# A Review of Genetic Transfer Therapy Trials from a Regulatory Perspective for the Scientific Understanding

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## ABSTRACT

### A Review of Genetic Transfer Therapy Trials from a Regulatory Perspective for the Scientific Understanding

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The present work reviews the safety and efficacy concerns of the Recombinant DNA Advisory Committee (RAC) regarding Gene Transfer Therapy (GTT) clinical trials. The RAC, an advisory board established by the National Institutes of Health (NIH) is charged with reviewing and discussing GTT clinical trials and give regulatory guidance to the NIH for further proceedings. Considering the infamous past of GTT clinical trials, where malignancies have been found as a serious adverse event, one of the main concerns among regulators is the possible insertional mutagenesis (IM) caused by the viral vector used in this kind of trials. Many controls have been suggested to decrease this risk like fully characterizing the insertion sites (IS) of the vector used. The rest of the regulatory concerns are related to efficacy and safety of the whole therapy. This is no different from any other type of therapy or drug that is subject to FDA approval, however, there are some specific points for GTT clinical trials that must not be ignored. Based on these concerns, I include two case studies to exemplify how their findings would be reviewed from a regulatory perspective The first is a trial that uses an Adenovirus-associated virus vector to treat adult patients suffering from Hemophilia B. The trial is successful but they did not covered key efficacy and safety aspects that would fully convince a regulator to approve this kind of therapy. The second trial uses a lentiviral vector to transduce ex-vivo hematopoietic cells to correct the condition known as X-linked adrenoleukodystrophy (X-ALD), which affects children from 5-12 years of age and results in mental retardation and if not treated can cause death. The researchers on this trial took a lot of measures and controls to ensure the safety of the therapy however they failed to include a statistically significant population that would describe the efficacy of

the therapy over the standard of care. I hope this can serve as a guide to harmonize the understanding of regulatory requirements from the science community and work together for a better drug development process.

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I would also like to thank Dr. Carol Lin for her guidance and support on the writing of this project and during the whole program at Columbia.

To my parents, my sisters and that special one that shaped me the way I am today.

## CHAPTER I

## INTRODUCTION

Gene transfer therapy refers to the introduction of genes into human cells to restore the gene function which was impaired due to a genetic lesion or an infectious disease (1). The expression of the new introduced gene, also called transgene, can be transient or durable, depending on the type of disease which the therapy aims to treat.

The long-term expression of the transgene can be achieved by several gene delivery methods that integrate their genome into the target cell. These delivery systems are roughly categorized into viral and non-viral methods. The non-viral methods are highly complex and they include liposomes, plasmids, nanoparticles, etc.

The most common and widely used are viral vectors and they can be derived from retroviruses, lentiviruses, adenoviruses and adeno-associated viruses (AAV). These viral vectors based their delivery method in the integration of their genetic material into the host genome. However, this integration is not highly controlled and sometimes can integrate in the cell genome altering the natural sequence and deregulating the expression of certain cellular oncogenes or truncating tumor suppressor genes that leads to the development of cancer (2).

Currently in the US, there are several gene transfer therapy (GTT) trials that are being conducted. Most of them aim to cure monogenetic diseases that require a long-term expression of the corrected gene version. There also GTT trials that aim to restore function of a tumor suppressor gene in a particular type of cancer (3-12).This type of trial requires a different type of endpoint that is very complex and hard to measure, since cancer is the consequence of accumulated genetic lesions and mutations.

Another feature of current GTT trials is the type of gene delivery system they use. The majority of them use viral vectors of all varieties; however, there are also trials

that use non-viral vectors. The use of non-viral vectors represents a whole different array of efficacy and safety endpoints to be defined in each trial.

Gene therapy has been evolving slowly since 1989 when the first gene transfer in the United States took place. This trial used a retroviral vector to correct an adenosine deaminase (ADA) deficiency which causes an immunodeficiency n two pediatric patients. The investigators on this trial transduced *ex vivo* the non-mutated copy of the ADA gene to hematopoietic stem cells. The immune system on both patients was restored (2).

Following the release of the results in that first trial, many other gene transfer trials were submitted to the FDA; however, this new fever resulted in careless planning of clinical trials that gave disappointing results. Many aspects were not considered, like immune response against vectors, which unfortunately caused the death of a young patient that was enrolled in a trial to restore ornithine carboxylase deficiency. This patient had a severe immune reaction to the vector (2). This trial was conducted in 1999 and represented a wakeup call to regulatory agencies all over the world.

The scientific community stopped and went back to *in vitro* testing of all these issues observed in the viral vectors. Unfortunately, the most tragic adverse event of early GTT trials had not yet happened. In a trial that occurred in France for the treatment of X-linked severe combined immune deficiency (X-SCID), after a successful correction of the disease, years after the treatment, some patients developed leukemia. This was attributed to a faulty integration of viral DNA within the LMO-2 locus which caused its deregulation that led to a T-Cell over proliferation, eventually causing leukemia (13)

After this tragic event, regulatory agencies all over the world established advisory committees for the careful review and discussion of gene transfer trials.

