMT score change) from

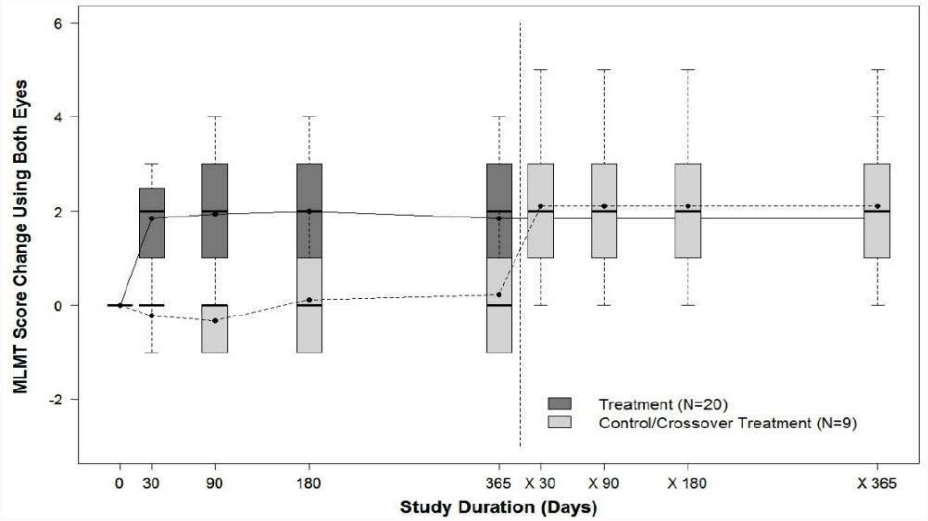

Figure 1: MLMT time-course over two years (using both eyes).

Table 3: Magnitude of MLMT score change using individual eyes at year 1 (ITT).

Change Score	First-Treated Eye (N=21)	Control (N=10)	Second-Treated Eye (N=21)	Control (N=10)
-1	0	1 (10%)	0	2 (20%)
0	4 (19%)	6 (60%)	2 (10%)	5 (50%)
1	2 (10%)	3 (30%)	4 (19%)	3 (30%)
2	8 (38%)	0	8 (38%)	0
3	6 (28%)	0	5 (23%)	0
4	1 (5%)	0	1 (5%)	0
5	0	0	1 (5%)	0

Source: FDA Statistical and Clinical Reviews

baseline to year one are summarized in Table 1. A median MLMT score change of two and zero were observed in the Luxturna treatment group and control group, respectively, independent of the use of both eyes or the first-treated eye. An MLMT score change of two or greater is considered a clinically meaningful benefit for functional vision.

Table 2 and Table 3 illustrate the number and percentage of patients with different magnitudes of MLMT score change using both eyes and individual eyes, respectively, at year 1. If we consider both eyes, eleven patients (11/21, 52%) in the Luxturna treatment group had an MLMT score change of two or greater, while only one patient (1/10, 10%) in the control group had an MLMT score change of two (Table 2), (Figure 1).

On the other hand, if we consider the first-treated eye and second-treated eye separately, fifteen patients (15/21, 71%) in the Luxturna treatment group had an MLMT score change of two or greater, while no patient in the control group had a score change of 2 or greater (Table 3).

Safety assessment: Twenty-seven (27/41, 66%) of the 41 patients (12 from phase 1 and 29 from phase 3, totally 81 eyes) receiving Luxturna had ocular adverse reactions in 46 injected eyes (46/81, 57%). The most common adverse reactions (incidence ≥ 5%) were conjunctival hyperemia, cataracts, increased intraocular pressure, retinal tears, dellen, macular hole, eye inflammation, macular breaks, subretinal deposits, eye irritation, eye pain, and maculopathies (Table 4). Whether these ocular adverse symptoms are associated with injection of Luxturna is not clear. However, most of these adverse events were temporary and controllable. There were no deaths in the clinical studies. There were two serious adverse reactions, including (i) endophthalmitis (infection inside of the eye) with a series of subsequent complications, and (ii) loss of vision due to fovea thinning as a result of the subretinal injection. Systemic adverse events included hyperglycemia, nausea, vomiting, and leukocytosis. These systemic events were likely caused by systemic corticosteroid use and reactions to anesthesia.

