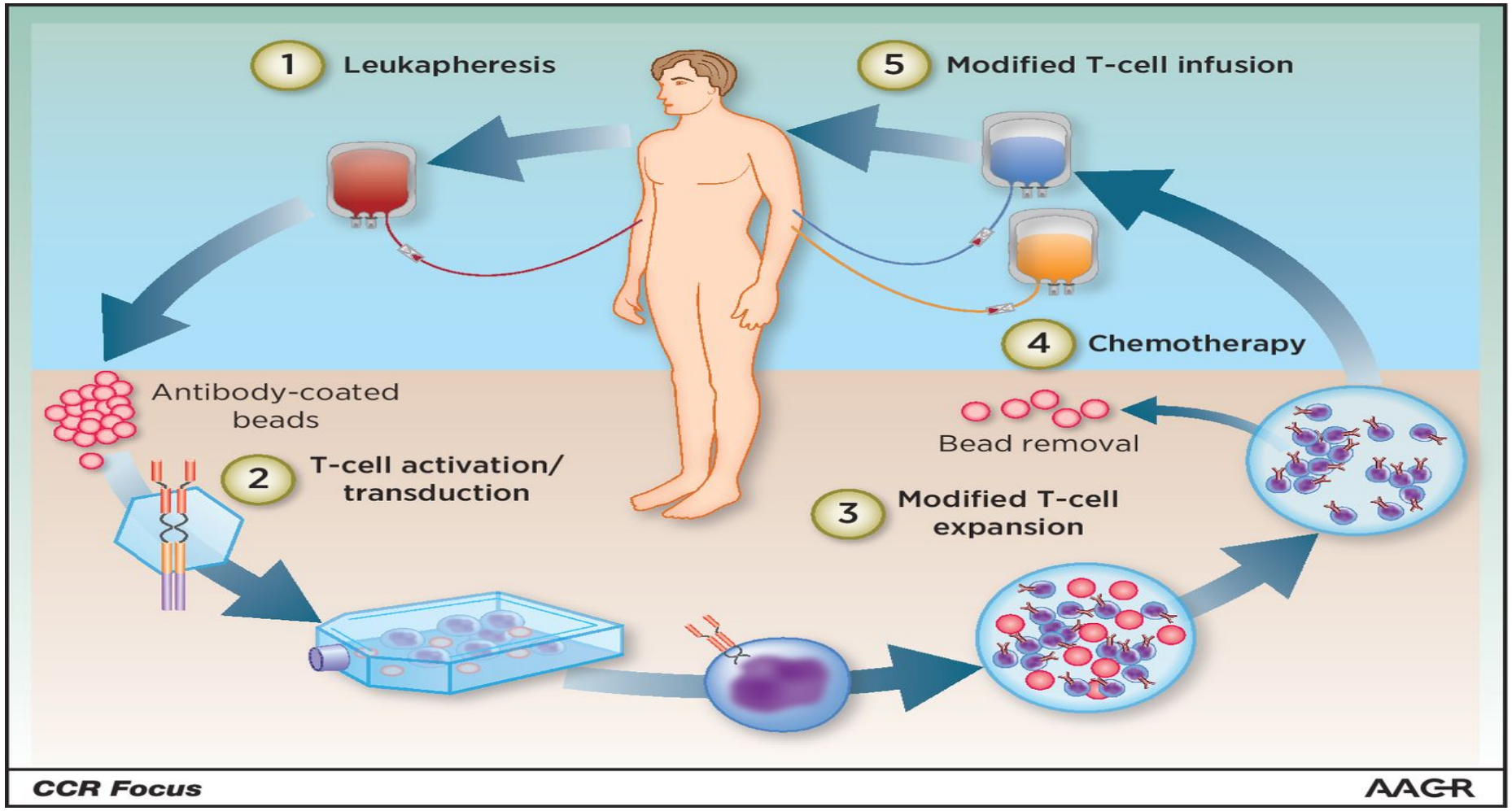
WHEN THE CELL IS THE DRUG, CHALLENGES FOR BIOANALYSIS

21 Nov 2018

CHIMERIC ANTIGEN RECEPTOR THERAPY

A brief overview



Manus et al, 2016 *Clinical Cancer Research*