In the next chapter, I will provide a description of the regulatory system in the US and how these GTT trials are evaluated and approved.

# CHAPTER II

## REGULATORY CONCERNS

### United States Regulatory System

In the United States of America, the Food and Drug Administration (FDA) is in charge of the overseeing and approval of all clinical trials of investigational drugs. Within the FDA, the Center for Biologics Evaluation and Research (CBER) is in charge of regulating human gene therapy research and any products that may be submitted for commercial approval.

The CBER defines a human gene therapy product as products that introduce genetic material into the body to replace faulty or missing genetic material, thus treating or curing a disease or abnormal medical condition (14).

Although there has not been any genetic therapy products approved by the FDA to this date, there are many GTT research trials in the US and CBER, working with the National Institutes of Health (NIH) they launched a system to keep track on all these trials and oversee their compliance to regulations. This system is called the Genetic Modification Clinical Research Information System (GeMCRIS). This system tracks all trials that have been approved and registered with the NIH and CBER and provides information on trial location, investigators conducting the trial, gene products used, vector used and a summary of the protocol (15). This system provides useful information to overseeing regulatory agencies, patients, physicians and sponsors, which is displayed as preformatted reports easy to search and read.

In turn, the NIH also has appointed the Office of Biotechnology Activities (OBA) to be in charge of the oversight of these GTT trials. The NIH has also created the Recombinant DNA Advisory Committee (RAC) which is composed by experts in many disciplines, physicians and members of patient organizations that get together as a public forum for the discussion of the scientific, regulatory, ethical and legal issues

associated with this kind of trials. They issue reports that signal their concerns and provide advice to the NIH. Further, they have developed a set of guidelines that regulate the safe conduct of GTT research trials that are continuously evolving over time with every new finding that the trials might bring (16).

**RAC meetings**

Approximately four times a year, the RAC assemblies to review and discuss trials that have reported important scientific results or that have raised ethical or safety issues. These meetings are open to the public, its minutes available through the OBA website and the live webcast of the minutes is available online. The NIH takes into serious consideration all the recommendations and advice the RAC provides from these meetings, which is one of its main purposes. Therefore, even though the RAC is just an advisory committee, I assume that their concerns would be the same concerns the regulators would have from GTT trials (16).

I have analyzed their reviews and comments from meeting minutes from ten meetings held from March 2010 to June 2012 with the intent to summarize the current most common concerns within the regulatory community (3-12). However, for the purpose of this project I only focused on the concerns regarding gene transfer trials that involve gene correction of a known monogenetic condition and use viral vectors for this purpose. Like I mentioned in the introduction, there are currently several other GTT trials that use non-viral vectors and many other trials that use viral vectors but they aim to restore gene function in cancer and therefore the concerns for this type of trials are different and vast; perhaps in another project I can address these problems with more detail.

**Insertional Mutagenesis**

One of the most common concerns that I observed in almost all the trials reviewed in the RAC meetings was the fundamental problem of insertional mutagenesis using viral vectors. Viral insertional mutagenesis refers to the mutagenesis of the genome caused by the integration of the viral DNA in a way it interferes with the natural gene arrangement of the target cell and in consequence, start a series of events which will result in the transformation of the cell (2). This can also happen when the viral promoter contained in the viral DNA integrates near a cell oncogene and upregulates its expression.

Even though there is extensive experimental evidence of the safety of some types of viral vectors, the concern for insertional mutagenesis remains. In all the meeting minutes, there is always at least one investigator concerned with this problem (3-12).

The reason for concern is not unfounded, since the infamous clinical trial in France where patients developed leukemia after being treated for SCID-X1 using a retroviral vector for the gene transfer (13), regulatory agencies all over the world have been extremely cautious with viral vectors used in gene transfer. The scientific community has worked very hard to develop a safe viral vector that guarantees no insertional mutagenesis, unfortunately there hasn't been any solid experimental evidence to prove this and the fear of viral vectors causing malignances due to insertional mutagenesis is still present in the minds of both regulatory agencies and scientific community.

Efforts have been directed to the use of non-viral vectors in gene transfer, however, the transgene expression rates have not matched yet the efficiency viral vectors achieve (17).

Investigators have also been focusing on this problem developing tests and evaluations to establish a more accurate safety profile that can convince the scientific community that no transformation events will occur due to the insertional mutagenesis problem. In the next chapter, I will describe a variety of methods that can be applied to achieve this and provide the regulators with solid safety evidence.

