



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

16 March 2018
EMA/CAT/60852/2018
Inspections, Human Medicines Pharmacovigilance and Committees Division

Report from the CAT expert meeting

Scientific and regulatory considerations for adeno-associated viral vector (AAV)-based gene therapy

On 6 September 2017, the Committee for Advanced Therapies (CAT), one of the Scientific Committees of the European Medicines Agency (EMA), organised a one-day meeting on scientific and regulatory considerations for recombinant adeno-associated viral vector (rAAV)-based gene therapy medicinal products. The meeting was held in the premises of the EMA in London and was attended by the members of the CAT, members of other EMA scientific committees and working parties, and invited experts, who are leaders in the field of AAVs.

Background

An increasing number of recombinant AAV-based gene therapy medicinal products has entered clinical development. Different AAV subtypes including AAV 1, 2, 5, 6, 7, 8, 9 and engineered derivatives are explored in various therapeutic areas, including ophthalmology, neurology, haematology, metabolic disorders and muscular disorders. It is foreseeable that AAV-based advanced therapies will become part of the therapeutic armamentarium in rare genetic disorders.

The aim of this expert meeting was to get expert opinions on various scientific aspects of rAAVs such as clinical grade manufacturing, virus-host interactions in animals and humans, immunity, persistence, toxicity and clinical development.

Introduction

The regulatory framework for Advanced Therapy Medicinal Products (ATMPs), which include AAV-based gene therapy medicinal products, and an introduction to the role and responsibilities of the CAT were presented.

Regulation (EC) No 1394/2007 (ATMP Regulation) defines the different ATMPs (gene and cell therapy medicinal products and tissue engineered products) and sets up a dedicated committee, the Committee for Advanced Therapies (CAT). All member states are represented in CAT, as well as patient organisations and physicians. There are 5 members that are both members of CHMP (Committee for Medicinal Products for Human Use) and CAT. CAT has close interactions with other committees and Working Parties, especially Biologics Working Party (BWP) and the Scientific Advice Working Party



(SAWP). Main tasks of the CAT are: evaluation of marketing authorisation applications, ATMP certification and classification and scientific advice for ATMPs.

CAT also has the responsibility to develop and keep its guidelines on ATMPs up-to-date. Therefore, CAT is following closely the scientific developments in ATMP. This expert meeting was organised especially with that goal in mind: to get the experts' input on the ongoing scientific developments in the field of rAAVs. The meeting is timely, as CAT sees a lot of developments involving rAAVs.

Scientific considerations for rAAV-based therapy. Current status.

Review on AAV-host interactions and how to adapt vector tropism by capsid engineering (speaker: Hildegard Büning; Institute of Experimental Hematology, Laboratory for Infection Biology & Gene Transfer, Hannover Medical School, Germany)

AAV is an adeno-associated virus, member of parvovirus family. The outer part is a non-enveloped capsid. AAV as a virus has two life cycles, the lytic and the latent one, the former being the reproductive life cycle, which depends on coinfection with helper virus (e.g. adenovirus). In recombinant AAVs, all virus and capsid genes (responsible for replication and encapsulation) are removed from the virus.

Endocytosis is the main entry pathway for AAV followed by release from the endosomes, whereafter AAV enters the nucleus and the genome is transcribed. There could be secondary entry mechanisms depending on the serotype and cell type. None of the different serotypes is specific for one single tissue. Mutations in the capsid can modify the entry or degradation of the capsids. In addition, capsids can be engineered for example by inclusion of specific ligands (e.g. binding to tumour receptors) resulting in a different tropism of the rAAV. A lot of research is ongoing to engineer the capsid and new, customised rAAVs will enter in clinical research in the near future.

Persistence of the AAV (as episome) depends on the cell turnover in the target tissue and the number of AAV genomes in the cell. Long persistence (over 1.5 years) of AAV episomes have been reported. For the chromosomal integration process of the wildtype virus, an integrase (AAV-Rep) is needed, which is no longer present in the rAAV vectors. There are currently insufficient data on the chromosomal integration for the establishment of clinical relevance.

Review of immunology in AAV-gene therapy (speaker: Anne Galy; Genethon, France)

AAV induces innate, humoral and cell-mediated immune responses. The most measurable aspect is the adaptive immunity; many persons have pre-existing neutralizing antibodies (nAb) due to natural exposure (e.g. up to 70 % of the population has anti-AAV2 antibodies), making them non-eligible for treatments with AAV of a certain serotype. It is known that some routes of administration (e.g. in the eye or brain) are less susceptible to neutralizing antibodies.

Seronegative patients can develop anti-capsid antibodies. Capsids are taken up by antigen presenting cells. The immune response is CD4 and B cell dependent.

Human immune response cannot be fully predicted from studies in mice.

