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AAV Gene Therapy trials

Considerations for safe implementation at GOSH

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Intro

These guidelines are the results of an expert panel discussion at GOSH / UCL. They have been written following a series of meetings organised in 2019 between PIs and clinical researchers who have shared their experiences and lessons learned from their direct involvement with AAV gene therapy trials. Special consideration has been given to

- Adverse events and SUSARs reported so far in the various AAV trials
- Immunosuppressive regimens used: differences, pros and cons
- Next steps

Background

Engineered AAV vectors are derived from an adeno-associated virus, a parvovirus which is dependent on a helper virus (eg Adenovirus) for replication. AAV is not known to be pathogenic in humans, and antibody titres are detectable in >60% of adults

AAV vectors used for therapeutic applications lack viral coding sequences but retain inverted terminal repeats, and are packaged into a viral capsid. Immune responses may arise to the capsid, to the transgene product or both over time.

Several hundred subjects had been infused with AAV without fatal outcome for a variety of conditions, using local or systemic routes. Nevertheless, concerns of toxicities have recently arisen in early phase human studies, and recent non-human primate studies, in particular where high AAV dosing has been used.

Risk Assessment considerations

Before starting any AAV gene therapy trials at your site please consider the following general aspects in order to assess the risk of handling genetically modified organisms:

- ACGM categorisation of the final GM vector (Genetically Modified Microorganism): i.e. *Class I or Class II*
  Facilities in which the proposed activities involving the GMM would take place, particularly during *predose assessment, dosing and postdose care*
- Who will receive and prepare the gene therapy agent*? i.e. Pharmacy trials team will receive the IMP, Gene and Cell Therapy laboratory technicians will prepare the IMP dose*
- Who will administer the gene therapy agent? i.e. Research nurses who are deemed competent to administer intravenous medication
- Qualifications/Training relevant to procedures involving GM material. *i.e. Protocol and relevant training carried out before administration.*

Humans at increased risk from the GMM

Patients receiving the AAV vector

The administration of AAV vector has the risk of causing immune-mediated hepatitis. For patients who have positive serology for hepatitis B or C, or have pre-existing liver conditions, administration of AAV vector may represent an unreasonable risk; therefore, negative serology testing and liver function must be confirmed prior to treatment.

Additionally, administration of AAV vector may represent an unreasonable risk for patients with positive anti-AAVX antibodies; therefore, appropriately low antibody titers must be confirmed prior to treatment.

Unintended human recipients.

Other humans potentially at increased risk are unintended human recipients (healthcare workers and close contacts of the patient). It is not expected that exposure would lead to adverse effects in healthy humans if neither wild type AAV nor IMP are known to be pathogenic. Studies have shown that vectors will be excreted from the body for up to a few weeks after injection/infusion; this is called "viral shedding". Vector shedding can be found in the blood, urine, saliva, and stool for up to a 4 weeks following injection. The risks associated with the shed vector are considered negligible as the vector used is non-pathogenic and cannot replicate. Because of this it is believed that shed vector is unlikely to result in clinically significant adverse effects. Regardless, instructions should be provided to patient families and care givers regarding use of protective gloves if/when coming into direct contact with patient bodily fluids and/or waste, as well as good hand-hygiene for a minimum of four weeks after the injection.

Will segregation from other patients/visitors be necessary after the gene therapy agent has been given? If yes, for how long?

Specific segregation will not be required. During the inpatient stay, personnel are required to follow appropriate safety precautions as per institutional standards for infection control. IMP infusion will be administered under sterile conditions in a PICU or other appropriate setting (e.g., interventional suite, operating room, dedicated procedure room) with immediate access to acute critical care management. For intravenous infusions, the IMP will be delivered one time through either a venous catheter inserted into a peripheral limb vein (arm or leg) or a central line at a specific dose. IMP should be slowly infused; the time of infusion will depend upon volume required, utilizing an infusion set and pump in accordance with the Pharmacy Manual. Other mode of administrations might be required for other specific indications (i.e. intrathecal). Additionally, patients are prohibited from donating blood for two years following the vector injection.

Following administration of gene replacement therapy, patients should return to an appropriate designated setting to ensure close monitoring of vital signs and adverse events. Vital signs will be continuously monitored throughout the gene replacement therapy infusion as described in the protocol. Patients should be maintained in the PICU or other appropriate setting for 24-48 hours after the start of gene replacement therapy.

Could the patient require emergency treatment for the condition being addressed with gene therapy and how would this be safely dealt with?

The patient must be dosed in the PICU or other appropriate setting with immediate access to acute critical care management. We would utilise the hospital emergency team should it be required.

Will there be any preliminary or concurrent treatment with other agents (eg, immunosuppressive therapy)?

AAV gene therapy can activate the immune system via multiple mechanisms, i.e. innate response (via TLR activation); and adaptive immunity.

