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In vivo gene therapy:

CMC Development and Regulatory Challenges

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Cell and Gene Therapy From Concept to Clinical Use: Drug Design and Development“

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Presentation Outline

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Introduction to Freeline

What is an ‘in vivo’ gene therapy

- AAV Gene Therapy & clinical experience

Challenges in CMC Development

- CQAs, Characterisation and QC
- Formulation Development
- Drug Substance & Product Manufacture
- Administration

Regulatory Challenges

- Opportunities for Advice
- Current ‘hot topics’

Clinical-stage, fully integrated, next generation, systemic AAV gene therapy company dedicated to transforming the lives of patients suffering from systemic debilitating diseases

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1. **Proprietary capsid with significantly higher transduction efficiency in the liver**
2. **High protein levels at low doses allows us to target diseases perceived as beyond the reach of AAV GT**
3. **Potential for a functional activity for Haem B* with FIX expression levels in the normal range**
4. **Fabry clinical program providing initial evidence of sustained αGLA activity levels**
5. **Proprietary analytics and CMC capabilities that can deliver high quality at commercial scale**
6. **Leadership Team with deep CMC, development & commercial expertise in GT and rare diseases**

FIX

αGLA

* Certain adult Haem B patients

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What is an ‘in vivo’ Gene Therapy?

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Where the DNA to be delivered is administered directly to the patient

- Typically using a viral ‘carrier’
- Gene of interest and DNA sequences enabling transcription *in vivo* are packaged into a viral capsid
- DNA resides in the cell nucleus in the form episomal concatemers enabling long term expression of the protein coded by the GOI
- The Freeline proprietary AAV capsid is designed to be liver directed and is administered intravenously

AAV Gene Therapy

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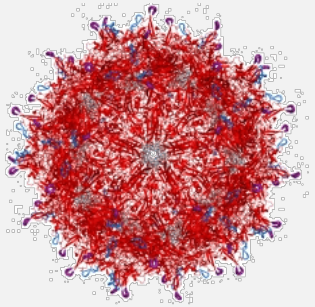
What is AAV (adeno associated virus)

- Single stranded DNA virus, ~5kb;
- Protein capsid; very small – ca 22nm and non-enveloped
- High proportion of adults show immune response to one or more AAV capsids, but infection not associated with any disease or symptoms
- Requires a helper virus (eg adenovirus) to provide machinery for replication

Recombinant AAV Vectors

- Engineered not to contain genes for chromosomal integration or replication
- Capsid confers specificity over which cells are transduced (ie targeting) e.g.
  - AAV5 -retina,
  - AAV8 - liver
- Capsid also determines immune response – previous infection with same capsid will reduce efficacy
- Promotor can also be engineered to be tissue specific
- Resides predominantly episomally
- Payload capacity of ca 4.5kb



Clinical Experience with AAV Gene Therapy

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AAV Vectors have been used in > 140 clinical trials with some patients followed for over 10 years

AAV Vectors generally considered to be extremely safe and well tolerated

- Three recent deaths in a gene therapy study for X-linked myotubular myopathy using an AAV8 vector by Audentes Therapeutics linked to very high dose and additional patient risk factors

Two licensed Products:

- Zolgensma®; approved in multiple territories including EU, US, Japan for SMA (spinal muscular atrophy)
- Luxturna®; approved in multiple territories including EU, US for inherited retinal dystrophy
- Glybera approved in 2012 for familial lipoprotein lipase deficiency but withdrawn in 2017 due to lack of demand

Zolgensma® (Onasemnogene abeparvovec)

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‘world’s most expensive drug’

Pharmaceutical Development & Administration Aspects

- Presented as Solution for Infusion (5.5 or 8.3 mL EV) at a concentration of 2 x 10^{13} vector genomes/mL
  - Primary pack – CZ polymer vials
  - Storage and shipment at ≤-60˚C
  - Maybe be stored at 2-8˚C for up to 14 days on receipt at dosing site
  - Excipients: tris, magnesium chloride, sodium chloride, poloxamer 188
- Dosed on a per kg basis
  - Customised kits based on patient weight
  - Table of body weight versus dosing volume provided
  - Administered via syringe pump over approx. 60 minutes
  - Infusion within 8 hours of syringe preparation

