Overcoming the quality control barrier in ATMP development: high throughput analytics
Overcoming the quality control barrier in ATMP development: high throughput analytics

Damian Marshall
Director – New Technologies
Gene therapy product release

Up stream processing
- Plasmid DNA
- WCB
- Cell expansion
- Cell expansion
- Virus production

Down stream processing
- Clarification
- Buffer exchange
- Purification
- UF/DF
- Sterile filtration
- Formulation
- QC release

Titre and Potency
- Physical titre
- Infectious titre
- Infectivity
- Transgene function
- Stability

Identity - Physicochemical
- Transgene sequence
- Vector proteins
- Vector integrity
- pH (EP 2.2.3)
- Osmolarity

Impurities
- Residual HCP, HC-DNA, plasmid DNA
- Large T antigen protein / DNA
- Benzonase
- Aggregation
- Empty vector

Safety
- Sterility (EP 2.6.1)
- Mycoplasma (EP 2.6.7)
- Endotoxin (EP 2.6.14)
- Adventitious viruses
- Replication competent viruses

Plasmid DNA
WCB
Cell expansion
Cell expansion
Virus production
Clarification
Buffer exchange
Purification
UF/DF
Sterile filtration
Formulation
QC release
Industry challenges:

- Large number of batches for release
- Complexity of analytical assays
- Requirement for rapid release

<table>
<thead>
<tr>
<th>Total yield TU (from 200L)</th>
<th>Target cells</th>
<th>MOI</th>
<th>Cell Number</th>
<th>Transductions</th>
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<tbody>
<tr>
<td>1.0x10^{12}</td>
<td>T Cells</td>
<td>5</td>
<td>3.0x10^9</td>
<td>67</td>
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<tr>
<td>1.0x10^{12}</td>
<td>CD34^+</td>
<td>100</td>
<td>1.0x10^8</td>
<td>100</td>
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<table>
<thead>
<tr>
<th>Gene Therapy</th>
<th>Condition</th>
<th>Serotype</th>
<th>~ total dose</th>
<th>Doses per 1000 L</th>
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<tbody>
<tr>
<td>RPE65</td>
<td>Retinal dystrophy</td>
<td>AAV2</td>
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<td>2400</td>
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<td>Factor IX</td>
<td>Hemophilia B</td>
<td>AAV5</td>
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<td>4</td>
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<td>SMA 1</td>
<td>Muscular atrophy</td>
<td>AAV9</td>
<td>1.0x10^{15}</td>
<td>6</td>
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</tbody>
</table>

July 16, 2019

*Kite Announces Plans to Bolster Industry-Leading Cell Therapy Manufacturing Capabilities With New Viral Vector Facility*

SANTA MONICA, Calif.--(BUSINESS WIRE)--Jul. 16, 2019 -- Kite, a Gilead Company (Nasdaq: GILD), today announced plans for a new 67,000-square-foot facility in Oceanside, California, dedicated to the development and manufacturing of viral vectors, a critical starting point for the production of cell and gene therapies.

Bluebird ramps up lentiviral vector production with Durham Facility

By Maggie Lynch


In an indication of where the growth in the pharma industry is developing, Bluebird Bio is the latest to complete a new viral vector manufacturing facility to produce the investigational gene and cell therapies it is working on.

The biotech is in the process of qualifying the 125,000-square-foot lentiviral vector facility in Durham, North Carolina, in which it invested $20 million. About 600 employees work there.

Novartis prepped for ‘unprecedented’ Zolgensma demand

By Dan Stanton

Thursday, April 25, 2019 4:37 am

With over one million square-feet of manufacturing space, Novartis says it is prepared for the imminent approval of AveXis’ SMA gene therapy Zolgensma.

Speaking during its Q1 2019 results, Novartis said it is set for the imminent arrival of gene therapy Zolgensma (onsenansenogene abaparvovec), added to the firm’s pipeline through the acquisition of AveXis. The one-time therapy targeting spinal muscular atrophy (SMA) Type
High production throughput needed

The centre provides access to the expertise, skills, facilities and equipment as the stepping stone needed for organisations to develop new technologies and systems for large scale manufacturing.

