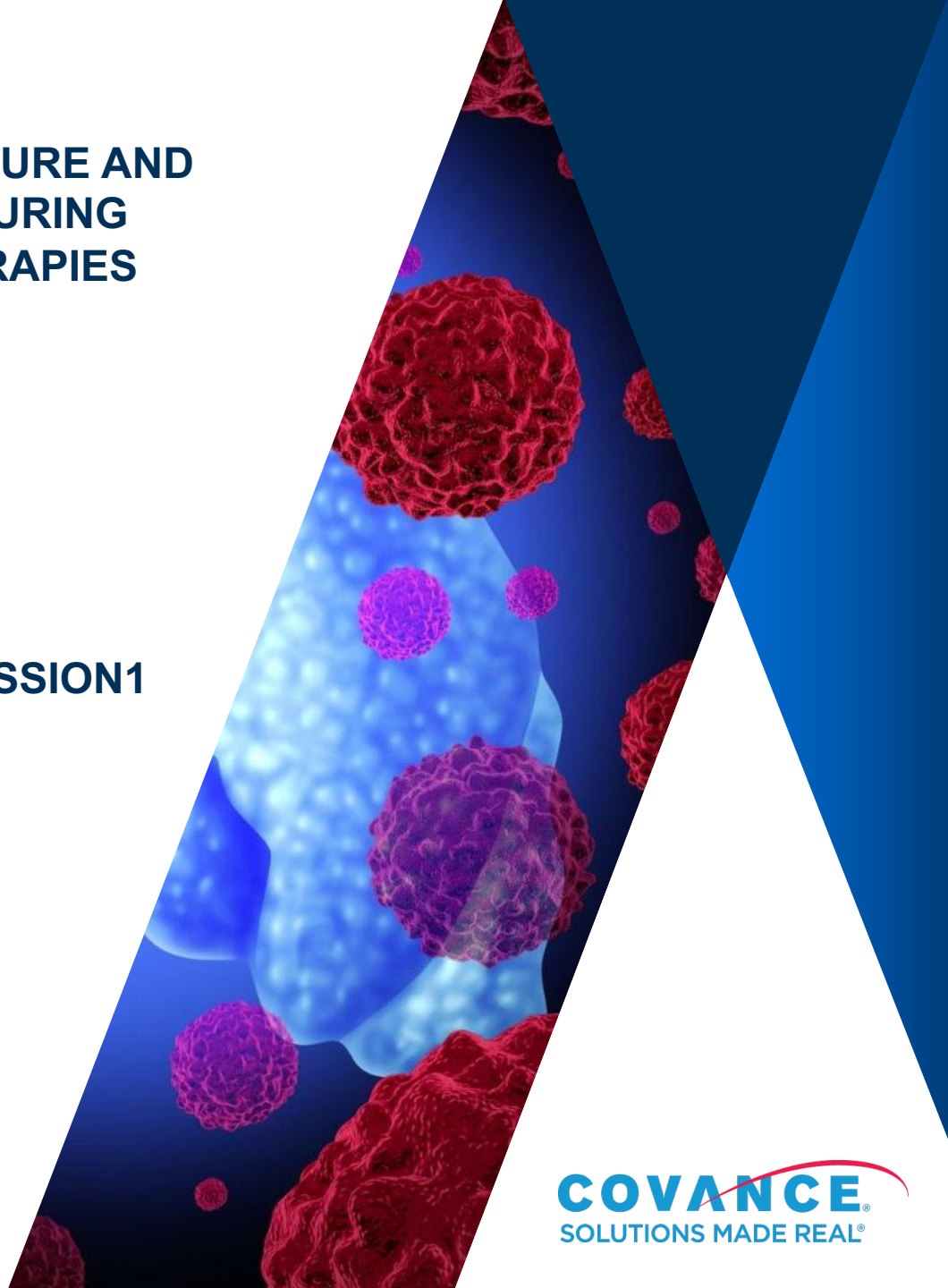
UNDERSTANDING HOW TO MEASURE AND DEMONSTRATE EXPOSURE DURING DEVELOPMENT OF CELL THERAPIES