Challenges in CMC Development - Quantitation

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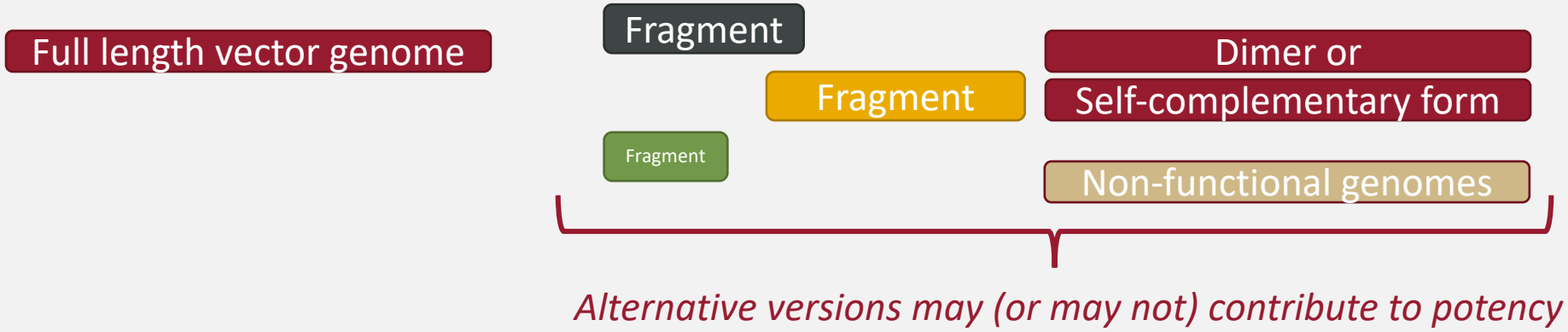
Even the dose is hard to define and specifically measure!

- Packaged DNA is highly heterogeneous:



Alternative versions may (or may not) contribute to potency

- Typical dosing qPCR or ddPCR assays monitor ‘vg/mL’ of a small portion of specific sequence (70-120 bp)
- Measured dose is highly dependent on choice of primer

➢ Orthogonal characterisation methods, e.g. DNA sizing assays and NGS are critical for supporting product understanding and development of an appropriate dosing assay

qPCR: quantitative polymerase chain reaction

ddPCR: droplet digital polymerase chain reaction

NGS: next generation sequencing

Challenges in CMC Development – Product Characterisation

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Viral vector gene therapies are complex biologics

DNA level

- Fragments
- Dimer/self-complementary
- Sequence integrity
- Unwanted plasmid or host cell sequences
- Methylation status

Protein Level

- Primary sequence + post-translational modifications
- Secondary & tertiary structure
- Ratio of VP1, VP2, VP3
- Fragments or truncations

Combined level

- “Full-to-empty” ratio
- Aggregation

Challenges in CMC Development – Product Characterisation

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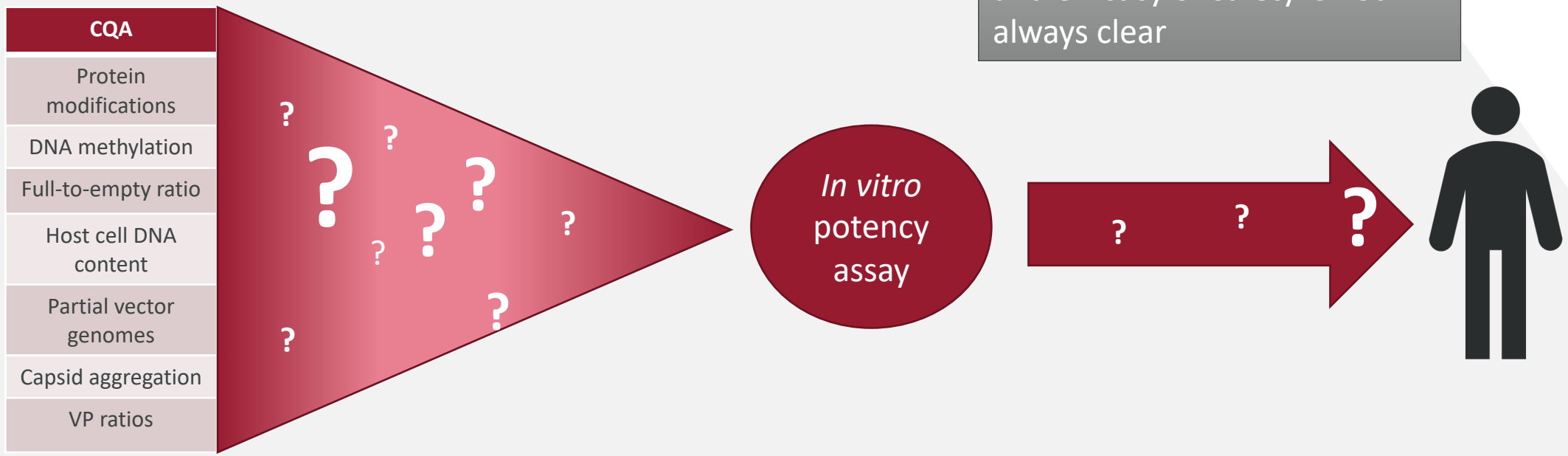
The relationship between CQAs and efficacy or safety is not always clear



