Success Story: Luxturna Potency Assay Development to Validation

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Analytical and Quality Control
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LUXTURNA: First Gene Therapy for a Genetic Disease Approved in the U.S.

- LUXTURNA® (voretigene neapvovec-rzyl) for subretinal injection
- For the treatment of patients with biallelic RPE65 mutation-associated retinal dystrophy who have viable retinal cells
- Adeno-associated virus (AAV) serotype 2 vector carrying the RPE65 transgene
- Formulated to a concentration of $5 \times 10^{12}$ vg/mL

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Viral vector is generated
- LUXTURNA is a suspension of an adeno-associated virus vector-based gene therapy for subretinal injection
- LUXTURNA is a live, nonreplicating adeno-associated virus serotype 2 that has been genetically modified to express the human RPE65 gene
- LUXTURNA is derived from naturally occurring adeno-associated virus using recombinant DNA techniques

LUXTURNA for subretinal injection

Transduction of RPE cells
- Injection of LUXTURNA into the subretinal space results in transduction of some retinal pigment epithelial cells with a cDNA encoding normal human RPE65 protein, thus providing the potential to restore the visual cycle.
Biological Products Safe, Pure, and Potent

All biological products regulated under section 351 of the PHS Act must meet prescribed requirements of safety, purity and potency for BLA approval; Federal Food, Drug and Cosmetic Act, (FDC Act), (21 U.S.C. 321 et seq.); (21 CFR 601.2).

Potency: 21CFR600.3(s) “The word potency is interpreted to mean the specific ability or capacity of the product(...) to effect a given result.”

Bioassay:
• Evaluate potency/activity of a drug for release/stability purposes
• Assay should reflect/mimic product’s known/intended Mechanism of Action (MoA)
“for complex molecules, the physicochemical information may be extensive but unable to confirm the higher-order structure, which, however, can be inferred from the biological activity"
Gene Therapy Mechanism(s) of Action

Multi-Step Process Based on “Central Dogma of Molecular Biology”

1. Gene Delivery
2. Gene Transcription to mRNA
3. mRNA Translation to Protein
4. Functional Protein
Infectivity Assay
Infectivity Assay
Infectivity Assay
Infectivity Assay
Infectivity Assay
Infectivity Assay
Infectivity Assay
Infectivity Assay

qPCR using Gene Specific Primers
### Infectivity Assay

Cells transduced with Serial 10-dilutions in 7 replicates of Test Article and Ad5

#### Cells lysed and Transgene specific Primers used for qPCR

<table>
<thead>
<tr>
<th>Cut off = 64 Copies</th>
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</thead>
<tbody>
<tr>
<td>Cut number</td>
</tr>
<tr>
<td>Log(Concentration)</td>
</tr>
</tbody>
</table>

#### Viral Particles Dilutions

- **B**: Logarithm of the dilution factor
- **S**: Logarithm of the initial dilution plus the sum of ratios of infectious-positive wells per total wells at each subsequent dilution
- **V**: Volume of the diluted Test Article used for inoculation

#### Table

<table>
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</tbody>
</table>

#### Calculations

1. **Infectious Titer (IU/mL)**: \(10^{\left(\log(D) + 0.5\right)} / V \text{ [mL]}\)

   - **D**: Logarithm of the dilution factor
   - **S**: Logarithm of the initial dilution plus the sum of ratios of infectious-positive wells per total wells at each subsequent dilution
   - **V**: Volume of the diluted Test Article used for inoculation

2. **Particle-to-Infectious Particle Ratio**

### Hypothetical Data

**M-GENE-US-00335**

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**Bioassay: Analytical Sciences**

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**WE DON’T FOLLOW FOOTSTEPS. WE CREATE THE PATH.**

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**Spark Biosciences**
Activity and Gene Expression Assay

Cytoplasm

Nucleus

M-GENE-US-00335
Activity and Gene Expression Assay
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Activity and Gene Expression Assay

