PRE-CLINICAL CHALLENGES AND TRANSLATIONAL SOLUTIONS

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C&GT Training Day, 16 September 2020

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PRECLINICAL OBJECTIVES FOR GENE AND CELL THERAPIES

One single objective!

Evaluate the risk - benefit ratio

Gene (protein) replacement

Gene Silencing

Genetically-modified cells

Gene Editing

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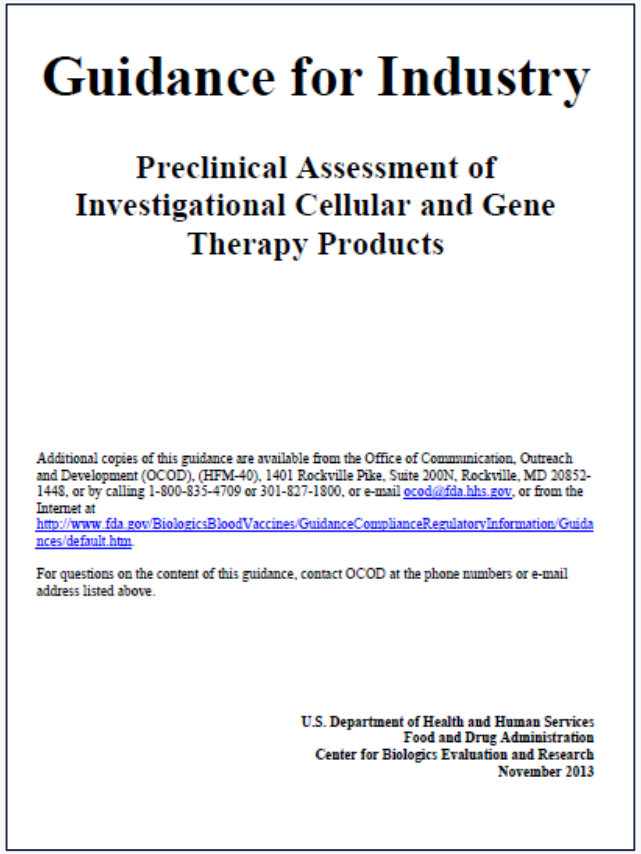
REGULATORY ENVIRONMENT

Current status

- **ICH guidelines**
  - S6 (R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals
  - New topics for ICH harmonization – announced June 2019
    - Q5A(R2) Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin Update (to include viral-like/vectored particles - detection and clearance)
    - S12 Non clinical biodistribution studies for Gene Therapy products
- **FDA: Center for Biologics Evaluation and Research (CBER)**
  - Office of Tissues and Advanced Therapies (OTAT)
  - 2013 - Preclinical Assessment of Investigational Cellular and Gene Therapy Products
    - 18 new Guidances in 4 years - 6 new Draft Guidance for GTs in July ‘18
  - 2020 - Long Term Follow-Up After Administration of Human Gene Therapy Products



Guidance for Industry

Preclinical Assessment of Investigational Cellular and Gene Therapy Products

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or e-mail ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

For questions on the content of this guidance, contact OCOD at the phone numbers or e-mail address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
November 2013

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EMA GUIDELINES – ATMP ACTION PLAN

2008 - Guideline on cell-based medicinal products

EMA ATMPs Guidances

- 2007 - Potency Testing of Cell-Based Immunotherapy MPs for Treatment of Cancer
- 2009 - Guideline on Xenogenic CBMPs
- 2010 - Reflection Paper on Stem-Cell Based MPs
- 2017 – Q&A on Requirements for Minimally Manipulated ATMPs
- 2018 - Guideline on Safety & Efficacy Follow-up – Risk Management of ATMPs
- In progress- Guideline on the Quality, Non-clinical and Clinical Requirements for Investigational ATMPs
- In progress - Guideline on MPs Containing Genetically Modified Cells
- Q4 2019 - Comparability for ATMPs

2018 – Quality, preclinical and clinical aspects of gene therapy medicinal products

