

# GENE REPLACEMENT THERAPY DEVELOPMENT FOR BARTH SYNDROME

*The responses in this document focus on gene replacement therapy (“gene therapy”) in which the non-functional gene is replaced with a healthy, normal copy of tafazzin. There are other approaches that include gene editing that are not the subject here.*

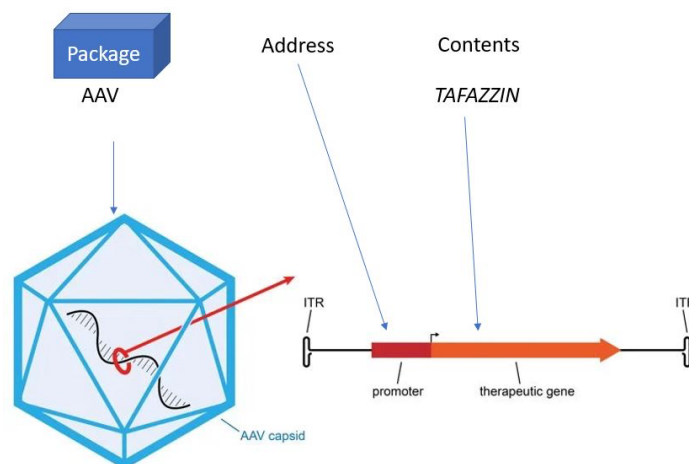
## BACKGROUND

### Why is Barth syndrome an ideal target for a gene therapy?

Barth syndrome happens when there is something wrong with the *tafazzin* gene. When a disease is driven or caused by a single gene, it is described as a monogenic disease and is an ideal indication for gene therapy – whose goal is to deliver a working copy of the gene into an affected individual. Fortunately for those with Barth syndrome, *tafazzin* is an exceptionally small gene and can easily fit into the capsule that delivers the therapy to the individual’s cells.

### How does gene therapy work?

In gene therapy for Barth syndrome, the goal is to deliver a new, working copy of *tafazzin* to prevent and reverse the impact of Barth syndrome on the heart and skeletal muscles. This can be achieved in many ways, with possible different “packages” and “addresses” to deliver the healthy copy of the *tafazzin* gene (see the diagram below). Generally, gene therapy is introduced into the body through an infusion that takes approximately 1 hour, during which the individual is monitored by a medical team.



*Barth syndrome gene therapy package*

### How is gene therapy delivered to the heart and skeletal muscles?

Based on the previous diagram, gene therapy for Barth syndrome would deliver the gene therapy “package” to the hearts and skeletal muscles of affected individuals. The promoter serves to make sure the package is delivered to the correct location (the “address”) in the genetic makeup of the cell. Currently, we are exploring using adeno-associated viruses (AAV) as the package, using only the capsid (the outer shell) of this naturally occurring virus. Different packages and addresses are under investigation and their resulting combination, along with alterations to the gene delivered, determine whether and how much of the gene therapy is delivered into heart and muscle cells.

### Which kinds of cells or symptoms of Barth syndrome will gene therapy NOT affect?

In the case of AAVs under consideration, the gene therapy package is not known to interact with white blood cells or the stem cells they originate from, so gene therapy as currently engineered is not anticipated to impact the neutropenia associated with Barth syndrome.

It also does not impact the nuclear genetic makeup of an individual. This means that the genetic information delivered through gene therapy will not be inherited by children of individuals who received the therapy. Therefore, a father with Barth syndrome, irrespective of whether he receives gene therapy, will still pass on the non-functional *tafazzin* gene on his X chromosome to his daughter (who would likely not experience clinical symptoms but be a carrier of the problematic gene).

### What work has been done to date to test gene therapy in Barth syndrome?

Presently, gene therapies for Barth syndrome have only been tested preclinically in animal models and human cells, but not yet in people. Two different Barth syndrome mouse models have demonstrated that delivery of a new copy of *tafazzin* can result in skeletal and heart muscle tissue/cells with improved mitochondrial function and structure, enhanced capacity for exercise and endurance measured via mouse activity, and preservation and restoration of heart function with reduced tissue scarring (fibrosis).

## GENE THERAPY CLINICAL TRIALS IN BARTH SYNDROME

### How likely are we to have a gene therapy clinical trial for Barth syndrome?

Based on the emerging science, there is a potential for multiple trials to test different approaches in Barth syndrome. These are not likely to be carried out simultaneously. Barth syndrome research teams are actively exploring multiple technical approaches to gene therapy to ensure individuals with Barth syndrome can safely receive the maximum clinical benefit for as much time as possible. The advancement of these approaches to clinical trials hinges on

overcoming technical challenges, external funding, regulatory approval, and the capability to manufacture the gene therapy.

### What are the costs of conducting gene therapy clinical trials?

Gene therapy is currently VERY EXPENSIVE! It can cost \$14-22M USD to develop and make a gene therapy that is approved by the FDA. The major driver of these costs is the production of the gene therapy material, which must be tailored for a specific disease and meet the quality standards required for testing in humans. Much work is being done to identify ways to scale production and drive down costs, but it will likely be years before pricing is more reasonable. For this reason, BSF has been working with researchers to identify opportunities to work with the National Institutes of Health and pharmaceutical companies to help define and find funding pathways.