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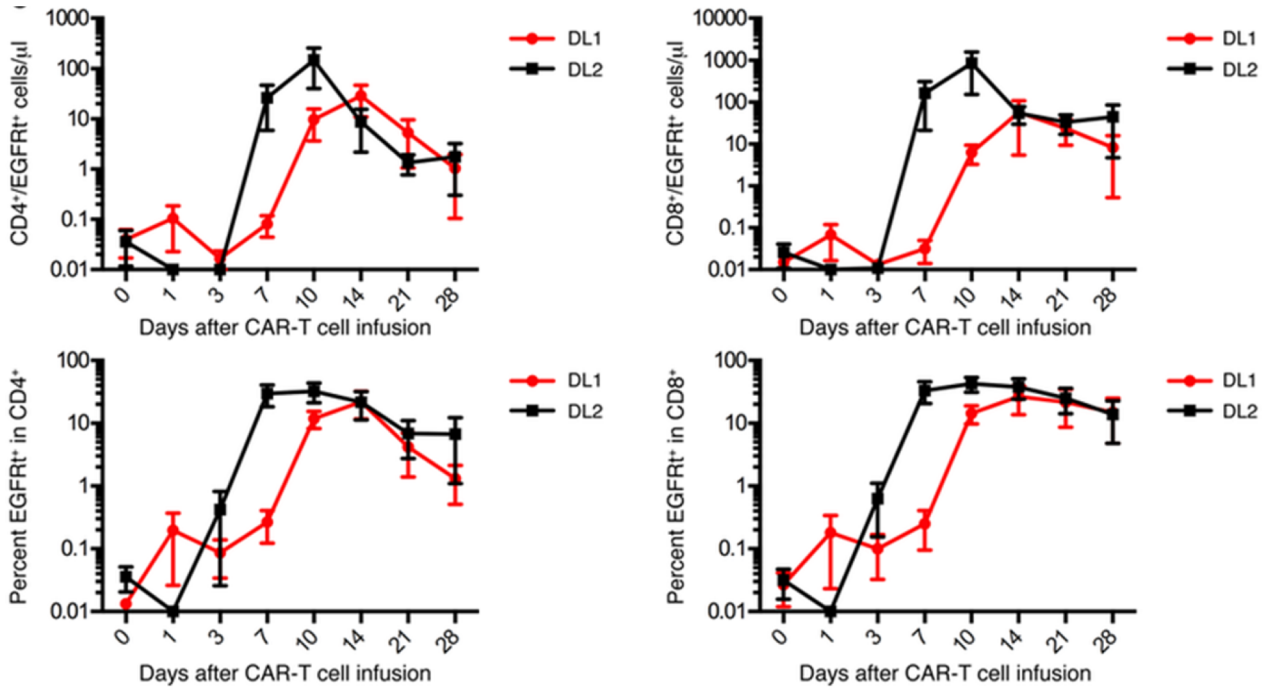
CHALLENGES OF CELL THERAPIES

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)