We can develop a method to quantitate CQAs of interest

... But we can't always say what impact that CQA has on patients

As development of AAV gene therapies progresses, knowledge regarding the biological impact of CQAs grows as well

CQA = critical quality attribute

Challenges in CMC Development – Product Characterisation

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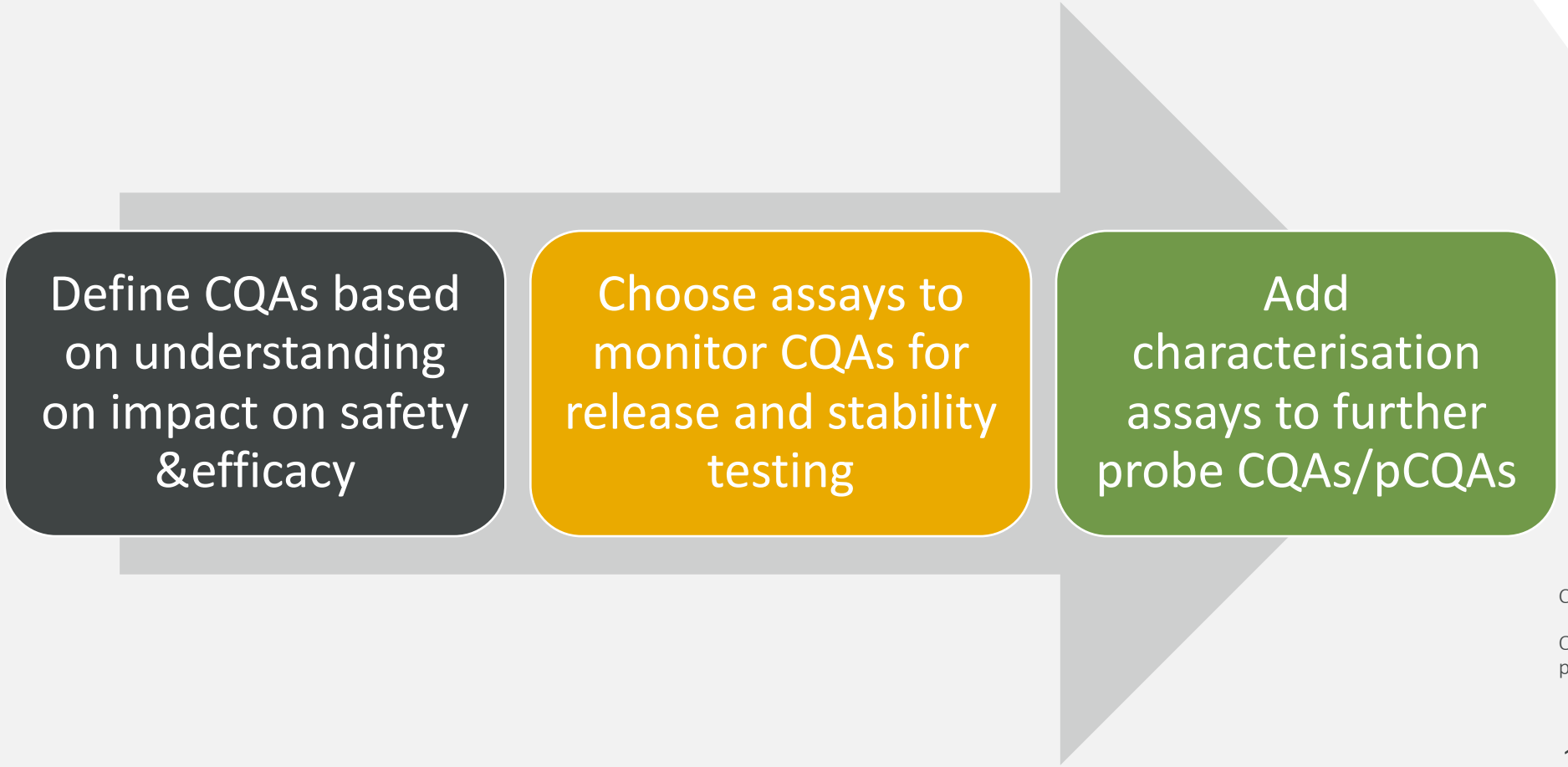