JAMES MUNDAY

15TH SEPTEMBER 2020

EBF C&GT TRAINING DAY – SESSION1



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Meeting Agenda

- Cellular therapies and changing bioanalytical needs for PK/PD
- Current regulatory guidance for demonstrating exposure for cellular therapies
- Techniques utilised for measuring cellular kinetics
  - Flow cytometry considerations with CASE studies
- Future perspectives – Importance of setting an appropriate cellular dose
- Q&A

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Traditional concepts for PK/PD analysis

- **Definitions of PharmacoKinetics (PK) / Pharmodynamics (PD)**
- PK is determination of the concentration of a specific drug in samples from specific tissue compartments
  - Concentration can be linked to safety/adverse events
  - Concentration data links to the dose and therefore the data can be used to select optimum dose levels for therapeutic effect
  - Concentration of a compound is governed by Absorbtion, Distribution, Metabolism and Excretion (ADME)
  - Controlled acute and repeated toxicokinetic animal studies are useful to identify a drugs biological persistence, tissue and whole body half-life, and its potential to bioaccumulate.
  - PK data are included in reports which regulatory authorities use to assess the suitability for new medicines to be produced for improved healthcare
- PD is the determination of the activity of a drug
  - Activity of a drug can be linked to efficacy/toxicity
- Relationship between PK and PD are key considerations for drug development
- PK/PD is complex for cell and gene therapies and not necessarily performed using traditional bioanalytical techniques

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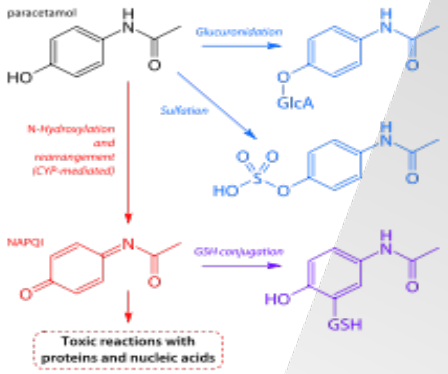
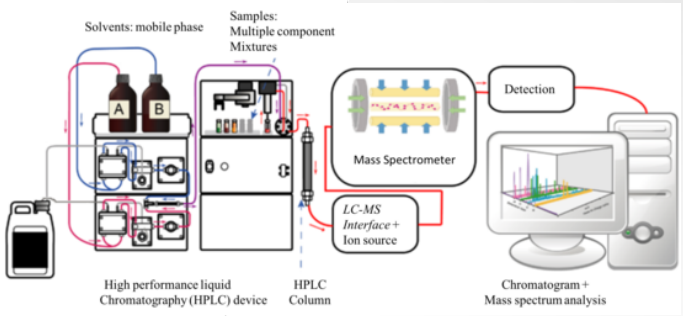
Traditional PK / PD approaches for Bioanalysis

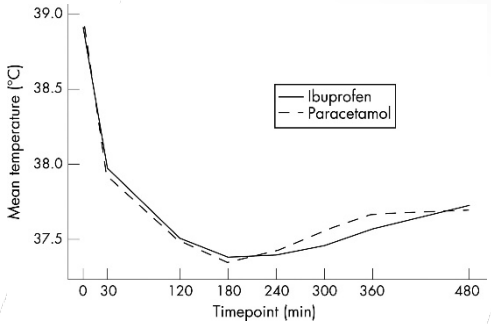
Defined chemical entity & metabolites (E.g. Paracetomol)



PK defined by Plasma concentration > LC–MS/MS



PD effects defined by basic temperature measurement (e.g. flu)



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Cellular Outcomes (E.g. Immune cells)

Challenges for PK/PD of cell therapies:

