Recommendations for risk assessment requirements (safety and toxicology) for animal models and nonclinical data

ASGCT Liaison Meeting Working Group

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Acknowledging the Challenge

Ever changing, ever advancing fields - with new advances in science and technological developments, growing breadth of disease states being addressed, and growing data base of outcome experiences as preclinical and clinical studies are conducted.

Acknowledge the tremendous effort that has been undertaken by FDA to provide the currently available guidance documents

Our group has brought our collective experience to the table and agreed on several areas where we feel current FDA guidance might be clarified or expanded.

Our thoughts on these topics are provided for consideration.

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Agreement among Working Group

- Consistency in FDA guidance is needed
- FDA might use existing pre-IND and clinical data to inform future guidance

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Outline of General Topics

- Specific areas where group has suggestions for modifications of existing guidance to provide consistency in recommendations across applications
- Requests for new guidance (such as for gene editing platforms, separate guidance document for cell therapies) or consolidation of existing guidance

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Specific Areas

- Gene or cell therapy characterization from research stage to clinical trials
- Bridging studies
- Dose selection for preclinical pivotal toxicology studies – need to identify minimum effective dose?
- Animal model selection – knockout rodents/large animal models
- Euthanasia time points (how many and longest required)
- Safety endpoints – inform from existing FDA safety/biodistribution/clinical trials data base
- Immunological endpoints – which, when assessed in development, and relevance to clinical trials
- Biodistribution/gene expression tissue collection and analysis

Vector Characterization

- Titer: (physical and infectious) to ensure consistency of dose throughout development process from proof-of-concept studies to the clinic. Research versus qualified assays and linking info to ensure consistency of dose
- Identity – capsid protein verification and vector genome sequence
- Purity: appearance, host cell impurities and presence of residual chemicals from purification process. Empty/full capsid characterization as this may affect potency of product although particle titer may remain constant
- Sterility and safety (endotoxin, mycoplasma, pH, osmolarity)
- Documentation bridging vector characterization when test article in preclinical studies expresses species-specific transgene, not human transgene.

Vector Titer Guidance – Research Vs. Qualified Assays

- Guidance
  - Research assay (i.e., PCR, UV/visible absorbance, dot blot or Southern blot) should accurately cover concentration range of vector formulation overdose range to be administered to research (proof-of-concept) animals. (Results not solid if dose not known)
  - **Suggest** qualified assay for product release should include PCR or ddPCR quantitation of vector, as well as microscopic examination of vector to assess integrity[1]
  - Guidance requested on translating from the initial developmental study research vector concentrations to the process comparable or clinical grade product, which depends on the original research vector and assays used to establish concentration/dose. May be difficult based on initial assays used on research product. **Suggest** ddPCR may help, as less sensitive to matrix effects/impurities.

[1] Accurate Quantification and Characterization of Adeno-Associated Viral Vectors
https://www.frontiersin.org/articles/10.3389/fmicb.2019.01570/full

Bridging Studies – *In Vitro*

- *In vitro* studies comparing original and new formulation of product where updates may include:
  - Changes in manufacturing process such as production cell line or scale-up (stacks to bioreactor?)
  - Change in purification process
  - Change in manufacturing materials
  - Change in vector formulation

Suggest needed bridging data: titer, purity, and potency (enhanced vector uptake by cells, enhanced gene expression, or improved full/empty capsid ratio)

Bridging studies using methodology used to develop original product can be effective whether or not mechanism of product action is known.

Bridging Studies – *In Vivo* Animal Models

- *In vivo* studies (efficacy/safety) in animal model of disease and/or normal animal model
  - Change in vector type (AD to AAV) or AAV serotype (at any stage of development)
  - Change in promoter or enhancer elements
  - Change in process enhancing relative numbers of full versus empty capsids that may impact needed dose for efficacy or lead to toxicity (transgene overexpression) – first identified from results of *in vitro* potency assays?
- If *in vivo* studies needed, how long duration to prove safety and what endpoints?
  - Based on previous animal study designs

Clinical Bridging Studies

- When?
  - Change in vector serotype, promoter, or route of delivery to enhance efficacy or improve safety
- How?
  - Demonstrate improved efficacy and safety in animal model(s) used to support original clinical trial before moving to clinic.
- Base the clinical dose escalation and endpoints on findings from initial clinical trial

Need for Identification of Minimum Effective Dose in Animals Models

- In some instances, FDA feedback to investigators in INTERACT or pre-IND meetings required the investigator to identify a minimum effective dose (MED) in proof-of-concept studies. However is this dose really needed to translate to the clinic?
- **Suggest** an identified MED is not essential to conduct preclinical pivotal toxicology and biodistribution studies, but at least two doses should be evaluated in early efficacy studies to help assess dose response for efficacy and potentially safety. These could form a basis for choosing clinical doses and doses for pivotal preclinical tox studies.

Animal Model Selection

- Model choice based on:
  - Comparable uptake and transduction of therapy in animal model and/or human cells or a qualified/validated potency assay
  - Desire or advantage for having comparable anatomy (i.e., pig or sheep for heart)
  - Availability and feasibility of using an animal model of disease
- When a rodent is the animal model of disease and initial research studies (proof-of-concept studies) show efficacy in the model, can pivotal preclinical toxicology studies be conducted in this rodent disease model, or the corresponding wild-type strain?
- When is use of a non-human primate or other large animal model necessary in pivotal studies?
- **Suggest** safety studies inclusive of biodistribution and gene expression in small animal model, with additional biodistribution and/or gene expression assessed in large animal model when the delivery method intended for humans cannot be sufficiently replicated in a small animal model due to anatomical feasibility.