CQA: critical quality attribute

QTPP: quality target product profile

Challenges in CMC Development – Assay Development

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Release Assays

- QC assays that ensure CQAs are within appropriate ranges
- Can be routinely run in QC environment
- Can be validated
- Specifications driven by process capability and understanding of impact on safety & efficacy

Characterisation Assays

- Provide additional information to increase product knowledge and understanding
- Opportunity to use novel or sophisticated methods not amendable to QC environment

Emphasis on analytical characterisation from early development

Challenges in CMC Development – Assay Development

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Safety	Basic Pharmacopoeial	Impurities	Product Specific
• Adevntitious agents • Endotoxin • Sterility • rcAAV	• Appearance • Subvisible particles • Osomlality • pH • Extractable volume	• Host cell DNA • Host cell protein • Other process impurities e,g, BSA, column leachables • Aggregates	• Dose (vg titre) • Capsid titre • Potency • Purity • Identity

Challenges in CMC Development - Formulation

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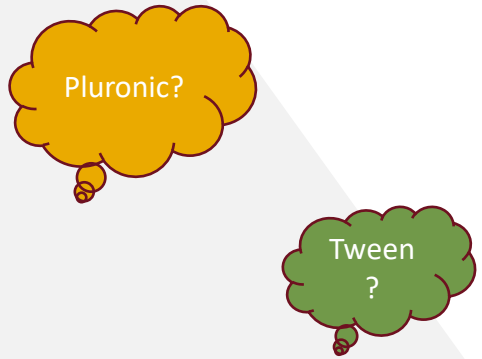
Historically viral vectors have been stored at low/ultra low temperatures

- Standard approaches for biologics e.g. surfactants to minimize adsorption
- Formulations to date relatively unsophisticated with reliance on low temperature storage



Challenged by lack of material/low batch sizes/cost

- Little material available for expansive formulation development studies

Cost drives conservative approaches and avoidance of processes which may increase risk e.g. lyophilisation

Challenges in CMC Development – DS Manufacturing

Highly complex processes for drug substance manufacture

- Ca. 5-6 week manufacturing time
- Multiple critical materials
- Multiple processing steps and process parameters; process characterisation critical
- Factors impacting DNA packaging may be difficult to understand/control

Regulatory expectations still evolving

- E.g. expectations wrt viral clearance

Relatively small number of CMOs with Viral Vector Manufacturing Capability

- Limited availability of slots/experienced staff

Relatively low yields

- Challenges with sampling etc

Very high cost

- > 1 million dollars per batch
- Depending on dose, this may result in single figure patient doses

Challenges in CMC Development – DP Manufacturing

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DP manufacture typically a standard biologics ‘fill/finish’ process

- Filtration through 0.2µm filters prior to aseptic filling

Labels applied either prior to freezing or using a dry ice process on frozen vials

- Label adhesion to frozen vials must be confirmed
- FDA require identity test on fully (clinically) labelled vials

Small batch sizes

- Design process to minimize losses e.g. in-line weight checks, low hold up volumes etc

Challenges in CMC Development – Sampling

Our sample is precious
Large amounts of a batch can be required for release and stability

Method selection

- Low volume methods can be selected for release and stability
  - SEC (µL) vs. AUC (mL)
  - BMI (µL) vs. light obscuration (mL)

Adapt stability strategy

- Number of batches
- Conditions tested
- Length of study

Re-use samples

- Sample from non-destructive methods such as visual inspection can be aliquoted following testing to be used in other methods

SEC: size exclusion chromatography

AUC: analytical ultracentrifugation

BMI: backgrounded membrane imaging

Challenges in CMC Development – Sampling

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EU GMP part IV allows some flexibility for Reference samples

12.18. As a general principle, a reference sample should be of sufficient size to permit the carrying out on at least two occasions of the full analytical controls on the batch foreseen in the marketing authorisation/clinical trial authorisation. However, it is acknowledged that this may not always be feasible due to scarcity of the materials or limited size of the batches (*e.g.* autologous products, allogeneic products in a matched donor scenario, products for ultra-rare diseases, products for use in first-in-man clinical trial with a very small scale production).

