1. Title of the clinical study

A Phase 3 Open-Label, Single-Arm Study To Evaluate The Efficacy and Safety of BMN 270, an Adeno-Associated Virus Vector–Mediated Gene Transfer of Human Factor VIII in Hemophilia A Patients with Residual FVIII Levels ≤ 1 IU/dL Receiving Prophylactic FVIII Infusions (Protocol number: 270-301)

A Phase 3 Open-Label, Single-Arm Study To Evaluate The Efficacy and Safety of BMN 270, an Adeno-Associated Virus Vector–Mediated Gene Transfer of Human Factor VIII at a dose of 4E13 vg/kg in Hemophilia A Patients with Residual FVIII Levels ≤ 1 IU/dL Receiving Prophylactic FVIII Infusions (Study 270-302)

2. Proposed period of release

From 31 May 2019 until July 2019

3. Name of company

BioMarin Pharmaceutical Inc.

4. Background

Hemophilia A (HA) is an X-linked recessive bleeding disorder that affects approximately 1 in 5,000 males. It is caused by deficiency in the activity of coagulation factor VIII (FVIII) gene that codes for FVIII protein, an essential cofactor in the coagulation pathway. This disorder can be either inherited or an acquired immunologic process, leading to insufficient quantities of FVIII or a dysfunctional FVIII, ultimately leading to a defective coagulation process. The clinical phenotype of HA patients generally correlates tightly with the level of residual FVIII expression. The clinical manifestations of severe HA are frequent spontaneous bleeding episodes, predominantly in joints and soft tissues, with a substantially increased risk of death from hemorrhage when the brain is involved. Subjects with moderate disease can exhibit manifestations similar to those seen in patients with severe HA, resulting in a comparable bleeding phenotype.

BioMarin Pharmaceutical Inc. is developing an experimental gene therapy AAV5-hFVIII-SQ (BMN270), an AAV5-based gene therapy vector that expresses the SQ form of human FVIII (hFVIII) under the control of a hybrid human liver-specific promoter, to deliver the sequence encoding hFVIII to the liver. AAV5-hFVIII-SQ will be delivered by single intravenous dose and is designed to achieve stable, potentially life-long expression of active hFVIII-SQ in the plasma, synthesized from vector-transduced liver tissue.

5. Information on the genetically modified organism (GMO)

5.1 Receptient organism:
Adeno-associated virus serotype 5 (AAV5)

5.2 Donor organism:
The inserted sequence is the human gene for clotting factor VIII under control of a liver specific promoter.

5.3 Methods used for genetic modification

The plasmids were constructed using standard molecular biological techniques for the precise excision and ligation of component elements using specific restriction enzymes followed by transduction and amplification in bacterial cells at each stage.

5.4 Resulting genetically modified organism

AAV5-hFVIII-SQ is a recombinant, replication-incompetent, adeno-associated virus serotype 5 (AAV5) vector containing a DNA genome, hFVIII-SQ.
The outcome of the genetic modifications is to remove the virus rep and capgenes coding sequences, leading to the loss of replication ability, and the insertion of the human Factor VIII transgene expression cassette leading to the expression of functional hFVIII-SQ in the liver.

A new characteristic is that AAV5-hFVIII-SQ cannot replicate even in the presence of helper virus because 94% of the viral genome is absent. Another new characteristic is that if AAV5-hFVIII-SQ enters a cell it cannot integrate into the genome because sequences responsible for integration have been removed.

6. Description of clinical study

The release of the GMO will be made in the context of the clinical trials with protocol numbers 270-301 and 270-302. Both studies are Phase 3, single-arm, open-label studies designed to assess whether, in an expanded sample, AAV5-hFVIII-SQ can safely alter the clinical phenotype of hemophilia A patients with residual FVIII activity ≤ 1 IU/dL.

Preparation and administration of the investigational product will be made by authorized trained personnel at the study dosing centers in Leuven, Brussels and Edegem.

Monitoring of the direct and indirect effects of AAV5-hFVIII-SQ in subjects will be achieved by the clinical assessments defined in the clinical trial protocol. Study investigators will monitor subjects throughout treatment and will report adverse effects according to the requirements stipulated in the protocol. Monitoring will occur throughout a subject's participation in the study, including a 5-year period of safety follow-up.

Vector shedding will be monitored at several timepoints after administration utilizing PCR.

7. Assessment of possible risks to human health and the environment

AAV5-hFVIII-SQ is a recombinant, replication-incompetent, adeno-associated virus serotype 5 (AAV5) vector containing a DNA genome coding for the SQ form of human Factor VIII protein. The intended use is limited to 3 hospital centers and the number of patients to be treated is limited (a maximum of 7 patients will be treated in Belgium in Study 270-301, and up to 3 patients will be treated in Study 270-302).

AAV is already widespread in the community and does not cause disease. The AAV meets the definition of biological agent of Risk Group 1 according to Directive 2000/54 / EC ("biological agent that is unlikely to cause human disease").

Exposure to AAV5-hFVIII-SQ is not expected to have any deleterious effects on health of humans, other species or the environment. If a person were to be inadvertently exposed, while unlikely, the dose received would be orders of magnitude less than the non-efficacious dose in the ongoing first-in human study of AAV5-hFVIII-SQ (Study 270-201). Of note, the subject who received AAV5-hFVIII-SQ at this non-efficacious dose did not experience any adverse events.

AAV5-hFVIII-SQ is a replication-incompetent virus derived from AAV5. The genetic modifications do not affect its natural host and tissue tropism. No transfer of genetic material between the GMO and other organisms is predicted.