Possible ways to overcome the problem with nAb include: development of synthetic AAV variants (modified capsids); use of capsid decoys; immunosuppression (unpublished results in mice show that

strong immunosuppression is not needed to block the development of antibodies against the capsid to enable redosing); removal of nAb via plasmapheresis (not really effective); change to the route of administration; and from a pharmaceutical perspective, avoiding the presence of aggregates.

Clinical experience with AAV-based gene therapies in Haemophilia – do animal models help? (speaker: Amit Nathwani; Katharine Dormandy Haemophilia Centre, Royal Free Hospital and UCL, United Kingdom)

A Nathwani presented his experience with AAV 8 in haemophilia B and reviewed the data from preceding trials that used AAV2.

There are several animal models of haemophilia. AAV vectors have been shown to mediate stable expression of factor IX in mice, dogs and non-human primates. However, expression of transgene/pharmacodynamic effects in animal models is not always predictive of outcomes in humans. Similarly the transaminitis observed after liver directed or systemic delivery of AAV was also not seen in animals despite administration of significantly higher doses. Additionally dose extrapolation from non-human primates was not truly predictive of efficacy in humans.

Studies in mice and dogs indicated that AAV serotype could influence efficiency of gene transfer. Based on the data emerging from more recent studies such serotype differences have not been observed in humans. Additionally human studies do not show a great advantage of the use of self complementary vectors as steady state levels appear to be similar though there are differences in kinetics of transgene expression.

The pipeline for AAV-gene therapy: from academic proof of concept to commercial development (speaker: Michael Linden, independent expert, United Kingdom)

M Linden addressed the issue from the side of the drug developer, highlighting the complexities and challenges to bring AAV products to an authorised product. He argued that there is a need for scientific, regulatory and funding flexibility. Following issues were highlighted:

- Dose finding studies are challenging.
- How to regulate minute changes to the vector when going from academic development to clinical trials/Marketing Authorisation Applications? It has been reported that a small change in vector design can have a big impact on tissue tropism, retrograde transport and other therapeutically relevant characteristics.
- GMP production facilities are not (widely) available for academic developers.
- The Intellectual Property (IP) landscape is complicated.
- Need for standardisation, for example of the neutralization assay (used in the screening of patients for clinical trials).

rAAV in neurologic and metabolic diseases

Further to the presentation of the case study, the discussion with the experts focused on the use and usefulness of animal models when targeting the central nervous system.

Experts indicated that clear differences in species barrier have been reported when rAAVs are administered systemically: some rAAV will be able to cross the blood-brain barrier in animals, but not

in humans. Anatomical differences in animals might impact also the in vivo transduction. Also the type of disease will play a role.

For direct/local injection into the brain or the eye, it might be easier to extrapolate from animal models, but factors such as the volume that can be administered needs to be taken into account.

On the question, if animal studies need to be repeated for each change of the vector, the experts indicated that each serotype will show its own biodistribution and tropism (so extrapolation between serotypes may not be accurate). Extrapolation might be possible for variants of the same serotype, but on the other hand, as mentioned in the presentation of Dr Linden, a small change in vector design can have a big impact on tissue tropism and retrograde tropism.

rAAV mediated gene therapies in haemophilia

The discussions with the experts focused on the duration and height of expression, the impact and assessment of AAV immunity and the development of inhibitors.

Regarding pre-existing immunity, experts commented that young babies might have maternal antibodies (Ab), which decline over couple of months. They will develop their own AAV-antibodies later on (on contact with the wild type virus), leaving a window of opportunity for paediatric treatment with AAV based vectors. So far, in the haemophilia trials, neutralising Ab where detected against the capsid, but not against the transgene product (factor VIII or factor IX). It was noted that the patient treated in the trials are not naïve (have been treated with plasma derived or recombinant factors). There are non-clinical studies that show that (liver directed) gene therapy might induce immune tolerance, but this needs to be confirmed in the clinical setting.

Clinical experience so far shows that 6 weeks immunosuppression (60 mg corticosteroids, tapered off over a period of 6 weeks) is sufficient to control transaminitis (increase in liver enzymes potentially triggered by a cell-mediated immune reaction). A difference in immune response has been observed between patients with haemophilia A and B, in that for example transaminitis was linked to loss of expression in haemophilia B but not in haemophilia A. The underlying mechanism still needs to be elucidated. The transaminitis observed in rAAV-treated haemophilia A patients may not be related to an anti-capsid response, but could be an issue of cellular toxicity by the transgene.

Regarding the duration of expression, the experts noted that animal and clinical data showed a sustained expression over years. There have been reports on AAV integration in the host chromosome, but in the experts' view, this will be extremely limited and most AAV will remain episomal. Integration is not linked to or necessary for the sustained expression of the transgene.