**Durable Transgene Expression**

The main goal of gene therapy is to restore function of a defective gene. Most of the times it is a partial restoration, meaning that sometimes levels of the repaired protein are not brought back to normal but increased significantly compared to the disease phenotype, which in most of the cases is enough to increase a patient's life expectancy or quality of life. However, one the main concerns of both scientists and regulators is the persistence of this corrected phenotype. A durable transgene expression is essential in GTT trials and the investigators should provide with the experimental evidence to demonstrate this. Since GTT trials intend to be curative, this has to be demonstrated.

Many RAC members expressed their concern on the length of transgene expression in patients enrolled in many GTT trials and demanded test results to support an appropriate expression of the corrected protein to be considered a therapy (3-12).

**Viral Vector Dose Determination:**

Many RAC members expressed their concern towards a safe and efficient dose determination for viral vectors in gene therapy (3-12). The concern mainly comes from the known fact that the number of insertion sites (IS) is directly related to the number of viral vector copies and a high number of IS is in turn associated with the generation of transformed clones (18).

For this reason, it is of utmost importance that the investigators define the dose carefully and considering the type of viral vector to use, since they have different insertional mutagenesis rate risks.

In the next chapters, a trial is described to calculate a safe and effective dose of viral vector in gene transfer trials.

**Immune Response against the Viral Vector**

In GTT trials that use viral vectors it is not uncommon to see the developing of an immune response to the vector since it is of viral origins. The investigator's in developing the test material, is responsible for characterizing the rate of immunogenicity the vector has and its impact on the therapy efficacy.

RAC members frequently stressed this concern to investigators and encourage them to perform the appropriate determinations and actions to prevent an exacerbated immune reaction (3-12).

**Risk/benefit Compared to Current Forms of Treatment**

One of the most important aspects of a new drug application is the stating and demonstration of a clinically significant benefit from the new drug. This is a key feature within the application and very often, scientist fail to clearly state it. They have all the clinical evidence to support it; however, the presentation of this evidence in a clear, transparent way is essential for a swift positive approval.

Many RAC members issued their concerns about this problem, stating that the investigators did not express all the features of their new proposed treatment compared to the current standard of care. They classify this as an ethical issue mainly because it needs to be included in the ICF so that patients that are enrolling to the trial know all the risks and benefits of the new therapy compared to what it is already out there (3-12).

**Manipulation and Testing of Biological Samples from Patients**

This is a common issue not only in GTT trials but in any other trial that requires biological sampling from patients. It amazes me that investigators fail to include this in their protocol description since it is an issue every regulator has in any type of clinical trial.

This is an issue that requires careful planning and justification since the FDA is very strict on biologic samples handling from patients. During my research I encountered a gene transfer trial to restore function of the tumor suppressor gene TUSC2(FUS1) gene in lung cancer patients using a nanoparticle gene delivery system (19) The trial is very interesting however, their study design required mandatory biopsies for analysis of gene expression on every patient to assess clinical efficacy. This mandatory biopsy sampling was strictly forbidden by the FDA and local IRBs. They do not explain why exactly this happened, but I suppose it was the consequence of a poor description of the purpose and sampling method by the investigators.

I strongly believe that if a good justification is provided, the FDA would allow the biological sampling needed for experimental evidence.

**Informed Consent Forms (ICF) Issues**

There was also a very common issue that is being seen not only on gene transfer trials but in any other clinical trial. However, since it was cited quite frequently in  in the RAC discussions, and considering the FDA always puts the safety and wellbeing of the patient before anything, I decided to include it and describe it briefly.

The main problem with ICF in GTT trials is that many of them refer to the research as “treatment” which could be misinterpreted by a patient as an approved therapy for the condition. This could cause confusion when comparing the research with actual approved forms of treatment for the condition. Since the FDA has not approved

any form of gene transfer therapy, an appropriate wording of this type of ICF should be enforced (3-12).

Many RAC members agreed upon this and all the investigators concurred with them, indicating that the appropriate corrections will be made.

## CHAPTER III

## CASE STUDY 1

This chapter is divided in three sections. In the first section I will describe the trial and its results providing with enough background information to understand their goals and study rationale. In the next section I will discuss the outcomes of the trial that may fuel regulatory concerns, taking into consideration what was discussed in the previous chapter. In the third section I will propose the next steps the trial should take to mitigate those tangible issues of patient concern in an understandable, transparent and efficient way.

The trial to be analyzed is the following:

Nathwani, A. C., Tuddenham, E. G., Rangarajan, S., Rosales, C., McIntosh, J., Linch, D. C., Chir, B., Chowdary, P., Riddell, A., Jaquilmac –Pie, A., Harrington, C., O'Beirne, J., Smith, K., Pasi, J., Glader, B., Rustaji, P., Catherine, Y.C., Kay, M.A., Zhou, J., Spence,Y., Morton, C.L., Allay, J., Coleman, J., Sleep, S., Cunningham, J.M., Srivastava, D., Basner-Tschakarjan, E., Mingozzi, F., High, K.A., Gray, J.T., Reiss, M.U., Nienhuis, A.W. and Davidoff, A. M. (2011). Adenovirus-associated virus vector–mediated gene transfer in hemophilia B. *New England Journal of Medicine* 365(25), 2357-2365.