As noted by the FDA, Luxturna subretinal injection should be given only to patients who have viable retinal cells as determined by the treating physician(s). Treatment with Luxturna must be done separately in each eye on separate days, with at least six days between surgical procedures. Patients should be treated with a short course of oral prednisone to limit the potential immune reaction.

Summary: The safety and efficacy of Luxturna were well established in a clinical trial with 41 patients aged between 4 and 44. All participants had confirmed biallelic *RPE65* mutations. The primary efficacy endpoint of Luxturna was based on the phase 3 study with 31 participants by measuring the change from baseline to one year in a subject's ability to navigate an obstacle course at various light levels. The treatment group demonstrated significant improvements in their ability to complete the obstacle course at low light levels as compared to the control group. The most common adverse reactions, such as eye redness, cataract, can be mitigated by medical care.

Advisory committee recommendations

The Cellular, Tissue, and Gene Therapies Advisory Committee

Table 4: Ocular Adverse Reactions Following Treatment with Luxturna (N=41).

Adverse Reaction	Patients n=41	Treated Eyes n=81
Any ocular adverse reaction	27 (66%)	46 (57%)
Conjunctival hyperemia	9 (22%)	9 (11%)
Cataract	8 (20%)	15 (19%)
Increased intraocular pressure	6 (15%)	8 (10%)
Retinal tear	4 (10%)	4 (5%)
Dellen (thining of the corneal stroma)	3 (7%)	3 (4%)
Macular hole	3 (7%)	3 (4%)
Subretinal deposits*	3 (7%)	3 (4%)
Eye inflammation	2 (5%)	4 (5%)
Eye irritation	2 (5%)	2 (2%)
Eye pain	2 (5%)	2 (2%)
Maculopathy (wrinkling on the surface of the macula)	2 (5%)	3 (4%)
Foveal thinning and loss of foveal function	1 (2%)	2 (2%)
Endophthalmitis (infection inside of the eye)	1 (2%)	1 (1%)
Fovea dehiscence (separation of the retinal layers in the center of the macula)	1 (2%)	1 (1%)
Retinal hemorrhage	1 (2%)	1 (1%)

Note: *Transient appearance of a ring-like deposit at the retinal injection site 1-6 days after injection without symptoms
Source: Modified from the applicant's BLA submission

(CTGTAC) evaluated the safety and effectiveness of Luxturna, giving several feedbacks. First, a 2-score increase of MLMT is clinical meaningful. Second, the potential risks of Luxturna are acceptable. Third, Luxturna may not work until 8 to 12 months of age because the retina has not been fully developed. Fourth, it should be considered if the efficacy of Luxturna declines over time. Consequently, the CTGTAC voted 16 (Yes) to 0 (No) in favor the approval of Luxturna.

Concluding Remarks

In this paper, a case study regarding Luxturna which is a gene therapy drug intended treating a rare disease of an inherited form of vision loss that may result in blindness. The FDA granted Luxturna (i) priority review, (ii) breakthrough therapy and (iii) orphan drug designations to assist the sponsor in Luxturna development. As discussed in Section 4, FDA's recommendation for regulatory approval of Luxturna was made based on a phase 1 clinical study and a phase 3 clinical study.

Although the CTGTAC voted for approval of Luxturna, several questions and concerns remain. These questions, which are likely to occur due to small sample size, include, but are not limited to (i) the treatment effect may have been contaminated by some covariates and/or interaction/confounding factors, (ii) no scientific/clinical or statistical justification for the selection endpoint and/or clinically meaningful difference, (iii) it is not clear that the study design can provide an accurate and reliable assessment of the treatment effect, and (iv) statistical methods for assessment of treatment may not be adequate (e.g., shift analysis before and after treatment was not done). In addition, one of the major concerns is probably that whether the performance of Luxturna based on limited number of patients is not by chance alone and hence is reproducible. Based on a quick assessment, the reproducibility probability was calculated based on the observed responses and the variability associated with the responses of the 31 subjects. The result indicates that there is a less than 60% probability of reproducibility if the study is conducted under similar experimental conditions in the future.

Thus, it is suggested that statistical considerations as described in Section 3 and some innovative thinking regarding study design and analysis should be taken into consideration when conducting rare diseases clinical trials.

Author Contributions

SCC conceived the idea and designed the work; WZ and CZ drafted the manuscript. CZ and WZ critically revised and worked in ensuring the accuracy or integrity of the work; all authors read and agreed to approve the final manuscript.

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