The transfer of genetic material is limited to the theoretical genetic exchange of DNA by homologous recombination with wild type AAV which could only occur if human cells were simultaneously infected with both wild type AAV and AAV5-hFVIII-SQ, in the presence of a helper virus. In the case of AAV5-hFVIII-SQ, such recombination could only result in the exchange of the hFVIII-SQ expression cassette with the rep and cap genes of the wild type virus. It is not possible for the AAV genome to contain both rep/cap genes and the transgene, as this is beyond the packaging limit of the virion.

Therefore the only mechanism by which the transgene could be mobilised is through a triple infection of the same cell by AAV5-hFVIII-SQ (containing the transgene), wild type AAV (providing the rep and cap functions) and a helper virus. This scenario is expected to be a rare event, and would only result in the production of more wild type AAV and more AAV5-hFVIII-SQ vector particles (which would still lack rep and cap genes and consequently could not be self-sustaining).

If AAV5-hFVIII-SQ enters a cell it cannot integrate into the genome because sequences responsible for integration have been removed. Integration of the AAV5-hFVIII-SQ genome has not yet been evaluated experimentally. Extensive studies with AAV2, AAV1, AAV8, and AAV5 vectors in rodents (, rabbits, nonhuman primates and in human subjects who were administered Glybera (an approved gene therapy product) lead to the estimation that the integration frequency of AAV vectors is several orders of magnitude lower than the spontaneous rate of mutation for human genomes so that the likelihood of insertional mutagenesis by AAV vectors is very low, although specific carcinogenicity studies have not been performed.

The only mechanism by which the vector could insert into a gene in a cell is a homologous recombination between the endogenous FVIII gene and the FVIII coding sequence of the vector. Such theoretical risk of recombination between the vector and the endogenous FVIII gene would be limited to dividing cells due to accepted mechanisms of homologous recombination during DNA replication. Thus, the actual risk of vector insertion into a gene of any cell is several orders of magnitude below the spontaneous mutation rate in human genes.

Given the nature of the product administration (intravenous) and the transient/low levels of shedding expected, the risk of unintended exposure to AAV5-hFVIII-SQ to humans and other biota is minimal. Nearly all of the low level of vector genomes that are present in body fluids appear to be cell associated, and not present as free vector particles. This makes it even less likely that there could be horizontal transmission of infectious genomes to others. Also, none of the vector genomes are detected in sperm so there is no likelihood of vertical transmission. Furthermore, on the basis of the vector construct, it is considered that AAV5-hFVIII-SQ vector construct has been appropriately engineered with characteristics such that the adeno-associated viral vector has been sufficiently attenuated resulting in disablement of the vectors ability to infect, replicate or survive outside of humans.

Preparation and administration of AAV5-hFVIII-SQ will take place in an approved hospital environment. Only qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment will undertake the preparation, handling and safe disposal of AAV5-hFVIII-SQ.

All disposable materials (including but not limited to gloves, masks, syringes, needles, catheter and tubing) that come into contact with the investigational product will be disposed of as biohazardous waste, according to national and regional regulations on hazardous and sanitary waste. They will be disposed of in accredited biohazard containers for solids or sharps and decontaminated by either by incineration or by saturated steam autoclave using validated fractional vacuum cycles by an authorized hazardous waste management company.

The used/partly used vials will be disposed as biohazardous materials immediately and drug accountability will be performed based on the logs. Any unused AAV5-hFVIII-SQ should be retained at the dosing facility for investigational drug accountability and monitoring. The unused material will be disposed as biohazardous materials, following the same procedure indicated above. Materials, equipment and non-disposable surfaces will be decontaminated by spraying with broad-spectrum disinfectants with proven activity against non-enveloped viruses. Solutions such as Surfa'safe Spray (didecyldimethylammonium chloride) or Umonium (benzalkonium chloride, isopropilic alcohol and lauromyristic alcohol) may be used.

The chance of dissemination of the vector is negligible outside of the location of IMP administration. The only plausible route via which harm could occur would be if there was inadvertent exposure of people undertaking the dealings to GMOs. This is considered unlikely or highly unlikely. Inadvertent exposure would require a skin-breaking injury (e.g. needle stick) or exposure to uncontained aerosols. Staff undertaking the dealings will wear gowns, gloves, protective googles and surgical masks, and the GMO will be double contained or prepared for infusion in appropriate facilities/clinical setting. There will not be work with any other GMOs by personnel in the work space during the conduct of this dealing. Labs for processing clinical samples, e.g. bloods etc. would use standard precautions for bodily fluids.

The only foreseeable case of unexpected GMO dissemination would be a spill during the preparation or administration of the product under study. This dissemination would always be contained within the room where the spill occurs. Should the investigational product be spilled or otherwise dispersed during the preparation or administration the procedures in the Study Pharmacy Manual, distributed to the dosing centers should be performed in accordance with standard practices for cleaning up biohazard waste spills, like those for treating potential blood borne pathogens.

- Notify others and isolate the area.
- If not already wearing, put on appropriate personal protective equipment: disposable aprons, gloves, particle protection facemask and safety glasses, face shield or goggles.
- Remove any broken glass or sharps with forceps or applicable tool and place into a sharps container.
- Decontaminate the area of the spill:
    - Place absorbent material over the spill.
    - Add disinfectant solution on the absorbing material and let it absorb.
    - Sweep up and place the absorbent material in infectious waste bag for disposal
    - Wash the area with broad-spectrum disinfectants with proven activity against non-enveloped viruses and dispose of all the used disposable materials as biological waste.

The research conducted within this framework adequately mitigates the risks of harm to the public health and to the environment.