- Cells are not defined
- Very complex metabolism driven by Biology

Necrosis

Loss of Cellular Integrity

Increased Permeability

Resting

Apoptosis

Apoptosis Marker

Annexin V/αPS

Activation

Morphology

Activation Markers

αCD25/αCD69

Chemokines

IL-8, MCP-1…

Proinflamm Cytokines

IL-8, MCP-1…

Proliferation

ZZZzz

Cellular Anergy

Functionally Impaired

Range of bioanalytical techniques to measure PK/PD relationships

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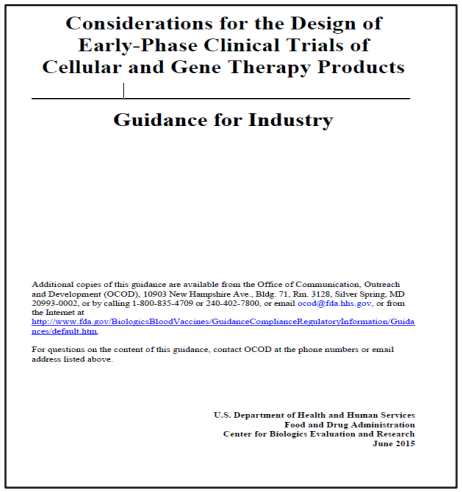
Limited regulatory guidance due to complexity and range of approaches



Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products

Guidance for Industry

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-7800, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
June 2015

EUROPEAN MEDICINES AGENCY
SCIENCE · MEDICINES · HEALTH

31 January 2019
EMA/CAT/852602/2018
Committee for Advanced Therapies (CAT)

Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials
Draft

Adopted by Committee for Advanced Therapies (CAT)	December 2018
Adopted by Committee for Medicinal Product for Human Use (CHMP) for release for consultation	January 2019
Start of public consultation	21 February 2019
End of consultation (deadline for comments)	1 August 2019
Adopted by CAT and CHMP	<DD Month YYYY>
Date for coming into effect	<DD Month YYYY>

Comments should be provided using this template. The completed comments form should be sent to AdvancedTherapies@ema.europa.eu

Keywords	***Investigational ATMPs, Gene therapy medicinal product, Cell therapy medicinal product, tissue engineered product, Exploratory trial, First in human trial, Confirmatory trial***

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 Facsimile +44 (0)20 3660 5555
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- Risk benefit approach general adopted for all regulatory documents
- FDA > Focus on Minimum Tolerated Dose when exploring appropriate dose in these instances
- FDA > Activity assessment > For CGT products, activity assessments might include specialized measures such as gene expression, cell engraftment, or morphologic alterations, as well as more common measures such as changes in immune function, tumor shrinkage, or physiologic responses of various types.
- EMA > Pharmacokinetics depend on the type of the Advanced Therapeutic Medicinal Product (ATMP) and include biodistribution (distribution and migration), as well as elimination parameters (persistence and clearance).
- EMA > For cell-based ATMPs, including genetically modified cells, distribution, migration and persistence of the cells should be understood in order to identify relevant risks related to unwanted biodistribution, and to focus the non-clinical safety studies to the aspects that are relevant for the intended clinical use.