On the question on the best time for treatment of children (in this setting of haemophilia), the expert indicated that treatment before any organ damage (joint damage) is preferred. Experts pointed out that treatment at age 3 (i.e. after the first rapid growth phase of the liver) could be beneficial. This would also allow offering therapy to a wider range of patients since pre-immunity would be less pronounced. However, as the liver is growing, transgene expression is expected to diminish over time, so a second gene therapy treatment might be needed in adolescence. Treatment with a different rAAV serotype could be a possibility, if one could avoid cross reaction between immune reactions towards different rAAV serotypes.

rAAV in ophthalmology

Following topics were discussed with the experts:

- Clinical efficacy: possibility of retreatment, timepoint for treatment, control group, dose, duration of expression, endpoints
- Clinical safety: risk of subretinal administration, immune reactions, distribution
- Non-clinical issues: dose selection on basis of animal studies, extrapolation of proof of concept (in animals, healthy eyes are treated: what will be the impact of overexpression of the transgene in healthy tissue?), difference in immunity between animals and humans.

With regard to the clinical efficacy questions, experts indicated that it is very difficult to generalise: each indication has its specific features, requiring different outcome measures. Differences between juvenile onset (severe disease) and adult onset (milder disease) and interindividual differences have to be considered. Not so much could be said about the dose: there is a lesser risk for immunogenicity in the eye, but prophylactic immune suppression is sometimes given (the eye is not an immune sanctuary – subretinal injection of rAAV can induce an immune reaction / inflammation). Single intraretinal administration of rAAV in early clinical trial phases very often excludes the affected eye from retreatment. This can be avoided by intraretinal application of rAAV into different areas of retina.

Regarding the safety of the subretinal administration, it was noted that the risk of macula detachment is disease specific, and that, as the administration is a surgical intervention, the adverse event profile is linked to the experience of the surgeons.

On the non-clinical questions, a single animal model will likely not give all the answers: one will have to take all non-clinical data into consideration, but also needs to apply a cautious interpretation (the animal model might not be a good representation of the human disease).

rAAV in muscle disorders /skeletal disorders

The discussion with the experts started on what rAAV serotype would be most appropriate for muscle targeted therapies. Experts noted that these are complex diseases where several tissues can be affected therefore different serotypes might be appropriate. However, there are possibilities to improve/change tropism (i.e. targeting capsids). Therefore, this should not represent a problem. Biodistribution is of importance.

With regard to the necessity to reach each target cell, there was an opinion that it will not be possible to reach the entire musculature/each myocyte. However, myocyte fusion plays a role, and targeting 20-40% of muscle cells seems to be sufficient to improve the quality of life of the patients. In addition, muscle fibres benefit may impact also on other cells.

In order to reach as many muscle cells as possible, very high doses are given, which creates problems from a technical / manufacturing (GMP) perspective. This dose is at least 2 logs higher than what is given for the treatment of haemophilia, so this could also increase the risk of side effects (immune response, liver toxicity, off target toxicity). Development of novel capsids / vectors / promoters is ongoing with the aim to reduce the dose.

Clinical grade manufacturing of rAAV

Following issues were discussed with the experts:

- What are the main product characteristics determining infectivity / potency of an rAAV product?
- How will different manufacturing processes (esp. upscaling) influence these attributes?
- What are the most reliable potency assays?

The experts indicated that there are no easy answers to any of the questions. There is no perfect potency assay, so multiple assays should be used, including an *in vivo* assay (specifically for characterisation / comparability purposes). The infectivity results in cell lines do not predict the *in vivo* activity.

The experts indicated that the manufacturing process directly impacts the biological characteristics and impurities of the drug product (such as the ratio between full and empty particles, the ratios of VP1/2/3). Variable levels of these impurities are present and the impact of these impurities is still unknown. There is a need to further discuss and agree, which impurities should be tested. The ratio between full and empty particle should be determined, as well as aggregation (known to impact infectivity). On a question on testing for host cell protein and host cell DNA, experts indicated that rAAV production is not yet as standardised as for biotech products.

Each manufacturing process will pose its own challenges: some processes are more difficult to upscale than others. For example, manufacturing using HEK-293 cells will result in a higher infectivity, but is more difficult to upscale. Some rAAV types are more difficult to produce in a baculovirus system, as this system is serotype specific. Self complementing rAAVs are more difficult to upscale and generally result in less homogeneous drug product.

Concluding remark

The CAT chair concluded the meeting, during which many of the aspects related to rAAV-based gene therapies that are relevant for regulators were discussed: quality/manufacturing issues, non-clinical testing and clinical investigations with rAAV-based products.

The CAT chair thanked all experts, moderators and case study presenters for their valuable input in the discussions and CAT members for their active participation.