### Trial Description

#### *Protocol Rationale*

Hemophilia B is a genetic disease where there is a defect in the gene coding for the coagulating factor IX (FIX), which is an essential part of the clotting cascade. Patients suffering from this disease present FIX values below 1% of normal levels, which is characterized by frequent bleeding episodes. The most common treatment for this X-linked disease is transfusions of FIX many times a week in a prophylactic manner. This treatment is very expensive and the infusion of FIX decreases the patient's quality of life. The idea of correcting the defective FIX gene would represent a curative therapy that would improve the patient's quality of life and economy. It has been demonstrated that values above 1% FIX are enough to cure the bleeding episodes.

The investigators in this trial used an adenovirus-associated virus (AAV) vector for the delivery of the corrected FIX version. They chose this vector because it has already demonstrated great efficacy in preclinical studies performed and this type of vectors can transduce nondividing cells. However in a previous phase 1 clinical trial, a version of this type of AAV (serotype -2), has only shown short-lived expression of FIX and they think it is related to a T-cell response to the capsid of this serotype and therefore against the transduced hepatocytes in the host. They have also observed that serotype 8 has a lower prevalence in plasma than serotype 2, which might reduce the T-cell mediated response against the vector.

For these reasons, the investigators have modified the AAV and combined serotypes 2-8 to create a fused vector that would still integrate with good efficacy but create a minor T-cell response to the vector. Additionally, the serotype 8 has an increased affinity for hepatocytes, which represents an advantage in the way that the vector can be administered intravenously in the peripheral vein. They also developed an expression cassette of the FIX gene that is packaged as complementary dimers within the vector and, based on previous experiments, has shown a greater rate of transgene expression compared to the single strand versions.

***Protocol design***

The investigators in this trial established some pre-requisites for people participating in this study which, in my opinion, were essential to circumvent some of the concerns the FDA may have. They enrolled patients that met the pre-requisite of not having neutralizing antibodies against vector serotype -8. After the pre-screening, 6 participants were chosen and they were provided with an Informed Consent Form.

The 6 patients enrolled were then divided into three cohorts: low vector dose (2x1011 vector genomes (vg) per kg of body weight), medium vector dose (6x1011 vg/kg) and high vector dose (2x1012 vg/kg).

To determine the safety profile of the product the investigators measured vital signs, anti-capsid and anti-FIX antibody levels, vector shedding, and cellular immugenicity. To evaluate efficacy of the therapy the investigators measured FIX activity and defined a lower limit of 3% of normal FIX levels for this matter, anything above this lower limit would be considered persistence of biological activity.

***Results***

Before the therapy started, the investigators assessed vital signs and FIX levels on all participants. They all showed FIX levels lower than 1% of normal values. The investigators also performed FIX gene sequencing to determine the type of mutation present in each participant. Four of them had mis-sense mutations, one had a null mutation and another had a mutation on the promoter. This sequencing confirmed the low FIX plasma levels observed in all participants.

The investigators did not observe any significant changes in vital signs after gene transfer, even though the vector was still present in plasma after 15 days. After 6 weeks of the gene transfer they identified 3 adverse events possibly related to the gene transfer. However, these were not classified as serious.

In general after gene transfer, all participants had an increase on their FIX plasma levels to an average of 3% of normal levels. These increases were directly related to the gene transfer and they all persisted for more than 16 months. This level increase also prevented any bleeding episodes in all the participants, where two of them suspended their prophylactic therapy and four of them increased their intervals between them. The only serious adverse event that was observed and it was attributed to the gene transfer was an increase in hepatic transaminases levels in both patients at the high dose cohort. The increase in transaminases was treated with prednisolone and was controlled without any complication. It was caused due to a T-cell mediated immune response against transduced hepatocytes. This immune response caused a decrease

on FIX plasma levels due to the elimination of transduced hepatocytes but after it was controlled with glucocorticoids, the FIX levels remained stable and above 3%, which is enough to maintain a bleeding-free phenotype. It is also worth mentioning that none of the participants developed neutralizing antibodies against FIX and the immune response observed was mainly cellular.

**Results Discussion from a Regulatory Perspective**

The results from this trial are very promising from a scientific perspective and they surely represent a good alternative for patients suffering from Hemophilia B. However, from a regulatory perspective, there are some issues that can be cause of concern within the FDA and that need to be addressed before continuing more clinical phases with this product.

In the next section I will suggest next steps to take on the researcher's part including evaluation methods, treatment modifications or trial amendments to be performed to ensure a swift FDA approval.