Turtle et al, 2016 *The Journal of Clinical Investigation*

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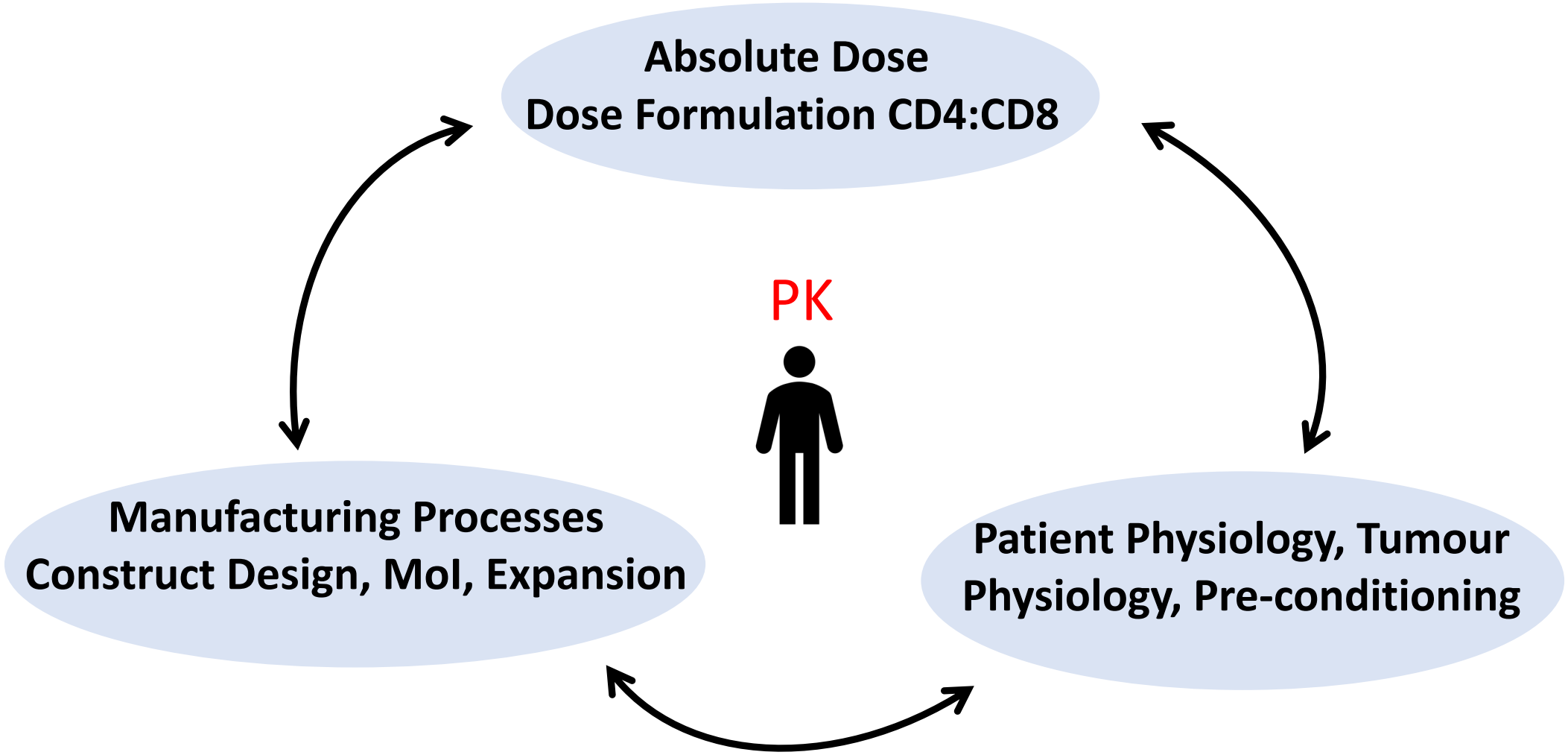
CHALLENGES TO PK ASSESSMENT



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Q-PCR METHODOLOGIES

Detection of Genetic Modification

Maps well to the Bioanalysis Guidelines

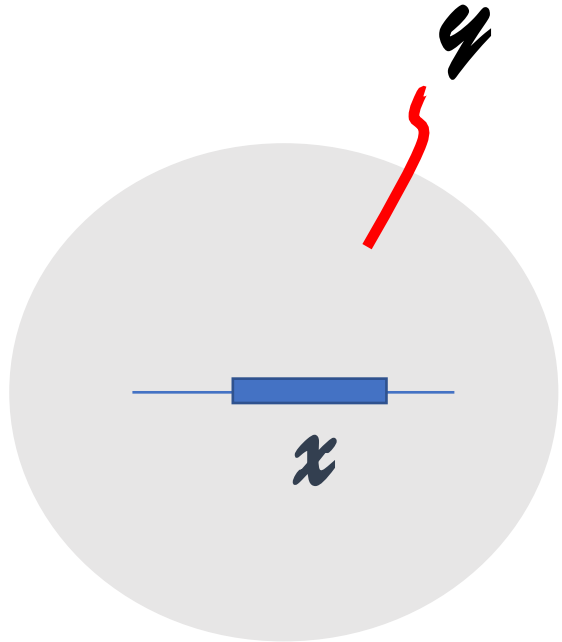
Batch analysis – single analysis for full PK profile

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

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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