### Who determines when a clinical trial can begin?

For new drugs and biologics (as in the case of gene therapy), the research team must obtain an investigational new drug (IND) approval by the U.S. Food and Drug Administration (FDA) to test a product in humans. In an IND submission, the research team must present preclinical data substantiating that the gene therapy product is ready to be tested in a human clinical trial. Sometimes, the FDA requires the team to conduct further tests in animal models before proceeding with a clinical trial in humans.

### How do research teams establish participants' eligibility for gene therapy clinical trials?

Each clinical trial is designed by a research team and based on the specific therapy being tested, the patient population, and the natural history of the disease. The age, weight, heart function, current drugs a patient is taking and location where they live may influence whether an individual is a candidate for a trial. While BSF and patients hopefully inform the selection of these parameters, the institution sponsoring the study ultimately sets the eligibility criteria, with input from Institutional Review Boards who monitor the ethics of testing drugs in people.

Additionally, anyone taking another investigational drug or who has received a heart transplant would likely not be eligible to participate due to possible dangerous or unknown interactions between the gene therapy and the medications an individual is taking.

Given AAV is a virus that is present in nature, previous exposure to AAV may also disqualify someone from participation, as this might increase the risk of a negative immune reaction. Much research is being performed to find ways to work around this limitation across diseases so that seropositivity, or having the antibodies that would react to AAV, does not prevent individuals from participating. More time is needed to resolve this challenge.

For the time being, ideal candidates will need to have low pre-existing antibodies to AAV such that they can receive the maximize clinical benefit from the therapy. They will also need to

demonstrate a willingness to participate in the study and the associated testing. Participation may require weeks or months residing at the testing site which may affect caretakers' and recipients' ability to work or go to school. BSF recognizes the potential concern and burden participating in a gene therapy trial may place on Barth families and has made a commitment to fund a current proposed clinical trial up to \$250,000 specifically to cover costs that families would otherwise bear.

What are the clinical trial sizes, age range of participants, and outcomes of gene therapy clinical trials to date?

There have not yet been any human gene therapy clinical trials in Barth syndrome.

What is the justification for whether to include pediatric participants in initial gene therapy trials?

For many therapies first being tested in humans, the general approach is to begin by assessing the new therapy in adults. Once the effectiveness and safety profile of the potential therapy are better understood, the therapy can sometimes then be tested in younger/pediatric participants. Usually, the FDA will only consider an initial pediatric clinical trial for diseases in which most individuals perish in the first stage of life.

How is the therapeutic dose of gene therapy for each participant determined?

Animal models help to establish the amount of gene therapy that needs to be delivered into the body to have a beneficial clinical outcome. For research focused on Barth syndrome gene therapy, mice that have received lower doses demonstrated some improvement in the skeletal and heart muscles but not much. However, when a higher dose was delivered, the mice experienced better ejection fraction and less scarring of the heart, or fibrosis. Thus, in gene therapy, a balance is sought between delivering enough therapeutic package so that it is effective, but no more than is needed to try keep the therapy as safe as possible. This amount is called the minimal effective dose and, in gene therapy, is often calculated based on the weight of a trial participant.

In a gene therapy clinical trial, would all the participants receive the treatment together at the same time or sequentially at different times?

During the initial stages of a human clinical trial, participants would likely be dosed sequentially, with weeks or months between each participant. The spacing between each participant would not be necessary once experience has been gained and the treatment is determined to be safe. Logistics of traveling to the treatment site would likely determine the order in which participants would receive the treatment.

How do we know if it is working?

Researchers have developed clinical endpoints to measure how hearts and other parts of the body of people with Barth syndrome are affected by gene therapy. Measuring these endpoints

may require that participants agree to blood tests, exercising, and/or imaging (such as echocardiograms or MRIs) conducted in context of a clinical trial.

#### How long might gene therapy work, or what is the durability of the therapy?

Gene therapy is not curative. However, studies in other diseases have provided insight into the length of time a gene therapy might work. For example, with the approved gene therapy used to treat spinal muscular atrophy (Zolgensma), data demonstrate durability of treatment at least 5 years post administration. In gene therapy studies in large animal models with muscular dystrophy, the positive effects of the treatment are visible 10 years after administration. However, given that animal and human cells continue to grow and divide over time, the therapeutic benefit of a single-administration gene therapy usually wanes over time. The issue of re-dosing later in life, similar to administering a vaccine booster, is a challenging but vibrant and active field of gene therapy research.

#### Is it known if age plays a role on the effectiveness?

Treating younger patients can halt progression of disease at an earlier stage. However, young gene therapy recipients may experience lessening clinical benefit as they grow and go through puberty.

For adults and individuals with advanced disease, current gene therapies under development may not be able to fully reverse damage to muscle tissue, specifically fibrosis of the heart muscle. Subsequently, gene therapy may not offer the same degree of initial clinical benefit that researchers anticipate observing in younger recipients.