Innate response

Both capsid and vector genome can interact with complement, TLR2 and TLR9. Double stranded RNA can also trigger activation of the innate immunity. The outcome of a robust innate response activation can be the complement activation, and trombocytopenia. Typically this innate response activation occurs within the first few days from the infusion. In severe cases, this has led for example to atypical hemolitic uremic syndrome requiring hemodyalisis, with eventual resolution of this complication.

Adaptive immunity

It is possible to observe antigen specific T-cell response to the AAV vector between 2 to 4 weeks following gene replacement therapy. One possible consequence to such antigen specific T-cell response is clearance of the transduced cells and loss of transgene expression.

In an attempt to dampen down the host immune response to the AAV-based therapy, different strategies have been developed, focused on the innate response and the adaptive response activation.
Eculizimab is recommended in some protocols in case of a robust early activation of the adaptive response with complement activation.

Corticosteroids. All patients may receive prophylactic prednisolone at the dose suggested in the protocol beginning 24 hours prior to gene replacement therapy until at least 30 days post-infusion. In a few protocols, the dose of corticosteroids is increased, for example triplicated, in the first 3 days after the AAV infusion. After 30 days of treatment, the dose of prednisolone can be tapered for patients whose ALT values, AST values, and T-cell response are ≤ 2X ULN (Upper Limit of Normal) for ALT and AST, and < 100 SFC/106 PBMCs for T-cell response.

If the AST or ALT values are > 2X ULN, or if T-cell response is ≥ 100 SFC/106 PBMCs after 30 days of treatment, the dose of prednisolone will be maintained until the AST and ALT values decrease below threshold. If T-cell response continues past Day 60, Investigator discretion should be used considering risk benefit for maintaining prednisolone. Variance from these recommendations will be at the discretion of the Investigator based on potential safety issues for each patient.

Additional immunosuppression has been proposed in some protocols should additional indication of T- cell mediated toxicity appear. This toxicity in some programs has resulted in signs (troponin elevation; CK elevation; severe transaminitis) and symptoms (cardiac rhythm disturbance; liver failure) and empirically required the addition of sirolimus and or mycophenolate mofetil, due to the inability of corticosteroids to control the immune response.

Interim Assignment of Containment Conditions to Protect Human Health

The research site will be provided with a spill kit as specified by the European Association of Hospital Pharmacists (EAHP) Guidance on the Pharmacy Handling of Gene Medicines. The Pharmacy Manual includes information on the storage of investigation product, preparation under aseptic technique, and handling and disposal.

If no specific containment requirements are recommended, conventional hospital facilities, good practice and implementation of the standard principles for preventing hospital-acquired infection will generally be adequate for the management of the risks associated with the IMP.

Emergency procedures- Follow local policy/protocol

According to the GOSH - Gene Therapy Lab SOP for spillage of biological material:

1. Evacuate area, remove contaminated PPE and transfer it to autoclave bags. Close and secure the bags for disposal as per SOP AQU002 Disposal of Laboratory Waste. Initiate spill response procedure.

2. Cover the spill with absorbent material. Starting at the edges and work towards the center.

3. Spray the affected area with a commercial preparation of >0.25% paracetic acid solution.

4. Allow contact period of > 5 minutes.

5. Use paper towels to wipe up the spill, working from the edge to center. Use tongs or forceps to pick up broken plastics, glass or other sharps that could puncture gloves

6. Discard absorbent material in autoclave bags. Close and secure the bags for disposal as per SOP AQU002 Disposal of Laboratory Waste.

In the Pharmacy: as per their trial specific spillage SOP.

In the Hospital and while being transported by pharmacy staff: per the Policy for handling Blood and Body spills.

Environmental Considerations

Dissemination of AAV-IMP would most likely only occur between human beings, if it is derived from human associated adenovirus. When the AAV-IMP is a replication-incompetent virus derived from a human associated adenovirus (AAVx), it is at a competitive disadvantage when compared to its parent strain / wild type AAV. The transgene should not be expected to confer any advantage to the GMO in terms of survival and selective pressure. AAV-IMP should be non-replicative by deletion of the rep and cap genes rendering it unable to replicate, even in the presence of a helper virus. Therefore, infection leading to replication of the GMO (and therefore potential for dispersal) is not possible under normal circumstances. AAV shows some species specificity, but can replicate in cells of a different species when infected with AAV in vitro, provided it is in the presence of a helper virus to which that species is permissive. It is not known whether zoonosis occurs in nature, nor whether other species can act as carriers or vectors under natural conditions. However, given the inability to replicate and site of administration, the possibility of exposure of AAV-IMP to non-humans should be considered negligible.