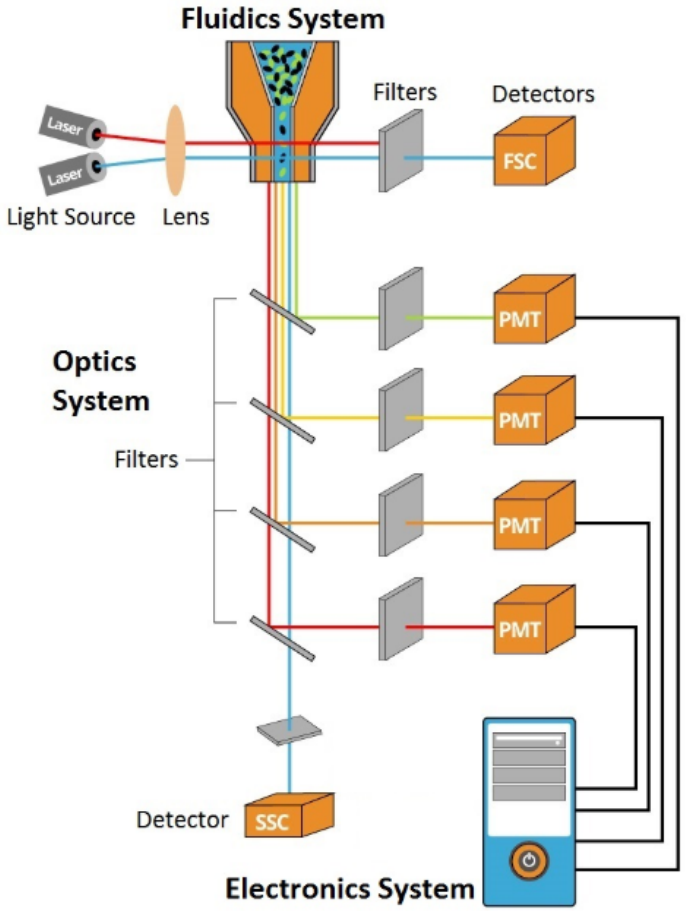
PROCESS STEPS IN FLOW CYTOMETRY

Panel Design

Sample Processing

Acquisition

Gating (Data Processing)
- Hand
- ‘Statistical’



No two cytometers are the same

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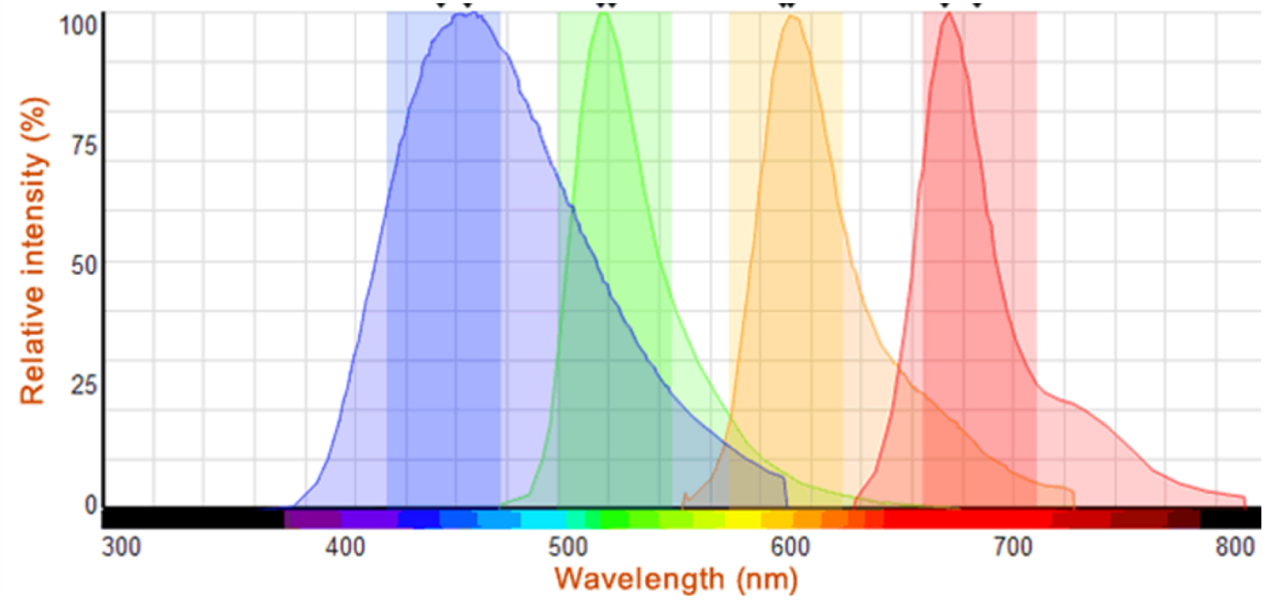
PANEL DESIGN FOR FLOW PK