- Quality control
- Qualified persons
- Operating policies
- Warehouse management
- Development assistance

Predicted Advanced Medicinal Product Output:
- 528 batches per year per module
- 5,280 batches per year for facility

Predicted QC sample output:
- >8,000 samples per year per module
- >105,000 samples per year for facility

Engineering maintenance:
- >3,000 Key equipment pieces for building function

Warehouse:
- >1.0M Material picks per year for facility
QC lab automation

• Automation can increase facility throughput and make QC faster, more agile, more compliant, and more efficient.

• Automation technologies already exist that could be used to streamline cell and gene therapy product release

• Up to 80% of QC laboratory tasks could be automatable

• Automation can also ensure better quality and compliance by reducing manual errors and variability, as well as allowing faster and effective resolution of problems.
Integrating new technologies

• Technologies for cell characterisation are advancing faster than ever before

• This presents an opportunity for technology integration to change the way product release is performed

• These include technologies for rapid analysis:
  • Rapid potency testing
  • Rapid viral characterisation
  • Rapid sterility

• Opportunities to incorporate lab-on-a-chip technologies
  • Sample miniaturisation
  • Multiparametric analysis

• High content technologies (single cell technologies)
Transformative approaches to QC

• Using state of the art technologies to support product release

• AR is increasingly being applied in the healthcare sector
  • AccuVein for visualising vasculature
  • brain tumour mapping
  • surgical training

• AR is also being investigated as a new approach to support GMP manufacturing by large pharma

• Are there opportunities for augmented reality in QC?
  • Advanced electronic data recording
  • Lowering skill barriers
  • Increasing operator output
Sensor technology integration
Real Time Release Testing

• RTRT is a framework to ensure the quality, safety and efficacy of the final drug product based on data generated during the process.

• This typically includes the measurement of CQA’s during the process in combination with real-time monitoring of process parameters

• RTRT can provide a higher assurance of product quality –
  • Real-time control of process
  • Enhanced process understanding
  • Operational flexibility
  • Framework for continuous manufacturing
  • Support of continual improvement
Summary

Data connectivity, advanced analytics, robotics and automation have the potential to revolutionise ATMP product release.
INTRODUCTION TO ANTHA

Lab automation as the key to realising an integrated and flexible digital strategy

Markus Gershater, PhD
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TRACKING LAB PROCESSES
AUTOMATED DATA STRUCTURING AND CONTEXT

Expansion and Transduction → Clarification / Buffer Exchange → Purification / UFDF → Sterile Filtration / formulation & fill finish

Analytical Development / QC
TRACKING LAB PROCESSES
AUTOMATED DATA STRUCTURING AND CONTEXT

Leukapheresis → Activation / Modification → Expansion → Harvest / Cell Washing and Formulation

Analytical Development / QC
TRACKING LAB PROCESSES
AUTOMATED DATA STRUCTURING AND CONTEXT

RUN A

RUN B

011010100001101
1010101100001101
1000011010111010
1010100001101011
1010111010000110
0110110000111010
0011010111010100
0001101011101010

0111010100001101
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1010101100001101
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0011010111010100
0001101011101010
AUTOMATED OPTIMISATION

High throughput DoE needed to rapidly explore the design space of each protocol
OPTIMISED: AUTOMATING DOE

Experimental Design File

Automated planning and programming

Planning

Hours of programming
OPTIMISED: AUTOMATING DOE

Watch video
Antha optimization of transfection gave 3-10 fold increase in viral titre, whilst providing 83% time and 32% resource savings.
Automatically generated array of liquid handling strategies for qPCR, tested over 4 replicates
Automatically generated array of liquid handling strategies for qPCR, tested over 4 replicates
AUTOMATED ANALYTICS

Robust protocols are automated flexibly to adapt any workflow without extensive reprogramming

Watch video
AUTOMATED DATA STRUCTURING
Automated integration of bioreactor, analytical and sample data
CONNECTED:
CASE STUDY: IN-LINE RAMAN
CONNECTED:
CASE STUDY: IN-LINE RAMAN

![Graph showing glucose concentration changes over time](image1)

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Sensor 1</th>
<th>Sensor 2</th>
</tr>
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<tbody>
<tr>
<td>count</td>
<td>114.60</td>
<td>114.60</td>
</tr>
<tr>
<td>mean</td>
<td>2.91</td>
<td>2.89</td>
</tr>
<tr>
<td>std</td>
<td>0.97</td>
<td>1.06</td>
</tr>
<tr>
<td>min</td>
<td>1.41</td>
<td>1.34</td>
</tr>
<tr>
<td>25%</td>
<td>1.88</td>
<td>1.87</td>
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<tr>
<td>50%</td>
<td>3.04</td>
<td>2.98</td>
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<tr>
<td>75%</td>
<td>3.83</td>
<td>3.90</td>
</tr>
<tr>
<td>max</td>
<td>4.19</td>
<td>4.16</td>
</tr>
</tbody>
</table>