The immediate cause of concern from the results presented in this trial is the T-cell mediated immune response which represents a safety risk to the patient and a possible efficacy decrease due to this elimination of transduced hepatocytes which already caused a significant decline in FIX plasma levels before it was treated with prednisolone. An immune response against a drug is always something to be considered because it can be the source of many serious adverse events that could lead to serious organ failure or even death if they are not treated in a timely manner. Additionally, the efficacy decrease needs to be further considered perhaps under the scenario of dose correction. The investigators should take corrective measures to address this issue that could represent a major delay in their drug approval.

The investigators did not mention any evaluation method to asses a safe integration of the gene within the host genome. It is important to remember that the patients that developed leukemia in the X-SCID trial developed it 2 and a half years after the gene transfer (13). From the results presented, there is not enough evidence to rule out any insertional mutagenesis event. In my opinion, a long follow-up of patients will not be enough to support a safety claim. In the next section I will mention some evaluation methods that can be performed to have more solid evidence that no insertional mutagenesis will happen in the patients transduced.

Another issue that may be important for the regulators would be the decline in FIX levels over time after gene transfer. From the results presented, it is clear a decline over time of the FIX levels that, so far have been stabilized at approximately 3%, however, in some patients the decline was pretty significant after a period of time where the investigators thought the FIX levels had been stabilized. How can the investigators make sure that the FIX plasma levels will not decrease further?

Based on the results showed, the determination of the dose was not completely clear. Even though there were some SAEs in the high dose cohort, better efficacy results were obtained. The investigators may need to perform another study to determine efficiently a dose where the maximum benefit is observed without or less SAEs. Additionally, the investigators did not show how they came up with the dose on all the cohorts. In my opinion, the investigators should include their rationale in calculating the doses to be used in each cohort.

Although the results look very promising from a scientific perspective, one problem between regulators and scientists is that the regulators need to see clearly the benefit over the standard of care. The investigators should present a clear table (a suggestion is included in the next section) that shows the advantages over the current standard of care as well as a risk/benefit analysis.

Regarding handling of biological samples and ICF, since it is not described in the published article, I cannot comment on its handling within the trial, but I would expect it to be completely transparent, in an easily understandable language that clearly states all the procedures and tests to be done to the patient and to any biological samples taken from him/her.

**Proposed Actions**

An immunogenicity tests to determine the cause of the T-cell mediated response is unnecessary at this point because the cause has already being determined. However, another study that could help correlate the grade of immunogenicity to the maximum plasma FIX levels would be very useful to address this issue. An amendment to the protocol could be done including a round of prophylactic glucocorticoids to avoid the triggering of the immune response from the beginning and therefore prevent the decrease in FIX plasma levels.

Regarding the main issue for viral vectors insertional mutagenesis there are many strategies to follow to minimize this risk. The main strategy would be developing a safer integrating gene delivery system, generating vectors that do not affect the natural genomic organization of the target cells, preventing faulty integration of viral promoters near oncogenes in the cell genome (2). In the particular case of this trial, the investigators could characterize and sequence the most common integration sites using 3' linear amplification-mediated-PCR (LAM-PCR). This new technology allows the identification and sequencing of unknown flanking DNA sequences in the cellular genome (20). This procedure can be really helpful to establish a safety profile of the viral vector knowing its integration pattern.

Since the human genome project was announced, the hope of an affordable genome sequencing technique has created a race in the scientific community to reach

the goal of a thousand dollars genome (21). Nowadays, this can be achieved and its clinical repercussions are unlimited. In this trial, a sample of transduced hepatocytes can be taken from the patients at different times after gene therapy to sequence and ensure non-transformant integration events. And in the case of finding a clone that could be potentially harmful, take the appropriate actions before the cancer develops fully.

Regarding the issue of a durable transgene expression, the only way to monitor this is with a long-term follow up of every patient subject to the treatment, measuring FIX plasma levels. However, with a careful dose calculation that ensures maximum transduction with minimal insertional mutagenesis risk, this can be achieved. The challenge would be maintaining the population of transduced cells in the patient. In the particular case of this trial, since there was a T-cell mediated immune response against the transduced hepatocytes, this represents a risk of losing these transduced cells and therefore losing efficacy over time. Perhaps a re-dosing of each patient would enrich the transduced cell population with the risk of enhancing the immune response. This alternative needs to be evaluated in a preclinical setting.

It has demonstrated in viral vectors that the transduction efficiency correlates directly to the number of vector integration events per cell (18). This was demonstrated using transduced primary hematopoietic CD34+ progenitors and cultured K562 cells with a reporter gene (enhanced green fluorescence protein EGFP), using a retroviral vector for gene transfer. They tested different multiplicities of infection (MOI) and analyzed the transgene expression levels in mass cultures and cell clones derived from each MOI. They concluded that limiting the insertion events to less than 3 per cell can help reduce the probability of encountering an insertional mutagenesis event (18). This type of approach can be performed to determine a safe and effective vector dose in future experiments.