Background is a key consideration – the negative population is important

Compensation

Spectral overlap

Specific challenges of tandem dyes – broad excitation/emission spectra



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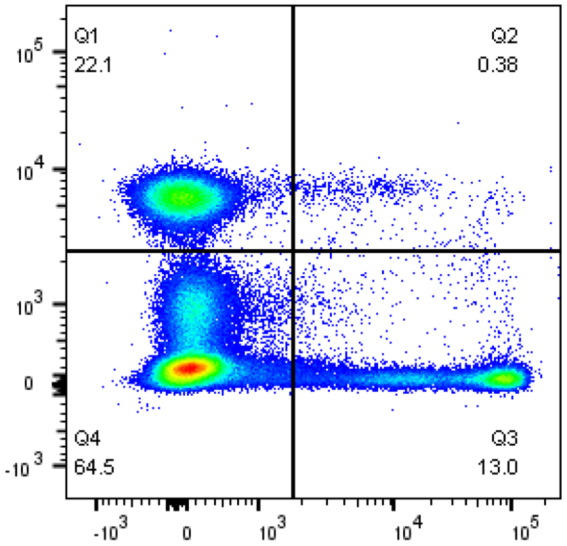
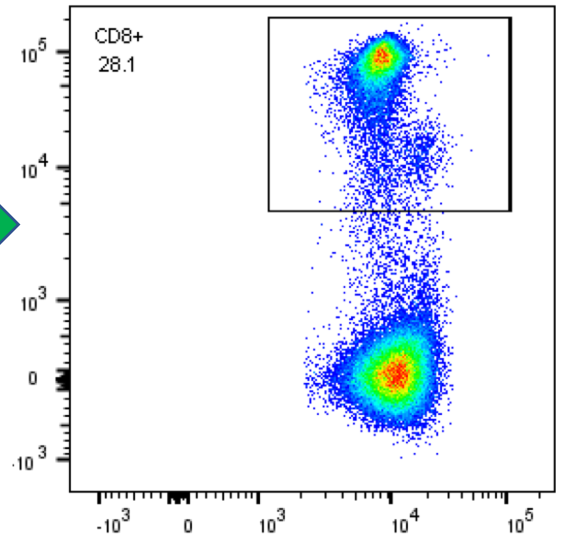
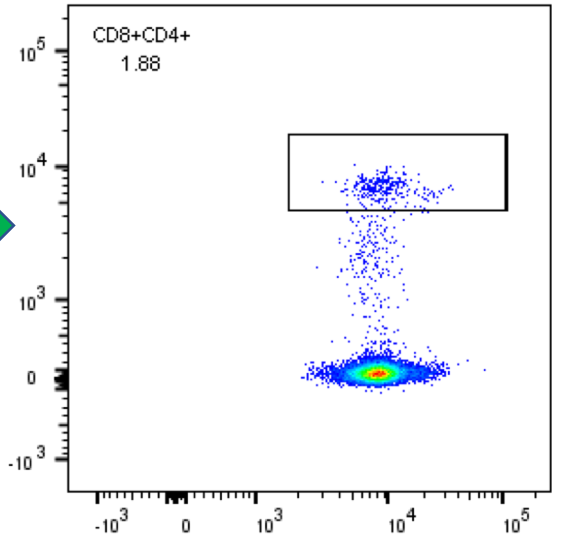
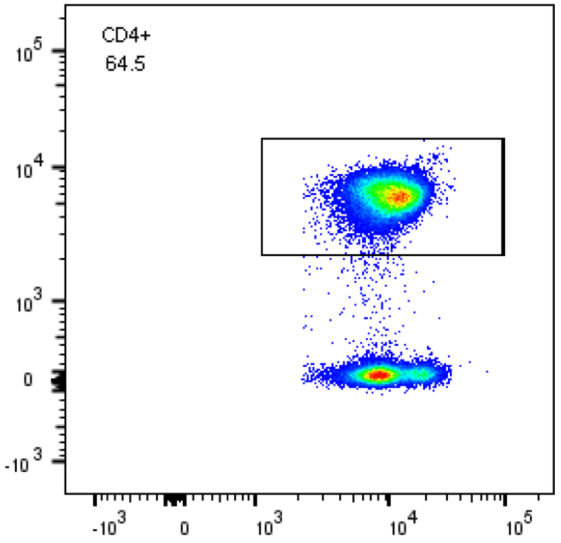
GATING