Enzyme Activity by Functional Assay

Protein Expression by ELISA
Method Development and Phase Appropriate Validation

**R&D Drug Discovery**

- **Analytical Method Development**
- **IND**
- **Pre-Clinical**
  - Phase 1
  - Phase 1/2 Validation
  - Phase 2
  - Phase 3 Validation
  - Commercial Validation

**Clinical Development**

- **Samples**
  - DS/DP samples from first manufacturing campaign
  - Representative DS/DP samples

**Reference Standard**

- Interim RS
  - Phase 1/2
    - Limited Quantitative information
    - Wider Acceptance Range
    - Assay Development/Qualification
  - Phase 3
    - Assay Validated
    - Narrower limits to ensure lot-to-lot consistency
    - Stability testing of validation lots to establish expiry dating prior to licensure

**GLP**

- cGMP

**Time (Years)**

- **Target Selection Optimization** 2-5
- **Toxicology Immunogenicity** 2
- **Safety** 2
- **Safety & Efficacy** 2-3
- **Bigger Cohort Safety & Efficacy** 2-5
- **FDA Filing/Approval & Launch Preparation**
  - Licensed Product

**Pharmacovigilance**

**Product Life-Cycle**
Luxturna Bioassay Timeline

Transfer of the technology from Children’s Hospital of Philadelphia (CHOP)

IND (2007)

Pivotal Phase 3 Clinical Trial Initiated (Nov. 2012)

In-vivo Potency Assay: Pupillary Light Response

Qualitative

Development

In-vitro Potency

Quantitative

Validation

In-vitro Potency

Phase Appropriate Method Validation Performed

Transition from Qualitative to Quantitative Potency Assay

**Phase I/II/III**

**Potency**
- In vivo Pupillary Light Response

**Commercialization**

**Potency**
- In vitro Potency Assay

- Animal-based
- Physiological Relevant
- Complex, Highly Variable
- Long Assay Time (4-6 Weeks)
- Qualitative

- Cell-based
- Less Variable Compared to animal-based assay
- Short Assay Time (1 Week)
- Quantitative
- Broad Dynamic Range
- Support Principles of 3Rs

### 4PL Fit

\[
y = \frac{(A - D)}{1 + \left(\frac{t}{C}\right)^B} + D
\]

- **A**: Upper Asymptote
- **B**: Slope
- **C**: ED50
- **D**: Lower Asymptote

**Potency**

\[
\text{Potency} = \frac{\text{Sample ED50}}{\text{Reference ED50}}
\]
The AAV2-hRPE65v2 Isomerohydrolase Activity Potency Assay


PLA-Analysis

LRAT: Lecithin Retinol AcylTransferase
CRBP: Cellular retinol binding protein
CRALBP: Cellular RetinALdehyde-Binding Protein
Development to Validation
Cell selection: Support MOA of the Drug

Development

- Cell number
- Dose-response
- Incubation time
- Critical reagents
- Readout
- Representative Sample/RS
- Identify variables

Validation

ICH Q2 (R1)

(1033) BIOLOGICAL ASSAY VALIDATION
(1034) ANALYSIS OF BIOLOGICAL ASSAYS

- Specificity
- Linearity
- Precision
- Accuracy
- Range
- Robustness


USP<1032> DESIGN AND DEVELOPMENT OF BIOLOGICAL ASSAYS

Cell Line Selection and Cell Bank Qualification

- Cell Line's History from Origin to Banking
- Sterile
- Mycoplasma Free
- Growth Characteristics
- Morphology

We don’t follow footsteps. We create the path.
Analytical method performance may drift over the time due to
- Critical Reagents Change
- Equipment Change
- Vendor Change
- Analysts

Proactive Assay Tracking/Trending/Monitoring is Important to Keep the Assay Performance in Controlled State
Summary

➢ Designed a statistically sound phase-appropriate validation
  ▪ Use historical data or development data for acceptance criteria
➢ Started early for development of qualitative potency assay
➢ Identified critical reagents and variables effecting the assay
➢ Established and characterized the cell bank
➢ Method life cycle management
  ▪ Methods evolve during product development and product life cycle
  ▪ Continuously monitor the assay performance, control chart
  ▪ Annual Method Review
Thank you!