![Graph comparing Glucose Raman vs. Glucose concentration](image2)

<table>
<thead>
<tr>
<th>Linear Regression Fit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression Coefficient</td>
<td>-1.0258</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.0877</td>
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</table>
CONCLUSIONS

Automation of lab and data processes

Rapid, comprehensive optimisation of automated analytics

Run automated analytics flexibly

Automatically structure data

Thanks:
Rapid Analytics

Shortening time for complex product release assays

Juan Miguel Sánchez-Nieto
Analytical Development Scientist
Challenge: reduce time between product formulation and patient administration

Day 1
Selection

Patient Material  Washing  Selection  Activation

Transduction

Day 1/3
Transduction

Day 1-10
Expansion

Day 10
Formulation

Wash Concentrate  Formulation  QC release

Identity
- Transduction efficiency
- Immunophenotype
- Appearance

Impurities
- Percentage non-CD3+ cells
- Large T antigen protein/DNA

Safety
- Genome viral copy number
- Sterility (EP 2.6.1)
- Mycoplasma (EP 2.6.7)
- Endotoxin (EP 2.6.14)
- Replication competent viruses

Potency
- Viable cell count
- CAR/TCR expression
- Cell killing activity
- Cytokine stimulation
Potency assays for immunotherapies
Current methods to evaluate T-cell potency

Chromium release

- Gold standard
- Limitations:
  - Time – leakage
  - Safety – use of radioactive material
  - Cell requirements – high effector to target ratios | physiological relevance
### Alternatives to Cr51 release assay

<table>
<thead>
<tr>
<th>Assay</th>
<th>Measure</th>
<th>Readout</th>
</tr>
</thead>
<tbody>
<tr>
<td>CytoTox - 96</td>
<td>LDH</td>
<td>Absorbance</td>
</tr>
<tr>
<td>Cell Titer-Glo</td>
<td>ATP</td>
<td>Luminescence</td>
</tr>
<tr>
<td>Calcein-AM</td>
<td>Dye release</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>Delfia EuTDA</td>
<td>BATDA release</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>Cytokine/cell death</td>
<td>Fluorescence</td>
</tr>
</tbody>
</table>
Solution: impedance – based potency assay

Real Time Cell Analysis system:

- Non-invasive system – electrical impedance
- Label free
- High throughput – 6x 96-well plates
- Flexible

Limitation:
- Optimisation required for each target cell line
How does the impedance-based potency assay work?

![Diagram showing the process of impedance-based potency assay over time. The graph illustrates initial cell attachment, continued attachment, max attachment, and cell death.](image)
Assay outline:

1. Target cells are pulsed for 2 hours with peptide prior to plating
2. Target cells are plated and allowed to attach for 4 hours – impedance readings are initiated
3. Cells are washed prior to killing assay
4. Transduced T cells are added
5. Killing response is measured every 15 minutes for up to 24 hours
Comparability between impedance and flow cytometry – TCR therapy

5:1 effector/target

- Effector only
- Non-pulsed
- Non Specific 1
- Non Specific 2
- Specific Peptide

Viability %

0 5 10

time (h)

Unpulsed  Effector Only  Non-Specific 1  Non-Specific 2  Specific Peptide

Impedance data  Live cells FACS
Correlation with impedance and quantitative image analysis – TCR therapy

Target cells | Effector Cells | Dead cells

Viability %

0h | 3h | 6h | 9h

Unpulsed
Effector only
Non Specific 1
Non Specific 2
Specific Peptide

Time (h)
Real time detection of product’s potency within 4h - CAR-T cell based therapy
Summary

- TCR and CAR-T immunotherapy potency can be reliably measured using impedance spectroscopy
- We have shown specificity of the assay independently of the therapy used
- Assay readout correlates with FACS analysis and image analysis
- The impedance assay is label free and provides **kinetic data** of cell killing
  - KT50
- This assay provides a **fast** and **high-throughput** alternative to current methodologies
Cell and Gene Therapy Catapult is committed to ensuring high standards of research integrity and research best practice in the activities we carry out. We subscribe to the principles described in the UK concordat to support research integrity.