EMA GTMP Guidances

- 2005 – Development and Manufacture of Lentiviral Vectors
- 2007 – Non-clinical Testing for Inadvertent Germline Transmission for Gene Transfer Vectors
- 2008 – Scientific Requirements for the ERA of GTMPs
- 2008 – Non-clinical Studies Required Before First Clinical Use of Gene Therapy Medicinal Products
- 2010 – Quality, Non-Clinical and Clinical Issues Relating Specifically to Recombinant Adeno-Associated Viral Vectors
- 2012 – Design Modifications of GTMPs During Development
- In progress- Scientific and Regulatory Considerations on Gene Editing Technologies (Committee for Advanced Therapies)

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KEY TAKEAWAYS FROM THE GUIDANCE DOCUMENTS

- Studies should be guided by traditional pharmacology/toxicology principles…BUT
- There is no standardized preclinical testing, each therapy is evaluated on specific product characteristics – which are unique
- Study objectives:
  - Consistent and well-characterized product is mandatory, when possible use product and administration device intended for patients
  - Use animal models of disease to provide insight into dose, activity and toxicity
  - Establish effective dose range, timing of product administration and dosing schedule
  - Characterize MOA and establish biomarkers relevant to pharmacology and toxicity
  - Identify toxicities that might arise in a clinical setting
  - Determine biodistribution and fate of product
  - Incorporate appropriate safety endpoints that capture full spectrum of acute and delayed-onset toxicities

CHALLENGES DURING PRECLINICAL STUDIES

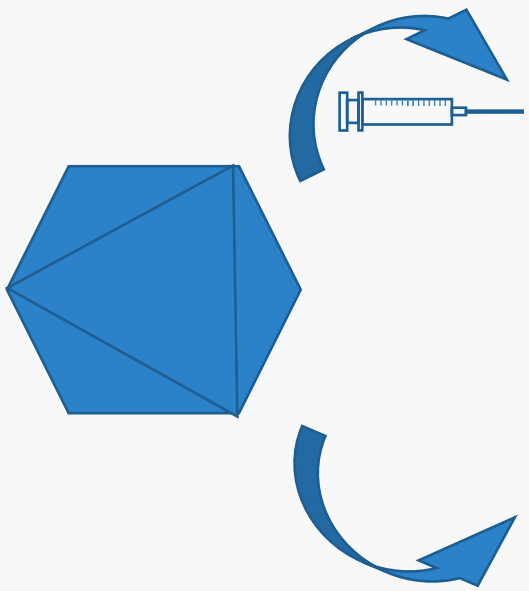
- Are preclinical models predictive of what will happen in humans ?
  - In terms of efficacy:
    - Pharmacology, transgene expression, MOA ?
    - Biodistribution: target organs and cells
  - In terms of safety:
    - Biodistribution (vector genetic material, transgene transcript)
    - Immune response against the vector and the transgene (innate and adaptive)
- Analytical challenges – linked to specific laboratory investigations for GCT

LABORATORY INVESTIGATIONS DURING PRECLINICAL STUDIES

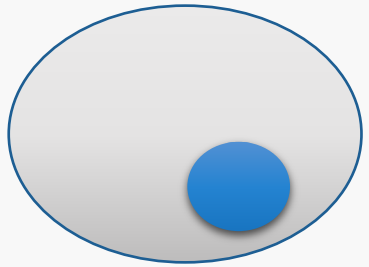
- Example for a Gene Therapy product
  - Product characterization
    - Vector concentration, full/empty capsid ratio, potency assay etc..
  - Pharmacology assessment: transgene expression in target and non target tissues
    - Vector biodistribution (qPCR)
    - Transgene expression (mRNA: RT-qPCR, Protein: ELISA, IHC…)
  - Immune response assessment
    - Humoral immune response:
      - Anti-vector antibodies (ELISA, ECL, cell-based assays - Nabs)
      - Anti-transgene antibodies
    - Cellular immune response: IFN-γ ELISPOT, ICS by flow cytometry
    - Inflammation: cytokines (multiplex)
  - Vector integration into host genome: LAM-PCR
  - Viral vector shedding: qPCR

