Development and qualification of a potency assay to support gene therapy

Hannah Maheno, CASSS Bioassay 2020
Outline

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Sanofi Method vs. Industry Requirements

1) Demonstrates MoA
- Mirrors infectivity, gene expression, and generation of functional protein
- Preferably a single assay
- Reflects pre-clinical & clinical response

2) Relevant cell line
- Needs to be relevant to the biological activity of the product (or bridged)
- Permissive

3) Quantitative Read-out
- Simple
- Platformable (across multiple serotypes)
- Robust
- Automatable
- Sensitive and quantitative analysis

Industry Average: Complex, semi-quantitative assay lacking robustness & precision
1. Internalization of viral vector
2. Vector trafficking to golgi
3. Capsid processing before nuclear entry
4. Nuclear entry of vector
5. Genome release
6. Nuclear entry of vector
7. Transcription/translation
8. Production of functional protein

4/27/2020
Development of a cell-based potency assay for an early phase gene therapy product
Objective

- Transition gene therapy potency method during GMP sample release and stability

- Evolve assay platform from a ELISA endpoint to a reporter gene endpoint via luciferase detection
Deliverables

1) Deliver a robust potency assay to support GMP release and stability

2) Cross-over data between the different platforms to bridge the on-going stability study

Both platforms target the same second messenger molecule response as a measure of potency
Reporter Gene cell line engineering

- **Cell line A engineered by Svar Life Science**
  - Stably transfected with activation protein (Sanofi plasmid with Gene Y)
  - Luciferase reporter system (proprietary) responsive to second messenger molecule

- **Characterization and manufacture**
  - Clone stability, cell viability, and second messenger response was evaluated during manufacture
  - Assay Ready Cells delivered
- *intracellular* reporter system

Transactivator exists as monomer

No expression of firefly luciferase activity
**+ intracellular reporter system**

Transactivator binds to transactivator, forming a homodimer that activates transcription of promoter. Expression of luciferase activity is directly proportional to the intracellular level of second messenger.
Confirming reporter gene cell line demonstrates MoA

A) AAV produced the highest luciferase signal T=72 hours post-infection.
B) Ad5 or proteasome inhibitors are not required to generate transduction-induced luciferase activity.
1. Seed

5E3 cells/well

37°C 5% CO2
6 hrs

2. Infect

Cell Line A
+ Gene Y
+ Luciferase Reporter

Seeding density
Flask Confluency

Dose Optimization

+ AAV

3. Measure

37°C 5% CO2
3 days

4. Data Analysis

BioSt@t
Parallel Line Analysis

Plate Uniformity

Bright Glo
(Promega)
Objective:

- Investigate the impact that flask confluency at time of seeding

Procedure:

- Examine multiple flask densities targeting 60, 70, 80, 100% confluency
- Perform assay from each flask on a single assay plate using same seeding density and optimal drug exposure previously defined

<table>
<thead>
<tr>
<th>Seeding Density</th>
<th>Cells/Flask</th>
<th>Targeted Confluency at Seed</th>
<th>Confluency (%)</th>
<th>Viable Cell Concentration (E5/mL)</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5E3 cells/well</td>
<td>5.70E+05</td>
<td>60%</td>
<td>65</td>
<td>2.5</td>
<td>93.5</td>
</tr>
<tr>
<td></td>
<td>7.10E+05</td>
<td>70%</td>
<td>75</td>
<td>3.2</td>
<td>95.2</td>
</tr>
<tr>
<td></td>
<td>8.50E+05</td>
<td>80%</td>
<td>80</td>
<td>4.0</td>
<td>95.2</td>
</tr>
<tr>
<td></td>
<td>1.00E+06</td>
<td>100%</td>
<td>95</td>
<td>4.7</td>
<td>95.9</td>
</tr>
</tbody>
</table>
A. An initial dose response curve was observed
B. Signal-to-Noise ratio was 600-fold for all conditions
C. Robustness with respect to flask confluency from 70-100%.
Objective:
• Determine the optimal infection incubation time
• Determine the optimal seeding density

Procedure:
• Seeding Density: 5E3, 1.25E4, 2.5E4, 1E5 cells/well
• Infection Incubation Time: 2, 3, 4 days
• Evaluate across a wide MOI series
Optimal Infection Day/Seeding Density