A presentation of an appropriate risk/benefit profile is essential in every regulatory submission. This helps the regulator to see the clear advantages of your novel therapy/product compared to the current standard of care. Even though a risk/benefit profile focuses mainly on clinically significant benefits and risks, it is highly recommended to include a pharmacoeconomical perspective in the profile since that aspect is part of a patient quality of life and it is very often ignored by the scientist part but should never be left out by the regulatory part of the industry. Table 1 presents a proposed comparison of benefits that could apply to what was presented in this trial.

| | **Proposed Novel Therapy** | **Current Standard of Care** |
|---|---|---|
| **Description** | AAV-mediated gene transfer of FIX gene to hepatocytes | Intravenous injections of FIX protein concentrate |
| **Frequency of administration** | One time administration | 2-3 times per week |
| **Type of treatment** | Curative | Prophylactic |
| **Reported Adverse Events** | T-cell mediated immune response to the viral vector resulting in decrease of FIX expression levels | Formation of neutralizing antibodies due to its exogenous nature |
| **FIX origin** | Endogenous | Exogenous |
| **Cost** | Unknown, but probably high cost | High cost and limited supply |

**Table 1.** Proposed comparison of novel therapy versus the current standard of care. This type of comparison must be much more elaborate and include as much information as possible that can help the regulator see the clear advantages of the novel therapy. It is very important to be transparent and clear, never hide or exaggerate any kind of information.

## CHAPTER IV

## CASE STUDY 2

In the same way as chapter III , this chapter is divided in three sections. In the first section I will describe the trial and its results providing with enough background information to understand their goals and study rationale. In the next section I will discuss the outcomes of the trial that may fuel regulatory concerns, taking into consideration what was discussed in the previous chapter. In the third section I will propose the next steps the trial should take to mitigate those tangible issues of patient concern in an understandable, transparent and efficient way.

The trial to be analyzed is the following:

Cartier, N., Hacein Bey-Abina, S., Bartholomae, C.C., Bougneres, P., Schmidt, M., Von Kalle, C., Fischer, A., Cavazzana-Calvo, M. and Aubourg, P. (2012). Lentiviral hematopoietic cell gene therapy for X-linked adrenoleukodystrophy. *Gene Transfer Vectors for Clinical Application* 507, 187-198

### Trial Description

#### *Protocol Rationale*

X-linked adrenoleukodystrophy (X-ALD) is a genetic disease that mainly affects male kids between 5 and 12 years old. It is caused by a mutation in the ABCD1 gene that codes for a transporter protein (ALD) which is involved in the metabolism of very long chain fatty acids (VLCFA). A defect in this gene would translate in low levels of ALD leading to the accumulation of VLCFA in plasma and specifically in the central nervous system where it causes demyelination which results in degenerative mental retardation until they reach a vegetative mental state or death within 2-5 years after diagnosis. There is also an adult version of the disease but it is milder and does not represent a very high death risk to the patient. The investigators focused this trial to the research treatment on the correction of ABCD1 gene in kids.

The current form of therapy for this disease is allogeneic hematopoietic stem cell transplantation (HCT) which has shown good results in demyelination progression on kids. However, as any other allogeneic transplantation, it faces two major challenges: the finding of suitable donor and the most significant, graft versus host disease (GVHD).

The investigators in this trial propose a new approach to the arrest on the disease which is transplantation of autologous hematopoietic stem cells (HSCs) transduced *in vitro* to express ALD and arrest the demyelination in the central nervous system. They propose using a lentiviral vector for the gene transfer into the HSCs. Lentiviral vectors same as AAV can transduce nondividing cells and there is preclinical data that shows more efficient gene transfer into HSCs than some retroviral vectors. Another advantage of using lentiviral vectors is that they represent a lower risk in the insertional mutagenesis problem with preclinical data to support it.

This initial trial was performed in France in 2005 and it was presented to the OBA in 2010 for review and approval of enrollment of American patients.

***Protocol design***

Two pediatric patients were enrolled in the study. They both had been diagnosed with a progressive cerebral neuroinflammatory demyelinating form of X-ALD determined by brain MRI. These two patients were eligible for allogeneic HCT but no suitable donor had been found at the time of enrollment.

The investigators obtained peripheral blood mononuclear cells (PBMCs) from the patients. From the PBMCs obtained, $CD34^{+}$ cells were purified and were enriched and finally co-cultivated with the lentiviral vector at a multiplicity of infection (MOI) of 25. This MOI was calculated previously to deliver approximately one or two integration events per cell and minimize the risk of insertional mutagenesis. After the cells were transduced, they were cryopreserved and stored for release testing (5% of transduced cells) and rescue transplantation.

The release testing consisted in a series of tests to determine the number of replication competent lentivirus (RCL) on transduced $CD34^+$ cells. The investigators used the permissive cell line C8166 to perform these assays. Three weeks after co-cultivation, RT-PCR and PCR tests were performed to detect viral mRNA and viral DNA but nothing was detected. Additional to the RCL assays, the investigators analyzed ALD protein expression and vector copy number. With these release testing results, the investigators proceeded to the administration of the cells to the patients.