Euthanasia Time Points

- There is a perceived trend in FDA expectations, relayed in pre-IND comments, toward increasing the number of euthanasia time points to three, generally extending the observation period to at least six months.
- Have longer observation time points identified potential toxicities of products that were not noted at earlier times – especially with AAV- based therapies?
- **Suggest** an acceptable minimum of two time points, with duration dependent on the specific therapy, disease indication, and potential for long-term effects.
  - Differentiate between gene and cell therapies?
  - Genome editing therapies vs. those with more established safety profile?

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Safety Endpoints

- Safety endpoints (noted in the guidance under “secondary considerations”) include hematology, serum chemistry, coagulation parameters and urinalysis.
- Included to assess health of animals and potential toxicities and comprehensive panels have been included in many pivotal study protocols.
- Need for these impacts number of small animals on study due to limited sample volumes.

Safety Endpoint Proposed Modifications

- **Suggest** FDA review the IND pivotal toxicology study database to evaluate whether clinical pathology data inform adverse outcomes in clinical trials. Extensive clinical pathology assessments in rodent models may at least double the number of animals needed and may not inform safety in humans.
- **Suggested** change in guidance language:
  - Limit clinical pathology endpoints to parameters relevant to the particular disease and therapy (i.e., coagulation parameters and CBC for hemophilia, CK when ROA is intramuscular, etc.), and basic liver and kidney endpoints in small animal models.

If limiting clinical pathology endpoints has no adverse impact on assessing safety, the modification would address RRR animal use.

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Immunological Endpoints

Given the limitation of animal models for predicting immunogenicity in the clinic:

- Samples from preclinical studies should only be tested if needed to understand safety or lack of transgene expression (impacting efficacy).
- If preclinical testing is needed (i.e., due to a safety or efficacy issue), the testing should be fit-for-purpose to address the specific issue.
    - I.e., antibodies or ELISpot against transgene protein

Integration Studies Not Needed in All Cases

- Based on collective data, the frequency and level of AAV integration is very low and is considered non-integrating[1] so integration studies should not be required
- We recommend that requests for AAV integration studies provide rationale
- If deemed necessary, a stage-appropriate evaluation of integration leveraging approaches below should be sufficient
- Leverage labeling, risk management and LTFU to properly inform patients and prescribers

Non-clinical integration study at clinical dose levels using target tissue from suitable canine or NHP model	Report the top ten integration sites and determine any potential clonal bias
Integration methods for AAV (e.g., TES or LAM-PCR) need to be sufficiently sensitive to detect low-frequency events (e.g., in liver target tissue detect </= 500 integration sites/reaction)	Check all unique integration sites for genomic distribution and any chromosomal hotspots within and across animals
Duration should cover acute malignancy risk by assessing integrations at a minimum of one time point within 2-6 months after dosing	Determine integration frequencies as IS/cell and IS/vg. Typical AAV integration frequencies are E-03 to E-05/cell and E-04 to 10E-06/vg. If clonal bias, genomic hotspots, or a higher than usual integration frequency is observed, then follow up using longer-term studies (>/= 6 months) with multiple time points may be needed
Methods should be characterized for their potential to generate false-positive detection events	[1] Long Term Follow-up After Administration of Human Gene Therapy Products https://www.fda.gov/regulatory-information/search-fda-guidance-documents/long-term-follow-after-administration-human-gene-therapy-products

Requests for Additional Guidance

- Circumstances – products requiring evaluation of insertional mutagenesis (Inconsistencies in FDA guidance – sometimes requested for AAV based therapies). Provide decision tree?
- Guidance on providing data identifying off-target sites for gene editing products – (Place in new guidance document for gene editing products?)
- Need for long-term follow-up studies to assess potential carcinogenicity or reproductive effects (What patient population and what stage of product development?)
  - **Suggest** for assessing potential reproductive effects using guidance consistent with EMA Guideline on Non-Clinical Testing for Inadvertent Germline Transmission of Gene Transfer Vectors[1]
- Guidance on single point of submission of all pivotal toxicology study data in one GLP final report (compared to sponsor submission of study data to FDA without disclosure of results to GLP study director).
  - **Suggest** latter should not be allowed as contrary to intent of the GLP guidance on study director being single point of control.

[1]Non-clinical testing for inadvertent germline transmission of gene transfer vectors https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-non-clinical-testing-inadvertent-germline-transmission-gene-transfer-vectors_en.pdf

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Request for Consolidation and Proposed Change in Guidance - Biodistribution

- Currently the preclinical assessment guidance[1] refers the reader to the 2006 Clinical Trials Long Term Follow up Guidance for tissues to assay for biodistribution and potential vector persistence.
  - **Suggest** guidance material be included directly in any updated preclinical guidance document.
- Section IV.B.2.c ii in 2020 long term follow-up guidance document states: “samples to be run in triplicate, with one of the triplicates being spiked.”[2]
  - **Suggest** guidance require an assessment of matrix effects on the assay and leave approach to the sponsor/investigator.

[1]Preclinical Assessment of Investigational Cellular and Gene Therapy Products
https://www.fda.gov/media/87564/download
[2]Long Term Follow-Up After Administration of Human Gene Therapy Products
https://www.fda.gov/media/113768/download

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Request for New Guidance Documents

- Genome editing products
- Separate gene and cell therapy guidance into two documents and update.
  - **Suggest** preparing separate guidance documents based on:
    - New information and experience in developing cell therapies (i.e., CAR T)
    - Updating and consolidating guidance on gene therapies – also based on new information gained since 2013

Thank you