The non-target organisms which could conceivably be affected are unintended human recipients (healthcare workers and close contacts of the patient). The most likely potential scenarios in which AAV-IMP may disperse from patients into the environment are via a spill, a needle stick injury during IMP administration, via blood following needle stick injury or via shedding directly from the patient. Routes of the virus dispersing from the test subject into the environment are via urine, stool, blood and saliva. It shouldn't be expected that transmission would lead to adverse effects in healthy humans if neither wild type AAV nor AAV-IMP are known to be pathogenic. It is possible the AAVx vector containing the SMN gene could interact with other viruses with which the patients come in contact, such as rhinoviruses, adenovirus, or herpes. If this happens, the AAVx vector could form a virus that causes infection if the healthy unintended human recipient and cells for rescue, replication, and packaging are also exposed to wild-type AAVx. The rescue, replication and packaging would stop; however, as the helper viruses, such as rhinoviruses, adenovirus, or herpes were cleared by the healthy unintended human recipient's immune system. In the unlikely event that transmission to a healthy unintended human recipient occurs, it is likely that the safety profile in healthy subjects would be at worst similar to those expected in patients.
As the GMO is replication defective and as the risk of shedding from patients is negligible, no special measures are foreseen in order to treat the patient for medical events related or not related to their disease. Isolation of

patients is not required. The GMO is a non-replicating vector; therefore it is not possible that it becomes persistent and invasive in natural habitats.

The hazard to the environment is negligible.

Considerations of possible immune responses to AAV

Three factors are likely to be important and should be considered by the investigators and sponsor. The vector, transgene and host.

The vector & transgene:

- What is the capsid type and is there any pre-existing immunity?
- How was it manufactured and is there any carryover of cell material, DNA or other contaminants?
- What is the transgene and promoter? Is the product expressed constitutively or is expression lineage restricted? What is the route of delivery?
- What is the dose and dosing schedule? In case repeated doses are considered, how are the immunological issues related to re-administration addressed?

Existing host immunity:

- General status: The underlying condition & comorbidities. Are there any active infections? Viral infections of the GI tract and Liver, Lung may be relevant.
- Determine seropositivity for 'helper' virus (Adenovirus, Herpes)
- Immunological status: Presence of anti-capsid antibodies; Use of recent or current immunosuppression; Recent live vaccines: avoid close temporal relationship between AAV GT and any vaccination

Host immune response following AAV:

Both innate & adaptive immunity is likely to be relevant:

- Generalised innate- immediate, to the capsid, may involve professional antigen presenting cells and release of cytokines
- Intercellular innate- upon target cell binding and entry, Toll like receptors and DNA sensing pathways
- Antibody- primary or secondary, against capsid or transgene; mediated by B cells with T cell help.
- T cell- In response to antigen presenting cells or target cells expressing antigens in the context of HLA; could be against capsid or transgene antigens.

Proposed assessments

These will be determined for the specific study, and will be influenced by the route of administration, dose, capsid, immunosuppression.

Baseline assessments

- FBC with attention to normal neutrophils and lymphocytes on the white cell differential.
- Renal and Liver function: for neuromuscular patients with constitutively elevated ALT and AST and CK levels GLDH should also be determined if possible (not influenced by muscle disease)
- BC, CRP,
- IgG,A,M
- Basic and memory Lymphocyte subsets
- Store serum (for cytokines, send to immunology)

- Store cells in liquid nitrogen (SCLN) for subsequent assessments such as ELISPOT (non routine, send to immunology)
- Anti-capsid Ab (non routine – store by immunology; refer to sponsor for analysis)
- Blood PCR for Adenovirus and Herpes virus
- NPA for respiratory viruses PCR
- Stool for enteric virus PCR

Routine assessments during study

- FBC, UE, LFT, CRP
- Store serial serum samples for cytokines- schedule dependent on study
- Additional viral monitoring in blood/CSF/tissue depending on immunosuppressive regimen

Additional suggestions

- Daily platelet counts for the first 5 days, then as specified by protocol
- complement (CH50, C3/C4)
- AST, ALT, GGT daily for first 5 days and, if possible, consider GLDH, then as specified by protocol.
- Coagulation parameters daily for first 5 days, then as specified by protocol
- Consider performing synacthen test if the child is on steroids for a long time

Additional samples in case of AE

- Store serum for AAV antibodies
- Store serum for cytokines
- Lymphocyte subsets, inc activation panel
- Complement levels
- SCLN for T cell studies
- Freeze blood / tissue/ CSF suitable for later DNA or RNA studies.

Factors to be considered during the set-up of an AAV trial:

- Consider initiating enhanced Immunosuppression for innate immunity activation and when platelet level <150.000. The costs may need to be negotiated with Sponsor during the set-up phase. The use of Eculizumab is particularly important to consider in advance as the cost is very high.
- Run a pre-treatment set of tests to determine prior immunity to various viruses (as in the pre-transplant guidelines) and consider vaccination if time allows. There needs to be a safe interval between AAV GT and the vaccination, which will depend on the age of the patient
- Share with families available recommendations on how to isolate their children from 1 week before to approximately 6 weeks after the treatment in order to avoid exposure to pathogens. Current GOSH recommendations for post-transplant BMT patients can be used as reference and can be found here:

https://www.gosh.nhs.uk/teenagers/your-condition/tests-and-treatments/bone-marrow-transplant