For all age groups, experts in gene therapy are determining the durability of effectiveness (i.e., how long it will last) by continuing to follow patients with other disorders who have been treated with gene therapies.

#### What is the age of the oldest person to be administered gene therapy?

We are unable to answer this question at this time due to the unavailability of the data. However, two of the three existing FDA approved AAV-mediated gene therapies, Luxturna for Leber congenital amaurosis (delivered to the eye) and Hemgenix for hemophilia B (delivered systemically), are both administered to adults.

#### What are the risk and safety considerations in gene therapy clinical trials?

Reported side effects may differ depending on the therapeutic package and mode of administration. Fever, chills, drops in blood pressure, nausea, vomiting, headaches, and muscle weakness are among the side effects reported. These commonly occur up to a few weeks following administration.

Severe adverse events associated with gene therapy, some so serious they have caused clinical trials to be halted, include liver problems (hepatotoxicity, elevated enzymes, acute injury), neurotoxicity, gastrointestinal infection or bleeding, thrombocytopenia, anemia, hyperbilirubinemia, kidney injury, cardiopulmonary insufficiency, sepsis, and even death also have been documented.

Many of these severe adverse events have been tied to the amount of gene therapy delivered and a resulting negative response by the immune system. Large investments in funding and research are being directed to solving this problem that is common across most potential gene therapies, including for Barth syndrome. Several different drugs are under investigation to determine whether they may play a role in suppressing the immune system before, during, and after infusion, and these could be important advancements to deliver safer amounts of gene therapy to patients.

How might Barth syndrome gene therapy change the practical, day-to-day life of an affected individual?

We expect that gene therapy (systemically providing a healthy copy of the affected gene – *tafazzin*) might stop disease progression and enable patients to regain some heart function and muscle strength, at least for a time.

How might gene therapy affect the nutritional and feeding challenges of Barth syndrome?

Without having yet conducted a clinical trial in Barth syndrome, it is difficult to predict the extent a gene therapy will impact these challenges. Current designs for Barth syndrome are focused primarily on heart and skeletal muscle. However, due to the systemic approach being proposed, some aspects of feeding and nutrition may also be improved.

What are the long-term anticipated effects of gene therapy?

Ideally, the individual will not endure a progression of Barth syndrome-related symptoms, and rather the heart and muscle function will improve. As a result, researchers also anticipate individuals will experience a reduction in fatigue and improved quality of life.

As has been described above, however, it is unlikely that a single gene therapy will address all the clinical symptoms associated with Barth syndrome. Researchers are also concerned that gene therapy is not a “one and done” and that individuals may need at least another infusion at some point in their lives to continue to experience the positive effects of the treatment. How that might be able to be achieved is still being worked on by many researchers around the world.

## BARTH SYNDROME EXPERTS WORKING ON GENE THERAPY

[Barry Byrne](#) MD/PhD – has been a longtime member of the BSF community and was first granted a BSF research award in 2010 to study Barth syndrome gene therapy. Alongside his certification as a pediatric cardiologist, is also one of the leading experts and primary investigators for completed and in-process gene therapy clinical trials in the US. [U. Florida](#)

[Todd Cade](#) PhD – a member of the BSF Scientific and Medical Advisory Board (SMAB) was first recruited by Barry Byrne and is an expert in measuring how our bodies convert food into energy. Starting in 2006, Todd has developed ways to measure how hearts of Barth syndrome affected individuals are unable to use the major fuel, fatty acids, to generate energy during physical activity. This measurement, known as  $VO_2$  max, quantifies the amount of oxygen that muscles can take up during exercise and can be very useful in quantifying the difficulties Barth syndrome affected individuals experience in utilizing fatty acids to generate energy during physical activity. [Duke U.](#)

[Christy Pacak](#) PhD – is an expert in the design of gene therapy products and testing them in the Barth syndrome mouse (knockdown model). Christy was the first to show that when a new copy of the *tafazzin* gene was delivered to Barth syndrome mice, their mitochondria looked healthier, and the mice not only moved more but also reached upwards, or “reared” more – a function that involves the use of many skeletal muscles. [U. Minnesota](#)

[Bill Pu](#) MD – a member of BSF’s SMAB and an expert in heart biology, animal and cell models, who is involved in several pre-clinical gene therapy efforts. His research demonstrated that gene therapy in Barth syndrome mice (knockout model), if delivered early, could prevent heart muscle disease. When delivered later in a mouse’s life, gene therapy resulted in improvements in heart scarring (though there was still some fibrosis) and improved function. [Boston Children’s Hospital](#)

[Hilary Vernon](#) MD/PhD – also a member of BSF’s SMAB, Director of the Barth Syndrome Clinic at KKI Johns Hopkins and the principal investigator for the completed TAZPOWER clinical trial for use of elamipretide in Barth syndrome. Her expertise in clinical management and metabolism, as well as her clinical trial experience in Barth syndrome (running the first-ever Barth clinical trial) make her a key member of the team. [Kennedy Krieger Institute](#)