Before HSC transplantation, both patients received full myeloablative procedures to favor engraftment of the transduced HSCs. The cells were infused at a dose of $4.6x10^6$ cells/kg for patient 1 and $7.2x10^6$cells/kg for patient 2.

Both patients were closely monitored for SAEs and disease progression. The hematopoietic cell line was recovered 13-15 days after transplantation and immunological recovery was achieved between 9 and 12 months.

***Results***

Before transplantation occurred, ALD protein levels were measured in the transduced HSCs. The transduction efficacy of $CD34^+$ cells before transplantation ranged between 33 – 50%. Two months after transplantation the ALD expression ranged from 20-33% of total PBMCs. These values decreased over time but finally stabilized at 10-13% after 16 months. These values remained stable up to 36 months after gene transfer.

The investigators also measured ALD expression in other cell subpopulations. They both had similar percentages of expression in granulocytes, monocytes, B lymphocytes and T lymphocytes, ranging from 7-14%. Expression in bone marrow $CD34^+$ cells was also measured and it ranged from 18-20 %.

They also performed Q-PCR to determine integrated vector copy number, these results correlated directly with the ALD protein expression levels observed in each cell

line. The mean number of integrated vector copies was 0.72 and 0.54 for each patient in transduced CD34+ cells. Additionally they performed a colony forming assay on CD34+ cells and they showed the obtaining of effective gene transfer into myeloid progenitors with long-term engraftment capacity.

What they did next, in my opinion is of high relevance from a regulatory perspective. They performed an integration site (IS) characterization and they discovered a high number of unique IS which was consistent to the polyclonal distribution of gene corrected hematopoietic cells.

They also sequenced the IS using third-generation sequencing methods to evaluate if the identical integration sites between lymphoid and myeloid lineages could mean a transduction of their progenitors and not that the transduction occurred after differentiation. They determined that the high number of identical IS suggested that it was not a random integration and it had a pattern, which could only happen in the progenitor HSCs.

This whole IS characterization represents a key element for building an appropriate safety profile of the product applying the best manufacturing practices (BMPs) industry protocol. As discussed previously, a good safety profile is something a regulator is always looking for and the more complete it is, the faster the product could be approved.

When they performed brain MRIs to determine progression of cerebral demyelinating lesions, for the first patient they observed that it was arrested 12-14 months after gene transfer and remained stable up to 36 months after therapy. However, they noticed a decrease in cognitive functions due to the 12-14 months that took before disease stabilization. They mentioned this is also observed in the current standard of care (allogeneic HCT). They performed intelligence quotient (IQ) tests to assess these decrease in cognitive functions. They measured verbal IQ, performance IQ. In the first

patient they noticed a slight decrease on PIQ but not VIQ. In the second patient they observed disease stabilization 16 months after transplantation. At this point they also observed partial vision that evolved slowly until 30 months after gene therapy. This patient's IQ scores were maintained normal up to 30 months after gene therapy but then declined due to the vision loss that occurred. Despite this vision impairment, this patient is performing normally for his age and remains stable in the progression of the disease.

The investigators demonstrated a successful lentiviral HSC gene therapy in both patients. They demonstrated long-term expression of ALD in many hematopoietic cell populations and characterized integration sites for transduced HSCs. However, they are aware that the percentage of transduced HSCs can be improved which would result in a reduction of the 12-16 month period during which the disease continues to progress after transplantation.

The investigators continue to follow-up the patients for long-term SAEs but they are still planning an extension of this trial to obtain more experimental evidence to support an application for therapy approval.

**Results Discussion from a Regulatory Perspective**

This is a very interesting trial with very promising results, however there are some issues that could fuel regulatory concern and therefore prevent its approval for further clinical trials.

One of the main concerns is the trial design. The results presented are not statistically significant, which raises the question of a true representation of the patient population in this kind of diseases. Perhaps there can be rare but serious adverse events that are not showing with this reduced patient population. Even though the trial aims to enroll more patients, the characteristic of a phase I study is to determine a safety profile for the product or therapy and it requires sufficient data to create a statistically

significant safety assumption. In my opinion, two participants are not enough to claim a safe and efficient profile for the therapy. A good example of this is the partial vision loss experienced by patient two, if this is an event attributed to the therapy, then it would be considered of high risk, however, with the few data available it is not possible to reach this conclusion.

Regarding, the risk insertional mutagenesis associated with the use of a lentiviral vector I think the investigators have presented enough evidence to minimize this probability of it happening. However, a careful follow-up of patients would be necessary to rule out any long-term malignancy that could arise. The investigators used a self-inactivating vector, which are vectors considered safer than their original particles. In these self-inactivating versions, the promoter regions in the LTRs have been deleted to minimize the probability of interactions between the vector DNA and the host genome (2). While this represents as a safe measure, it also represents a loss of transduction efficiency. A detailed analysis of the vector construction is provided by the investigators which represents more evidence to decrease the probability of insertional mutagenesis.