Upstream gating to CD3 Lymphocytes









Gating strategies are last in the workflow but must be decided first!

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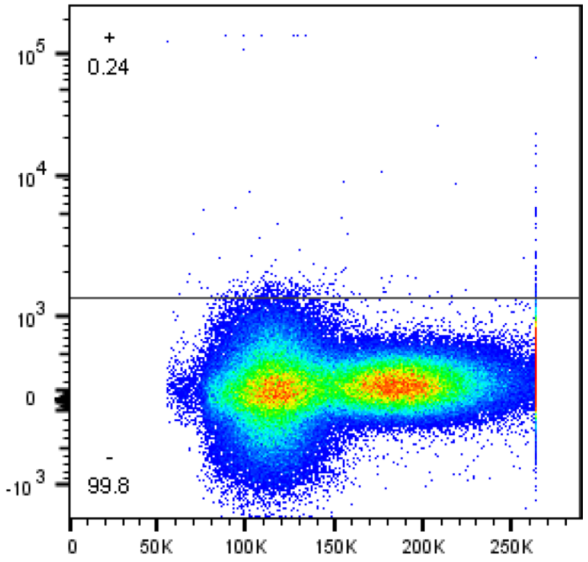
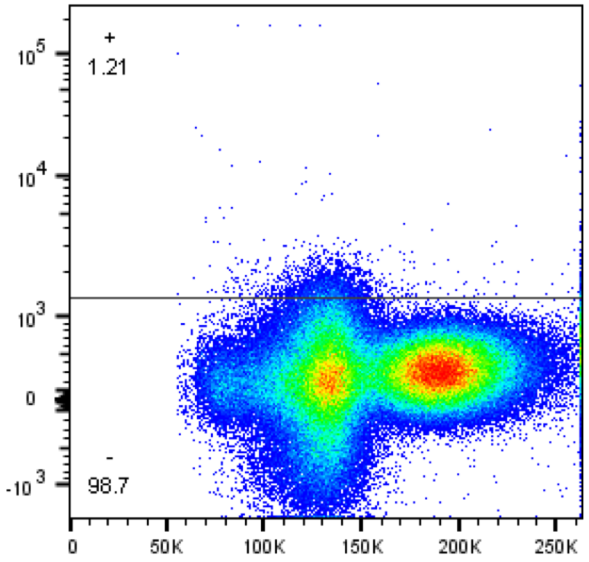
SETTING A STATISTICAL GATE

