WHEN THE CELL IS THE DRUG, CHALLENGES FOR BIOANALYSIS

21 Nov 2018
CHIMERIC ANTIGEN RECEPTOR THERAPY

A brief overview

Manus et al, 2016 Clinical Cancer Research
CHALLENGES OF CELL THERAPIES
PK and Bioanalysis for CAR Therapeutics

• Cell based therapies challenge our conception of a PK assessment
• Unlike conventional small or large molecules drugs, the initial dose is low, followed by *in vivo* cell population expansion (1000-fold) and then decline
• There are a number of bioanalytical techniques available to understand the behaviour of CAR cells following infusion
• As always the challenge is which questions you are trying to answer (and what answer you are looking for)
CHALLENGES TO PK ASSESSMENT

- Absolute Dose
- Dose Formulation CD4:CD8
- Manufacturing Processes
  Construct Design, Mol, Expansion
- Patient Physiology, Tumour Physiology, Pre-conditioning

EVERY STEP OF THE WAY
Q-PCR METHODOLOGIES
Detection of Genetic Modification

Maps well to the Bioanalysis Guidelines

Batch analysis – single analysis for full PK profile

DNA is stable, allows ISR

It’s a sensitive assay for pathological expansion

However, the CAR cell count is derived

It’s a surrogate measurement

X is DNA
Y is CAR
ADDRESSING GAPS IN Q-PCR PK

Direct measurement of CAR cells

Accurate quantification of CAR cells

Level of expression of CAR

Phenotype of CAR cells – both simple and complex
CHALLENGES AND BENEFITS OF FLOW CYTOMETRY PK

Does not map to the Bioanalysis Guidelines
Samples have a short analysis window
Sample analysis can only occur on a single occasion
Complexities in workflows and assay design
Data analysis (gating) presents specific challenges

Provides a direct CAR cell count
Provides phenotypic data
PROCESS STEPS IN FLOW CYTOMETRY

Panel Design

Sample Processing

Acquisition

Gating (Data Processing)
  Hand
  ‘Statistical’

No two cytometers are the same
Background is a key consideration – the negative population is important

Compensation

Spectral overlap

Specific challenges of tandem dyes – broad excitation/emission spectra
GATING

Upstream gating to CD3 Lymphocytes

Gating strategies are last in the workflow but must be decided first!
SETTING A STATISTICAL GATE

The negative population is critical in PK

- Critical populations should be gated statistically
- Mean + 4x Standard Deviation
- Changes in cytometer leads to shifts in the negative population
- Patient samples should be locked to one cytometer

\[
\text{Mean: } 149 \\
\text{SD: } 302 \\
\text{Boundary: } 1357
\]

\[
\text{Mean: } 246 \\
\text{SD: } 380 \\
\text{Boundary: } 1766
\]
WHAT ABOUT ACCURACY AND PRECISION?

Analyst 1

Analyst 2

Inter-Instrument assessments to assess impact

Instrument 1 → Instrument 2

Operational Controls
Exogenous spike into control matrix, absolute counts via ebeads, healthy donors

- Assay design must capture initial dose, expansion and decline
- Set an accurate range based on previous expansion data
- 2-150 cells/µL
- Inter Laboratory precision to assess changes in Test Site

How should a validation be related to realities of clinical sample analysis?
### CHOICES FOR FLOW CYTOMETRIC ANALYSIS

**A Kierkegaardian Approach to Bioanalysis**

<table>
<thead>
<tr>
<th>PK</th>
<th>Immunophenotyping (IMPT)</th>
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<tbody>
<tr>
<td>≤ 5 specific cell populations</td>
<td>≥ 5 different cell populations</td>
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- High laser count
- Low panel complexity
- Limited spectral overlap
- Use of baseline for gate setting
- Negative populations critical

- **Maximise accuracy/precision**
- **Sacrifice phenotype information**

- High laser count
- High panel complexity
- Compensation to manage spectral overlap
- Negative populations have low importance
- **Hand gating**

- **Maximise phenotype information**
- **Sacrifice accuracy/precision**