TRANSDUCTION ASSAYS FOR A GENE THERAPY PRODUCT

- Principle



Animal species



Cell

Transgene

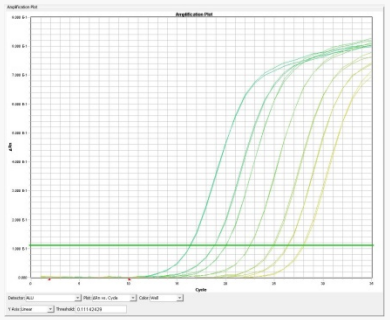
Reporter gene

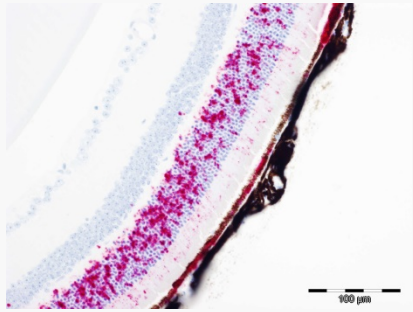
Transgene mRNA

RT-PCR, RT-qPCR
ISH

Protein

IHC
Flow cytometry
ELISA
Reporter gene readout (fluorescence, luminescence etc).





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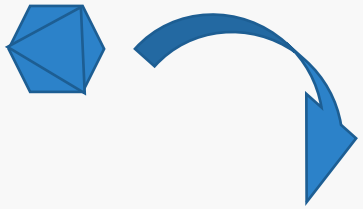
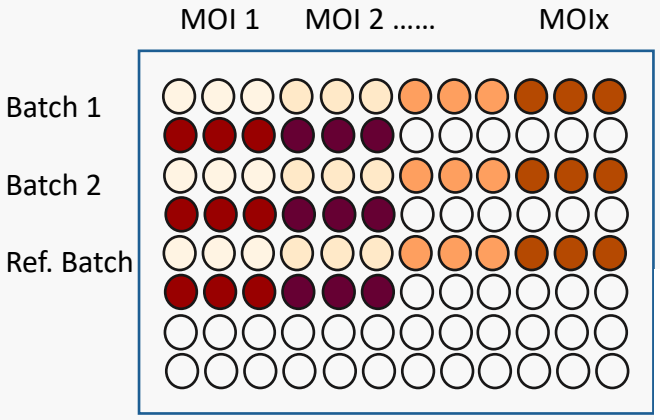
ANALYTICAL CHALLENGES FOR TRANSDUCTION-BASED ASSAYS

- Use of a reporter gene: no particular analytical challenge for the readout
  - Tissue or cell biodistribution: combinatorial AAV libraries screening, serotype selection
  - nAbs assays: animal screening for safety assessment, evaluation of the humoral immune response against the vector in preclinical setting
- Use of the final product candidate
  - Potency assay (Cell-based assay) development and validation
    - RT-qPCR assay validation prior to cell-based assay validation: no guideline, ie no consensus on practices: fit-for purpose validation
  - Pharmacology assessment
    - RT-qPCR assay validation

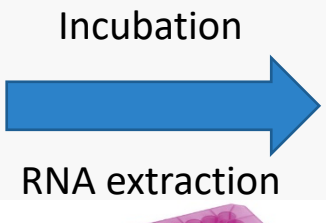
POTENCY ASSAY

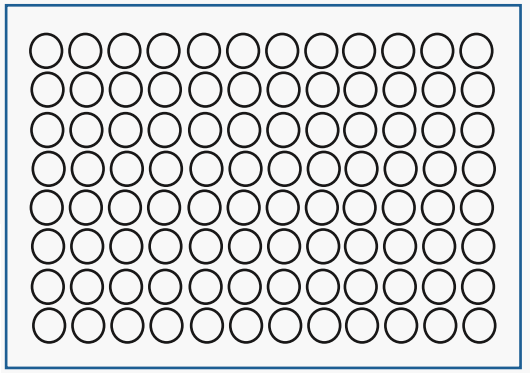
- Principle: measure transduction efficiency in cells
  - Example: AAV encoding for a soluble protein, downstream of a specific promoter for specific expression in target cells. Treatment of ocular disease, intravitreal administration