The investigators presented ALD expression measurement in many hematopoietic cell subsets which was sustained up to 36 months after transplantation. This gives evidence of durable transgene expression; however the levels of transduction are not ideal. Perhaps the investigators in a different trial can increase these levels and obtain better efficacy results.

The investigators did not include in their protocol design any immunogenicity assay to rule out any immune response against the viral vector. Immunogenicity determination is a key feature of any new drug application and it is very important to include an analysis in every trial. Neutralizing antibodies could be interfering with the efficacy observed or the time of disease stabilization after transplantation. The immune response is a very important factor to consider both for safety and efficacy reasons.

A very concerning issue in the results presented in this trial is the period between transplantation of transduced HSCs and the stabilization of the disease. The investigators mention this also happens in the current standard of care but as a regulator, I would like to see experimental evidence to this claim. There is a difference of 2 months between patient one and two. The investigators should gather more data to establish an average time of disease stabilization and then compare it to data described for allogeneic HST.

Regarding dose calculation, although the investigators explain their rationale in their dose calculation they did not present data from another dose escalation study to establish the appropriate dose with the highest efficacy and less SAEs.

A risk/benefit analysis should be included in the protocol in the ICF for patients. It is very important that the patients understand the clear risks and benefits of this therapy compared to the current standard of care (allogeneic HST). This represents an ethical concern that the Institutional Review Board (IRB) should have had addressed.

**Proposed Actions**

The main issue of this trial is the lack of statistical significance in their results. The only way to achieve this is to enroll many more patients to this study. However, since the target patient population is pediatric patients, special considerations need to be taken when doing research with this type of patients (22).

This study performed a good characterization of the IS in the transduced cells, which in my opinion presents good experimental evidence that reduces the probability of observing an insertional mutagenesis event. Solid preclinical data can be presented for this purpose. Preclinical studies to analyze described hotspots for each viral-derived vector systems integration sites is essential to determine any possible insertional mutagenesis-related event that could lead to the onset of malignancies in patients (2)

There are studies that describe the pattern of integration of HIV. In this article they describe the mapping of 524 integration sites in the human genome (23). Since this lentiviral vector was engineered based on HIV, I would consider useful to compare the IS analysis made by the investigators with this reported data for HIV.

In regards of analysis of durable transgene expression I think the investigators have made all appropriate measurements and have demonstrated expression of ALD protein over time. This expression was maintained up to 36 months after gene therapy and continues to be measured in the patient's follow-up. This represents a very good aspect of the trial since this long-term expression represents extended progression-free survival of patients affected, translating on good quality of life.

Immunogenicity tests for the vector can be performed with the use of an interferon- enzyme-linked immunosorbent spot (ELISPOT) assay. This type of assay can detect any T-cell mediated immune response against the viral vector (24). Neutralizing antibody detection can also be performed to determine any interference that may be happening against the viral vector.

The investigators need to collect data to support the claim that the same 12-16 month period before disease stabilization is also observed on allogeneic HST; this should be an important feature of any subsequent IND application for future trials. It is very important to include this kind of data in the ICF for patients, to ensure we are providing with all the facts on both the novel therapy and current standard of care before enrolling in the study. Since this is a pediatric study, this should be strictly enforced to help the parents or guardians make the best decision for the patient.

Clinical data from a dose escalating trial needs to be presented to confirm a safe and efficient dose establishment.

A risk/benefit analysis needs to be presented specifically in this novel gene transfer therapy against allogeneic HST. The most important feature to be included in

this comparison has to be graft versus host disease (GHVD), which is the main disadvantage of allogeneic HST. There are studies that fully describe the issue of GHVD in stem cell transplantation. There are many chronic events after allogeneic HST (25). The issue of finding a suitable bone marrow donor is another important disadvantage of the therapy.

## CHAPTER V

## CONCLUSION

The regulatory world is a very complicated environment where the main goal is the safeguarding of patients, ensuring that every drug and therapy available to them is safe and efficacious. As I showed in this work, there are currently many concerns regarding gene transfer therapy trials and they all have a rational origin. However, this becomes a problem when drug developers, the scientific community does not know how to interpret these concerns and makes the same mistakes over and over again.

Gene transfer technology has the vast potential of curing many diseases out there, including cancer. An understanding of both parts, regulatory and scientific, is key to allow access to patients to this amazing type of therapy.

I provided two examples to discuss and review from a regulatory perspective, taking into consideration all the concerns expressed by the RAC. I hope to have set a basic guide on how the scientific part can interpret their results and take the appropriate actions to make the regulatory agencies happy.

The tools are in place for both development, and adequate control. A harmonization between both parts is essential, and will benefit the patients infinitely.

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