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



Mean: 149
SD: 302
Boundary: 1357



Mean: 246
SD: 380
Boundary: 1766

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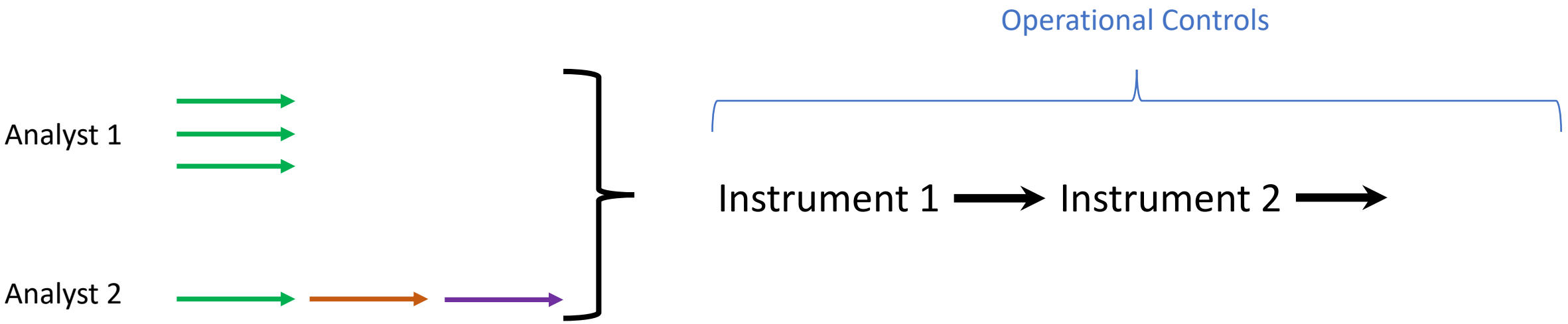
WHAT ABOUT ACCURACY AND PRECISION?



Inter-Instrument assessments to assess impact

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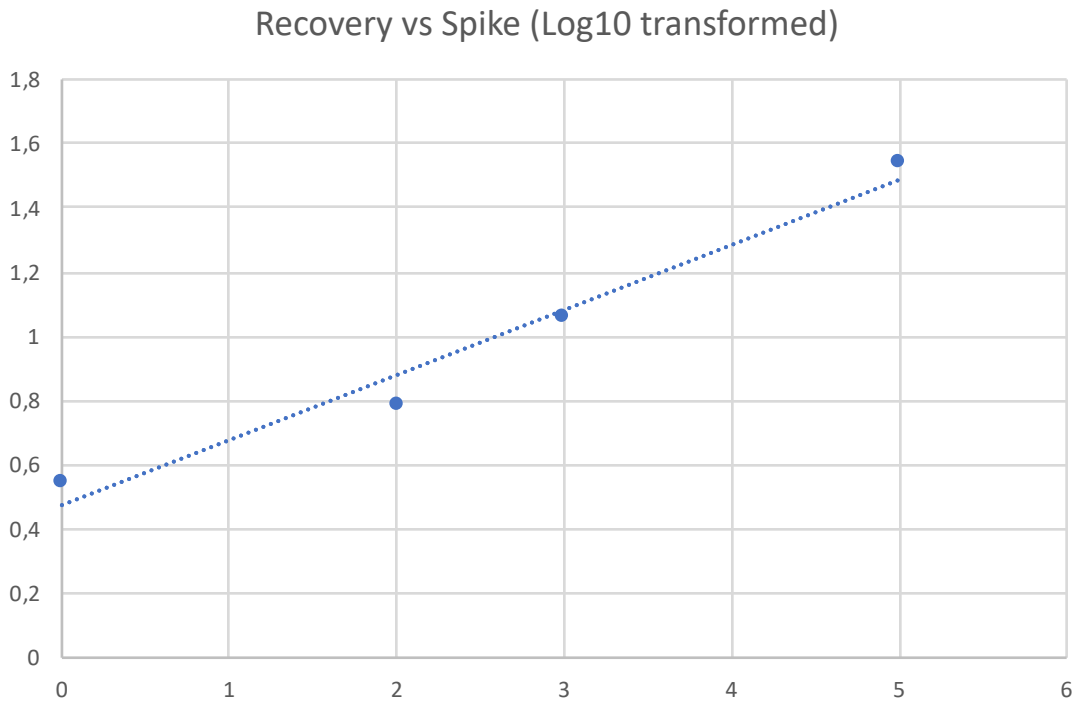
ACCURACY AND PRECISION

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?

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CHOICES FOR FLOW CYTOMETRIC ANALYSIS

A Kierkegaardian Approach to Bioanalysis

PK	Immunophenotyping (IMPT)
≤ 5 specific cell populations	≥ 5 different cell populations
High laser count	High laser count
Low panel complexity	High panel complexity
Limited spectral overlap	Compensation to manage spectral overlap
Use of baseline for gate setting	Negative populations have low importance
Negative populations critical	Hand gating
Maximise accuracy/precision	Maximise phenotype information
Sacrifice phenotype information	Sacrifice accuracy/precision

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