- CCI testing 'in lieu of sterility on stability'
  - Testing of buffer vials has been accepted for clinical trials material
  - Must be representative

Challenges in CMC Development – Sampling

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Advanced Therapies currently excluded from MRAs

Flexibility for exemptions

- Short shelf life
- Limited material
- Testing conducted to GMP

Exemption primarily foreseen for patient specific ATMPs

- But has been granted for Zolgensma

The Applicant has provided a justification, in accordance with paragraph 11.17 of Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products (Eudralex The Rules Governing Medicinal Products in the European Union, Volume 4, Good Manufacturing Practice), to rely on the finished product release testing performed in the US, claiming a limited amount of material available. The CAT agreed to the justification given by the applicant that in view of the current small batch size which is limited due to the batch allocation strategy, re-testing in the EU would consume a disproportionate amount of this batch size. Therefore, the current testing plan has been accepted for this specific small batch size. Should the applicant consider a batch upscaling or registration of new sites in the future, omission of the batch release re-testing in the EU will need to be re-considered.

Challenges in CMC Development - Delivery

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Emphasis on control of cold chain

- Reputable couriers
- Validated storage and transport conditions

Control of pharmacy procedures at site

- Defined thawing & preparation times
- ‘In use’ data supports accuracy of dosing and stability (inc potency)

Compatibility with Administration equipment

- Infusion sets, filters etc

Regulatory Challenges/Opportunities

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Regulatory standards not clearly defined and are constantly evolving

- Regulatory stance can change rapidly driven by events in the field
- Opportunity to influence direction by contributing comments on draft guidances

Opportunity (in theory) to benefit from enhanced opportunities for scientific advice through accelerated development schemes

- PRIME (EU)
- RMAT (US)

Other FDA advice schemes

- INTERACT – advice at pre-IND stage for innovative products
- CATT – opportunity to discuss new technologies

Opportunity to leverage platform approaches

Rapidly evolving field

Landscape constantly changing and bar does not stay still!

Sources of Regulatory Information

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FDA U.S. FOOD & DRUG ADMINISTRATION

Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)

Human Gene Therapy for Rare Diseases

And other disease specific guidance

Summary Basis for Regulatory Action

Date: May 24, 2019

From: Andrew Byrnes, PhD

BLA STN#: 125694/0

Applicant Name: AveXis, Inc

Date of Submission: October 1, 2018

Goal Date: May 31, 2019

Proper Name: onasemnogene abeparvovec-xioi

Proprietary Name: ZOLGENSMA

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products

31 January 2019
EMA/CAT/852602/2018
Committee for Advanced Therapies (CAT)

Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials
Draft

Questions and answers
Comparability considerations for Advanced Therapy Medicinal Products (ATMP)

Assessment report

Zolgensma

International non-proprietary name: onasemnogene abeparvovec

Regulatory ‘Hot Topics’

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‘Empty/full’ ratio – concern re potential for non-genome containing capsids to cause adverse effects such as reduced potency, immunogenicity

- Opportunities to remove ‘empty’ capsids during downstream processing
- Use of orthogonal methods to better characterise profile of empties/fully/partially full e.g. genome/capsid titre ration, AUC, cryo EM

DNA impurities - theoretical concern around delivery of oncogenes from ‘host cells’ in which viral vectors are produced

- Becoming increasingly important to characterise size distribution of packaged HCD; as a measure of potential functionality

‘Dosing’ assays – providing assurance that dosing is accurate and reproducible

- qPCR traditional method; field now moving towards ddPCR which is not dependant on a standard
- Expectation of validated assay even in early clinical development

Product potency – providing an *in vitro* measurement relevant to *in vivo* efficacy

- Validated potency assay essential in late development

Summary

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Product Characterisation

- Key to invest in analytical method development to develop product understanding and enable robust specifications

Manufacturing

- High cost/low yield
- Process knowledge and understanding critical to mitigate risk and ensure consistent product

Formulation and Delivery

- Critical to ensure patient receives correct dose & quality
- Opportunities to advance formulation strategies and move away from ultra cold storage

Regulatory

- Bar is rising
- Important to take advantage of opportunities for regulatory advice to maintain awareness of ‘hot topics