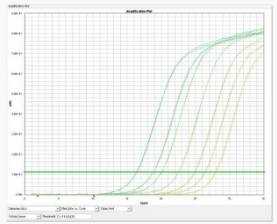


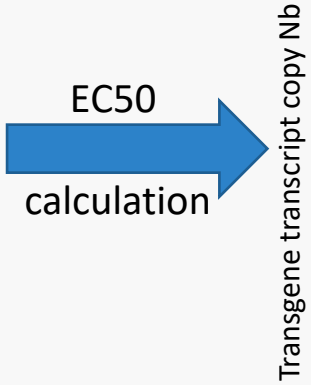
Cell line expressing adequate transcription factors
- Express the protein: ELISA not possible
- Have endogenous transcript levels

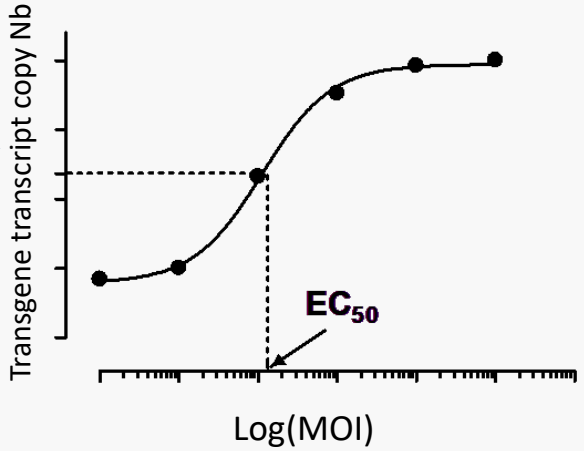




RT-qPCR for the transgene transcript copy number determination
- Codon optimized transgene
- Calibration curve and 3 QC levels







Curve fitting using a 4-PL regression model
Comparative EC50 (vs Ref. Batch)

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RT-QPCR ASSAY DEVELOPMENT AND VALIDATION

- No guideline for assay development and validation: fit-for purpose
  - Assay development – one-step RT-qPCR assay, absolute quantitation
    - Design and testing of 3 primer pairs and probe sets
    - Reference analyte: in vitro transcribed transgene RNA
    - Matrix: surrogate RNA
    - Calibration curve and 5 QC levels
    - Several annealing temperatures tested
    - RT-qPCR assay specificity in cell line RNA and NHP RNA from several tissues for transcript level determination in AAV DNA positive tissues during pivotal tox/biodistribution study

    → Selection of the best primer pairs and probe and optimal PCR conditions
  - Assay validation
    - Assessment of within- and between- run precision and accuracy (5 QC levels)
    - Determination of the dynamic range of the assay
    - RT-qPCR assay specificity and selectivity in cell line and NHP matrices
    - Recovery and stability in cell extracts and NHP tissue homogenates

POTENCY ASSAY DEVELOPMENT AND VALIDATION

- Guideline for Potency Assay design and validation: Potency Tests for Cellular and Gene Therapy Products, FDA Guidance for industry, 2011
  - Assay development: relative potency assay (comparison to a reference material)
    - Cell line to be used
    - Number of cells in wells
    - MOI range with a reference material
    - Incubation time

    → Selection of the optimal cell line and cell culture conditions
  - Assay validation
    - Assessment of within- and between- run precision with the reference batch (6 MOI levels)
    - Specificity
    - Assay Robustness (cell passages, cell culture medium batches, technician)
    - Determination of the acceptable range for reference material EC50 for sample testing

OTHER USES OF TRANSDUCTION ASSAYS

- Determination of the transcript levels in animal tissues:
  - Specific challenge: RNA content is different in every single tissue
    - Test of RT-qPCR assay specificity in all matrices
    - Evaluation of the matrix effect
    - Evaluation of the recovery and stability of reference analyte in tissue homogenates
  - Requires to have access to tox species tissues: ethical concern ?

- Neutralizing antibody assay using the specific product
  - Neutralizing antibodies assay results depend on full/empty capsid ratios
    - Using a vector encoding for a reporter gene with different ratio than the final product may not be fully adequate

TAKE-HOME MESSAGES

- No standardized preclinical testing, each therapy is evaluated on specific product characteristics and best science
- The selection of efficacy models and tox species is crucial
- Pharmacology and biodistribution assessments with adequate methods are mandatory
- Laboratory investigations require specific methods with no regulatory guidance: fit-for purpose method development and validation: requires anticipation !
- Seek expert advice from many disciplines and take preclinical plan to the regulatory agencies for scientific advice