Reporter cell line seeded at 5E3 cells/well, and infected for 3 days with AAV

4-Parameter Logistic Regression (4PL)
- One point in upper asymptote (3 is optimal)
Parallel Line Analysis

- Compare dose-response curves to reference in order to assess differences
- Shift relative to reference indicates change in potency that can be measured
Qualification: Reporter gene relative potency assay
Preliminary Qualification Results

\[ y = 1.0314x - 1.6699 \]
\[ R^2 = 0.9984 \]

**OBSERVED RELATIVE POTENCY**

<table>
<thead>
<tr>
<th></th>
<th>Qualification Parameter</th>
<th>Reporter Gene Potency Qualification Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td></td>
<td>75 – 118%</td>
</tr>
<tr>
<td>Intermediate Precision</td>
<td></td>
<td>17%</td>
</tr>
<tr>
<td>Repeatability</td>
<td></td>
<td>7%</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>20 – 200 %</td>
</tr>
<tr>
<td>Linearity</td>
<td></td>
<td>( R^2 = 0.990 )</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td>Method is specific to DS and DP samples vs. buffer only</td>
</tr>
</tbody>
</table>

**EXPECTED RELATIVE POTENCY**
### Comparing Workflows: ELISA vs. Reporter Gene endpoints

<table>
<thead>
<tr>
<th>Assay Step</th>
<th>Current Method</th>
<th>Reporter Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate format</td>
<td>24-well</td>
<td>96-well</td>
</tr>
<tr>
<td>Cell line</td>
<td>Parental</td>
<td>Reporter gene</td>
</tr>
<tr>
<td>Plasmid transfection</td>
<td>transient</td>
<td>Stable</td>
</tr>
<tr>
<td>Infection MOI</td>
<td>5 points in quadruplicate</td>
<td>6 points in triplicate</td>
</tr>
<tr>
<td>Ad5 required?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Sample Throughput</td>
<td>2 plates / 1 sample</td>
<td>2 plates / 4 samples</td>
</tr>
<tr>
<td>Infection time</td>
<td>3 days</td>
<td>3 days</td>
</tr>
<tr>
<td>Harvest/Lysis</td>
<td>1 day</td>
<td>Not required</td>
</tr>
<tr>
<td>Detection</td>
<td>ELISA</td>
<td>Luciferase</td>
</tr>
<tr>
<td>Data Analysis</td>
<td>PLA</td>
<td>PLA</td>
</tr>
<tr>
<td>Total Assay Time</td>
<td>6 days</td>
<td>4 days</td>
</tr>
<tr>
<td>Controls</td>
<td>• WRS</td>
<td>• WRS</td>
</tr>
<tr>
<td></td>
<td>• ELISA standard</td>
<td>• negative control</td>
</tr>
<tr>
<td></td>
<td>• negative control</td>
<td>• Positive Control</td>
</tr>
</tbody>
</table>
Cross over Strategy

• Generating similar results for platform comparability was not attainable because ELISA method was not robust

• Instead, selective historical data was used as a comparison
  • Lot 5 obtained lower potency compared to reference using the ELISA platform.
    • Can reporter gene method detect differences observed in potency?
Cross over Results

Graph showing relative potency (%) for different lots:
- Lot 1: ELISA 90, Reporter Gene 92
- Lot 2: ELISA 85, Reporter Gene 87
- Lot 3: ELISA 110, Reporter Gene 93
- Lot 4: ELISA 90, Reporter Gene 80
- Lot 5: ELISA 20, Reporter Gene 45

Legend:
- ELISA
- Reporter Gene
Final Conclusion

- Reporter gene assay was implemented to test DS and DP samples on release/stability
  - Replaced variable ELISA method
  - Increased method accuracy, range, throughput, and sensitivity

- Reporter gene assay not intended for early phase release testing but transitioned early due to program need
Value to Gene Therapy programs

- Potency is only method relevant to product efficacy in gene therapy and therefore has significant value

  - Structure/Function studies
  - Process characterization
  - Batch-to-batch consistency
  - Comparability studies
  - interrogates the product
THANK YOU

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