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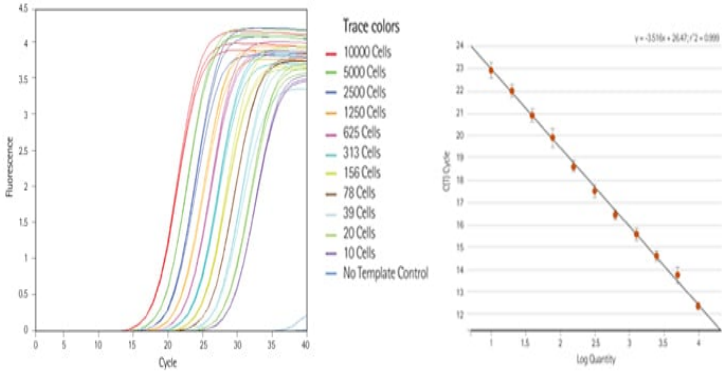
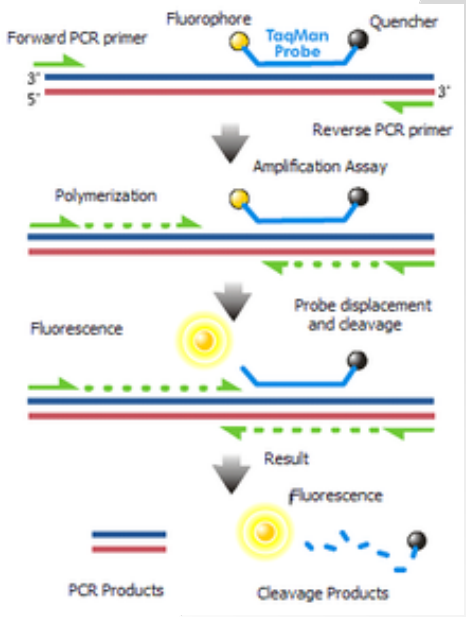
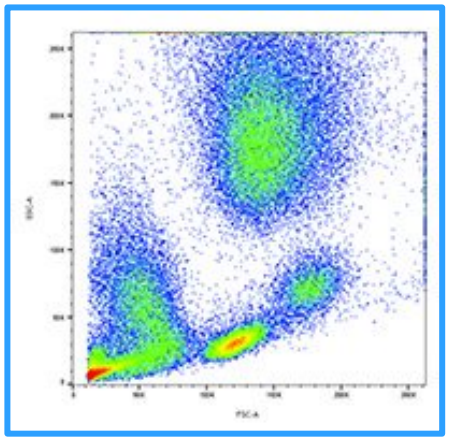
Techniques for measuring cellular kinetics and distribution

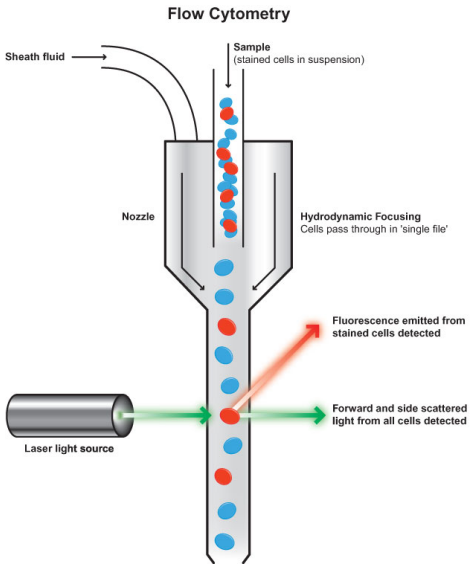
- qPCR





- Flow Cytometry





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13 Channels

2 HTS

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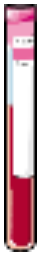
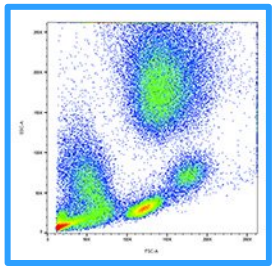
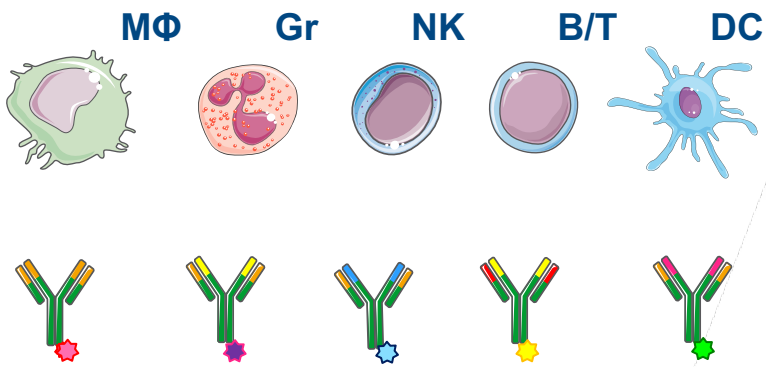
Flow Cytometry + Fluorochrome Conjugated MAbs to monitor Immune Cells subsets

Blood/Spleen: complex tissues composed by different immune cells directly/indirectly targeted by different therapeutics





Discrimination based on size and “complexity”



Additional layer of specificity with fluorochrome conjugated Mab

Cytoplasmic/Membrane proteins

Phenotypical Characterisation (Resting/Activation)

Concentration (Cells/mL)

Frequency (%)

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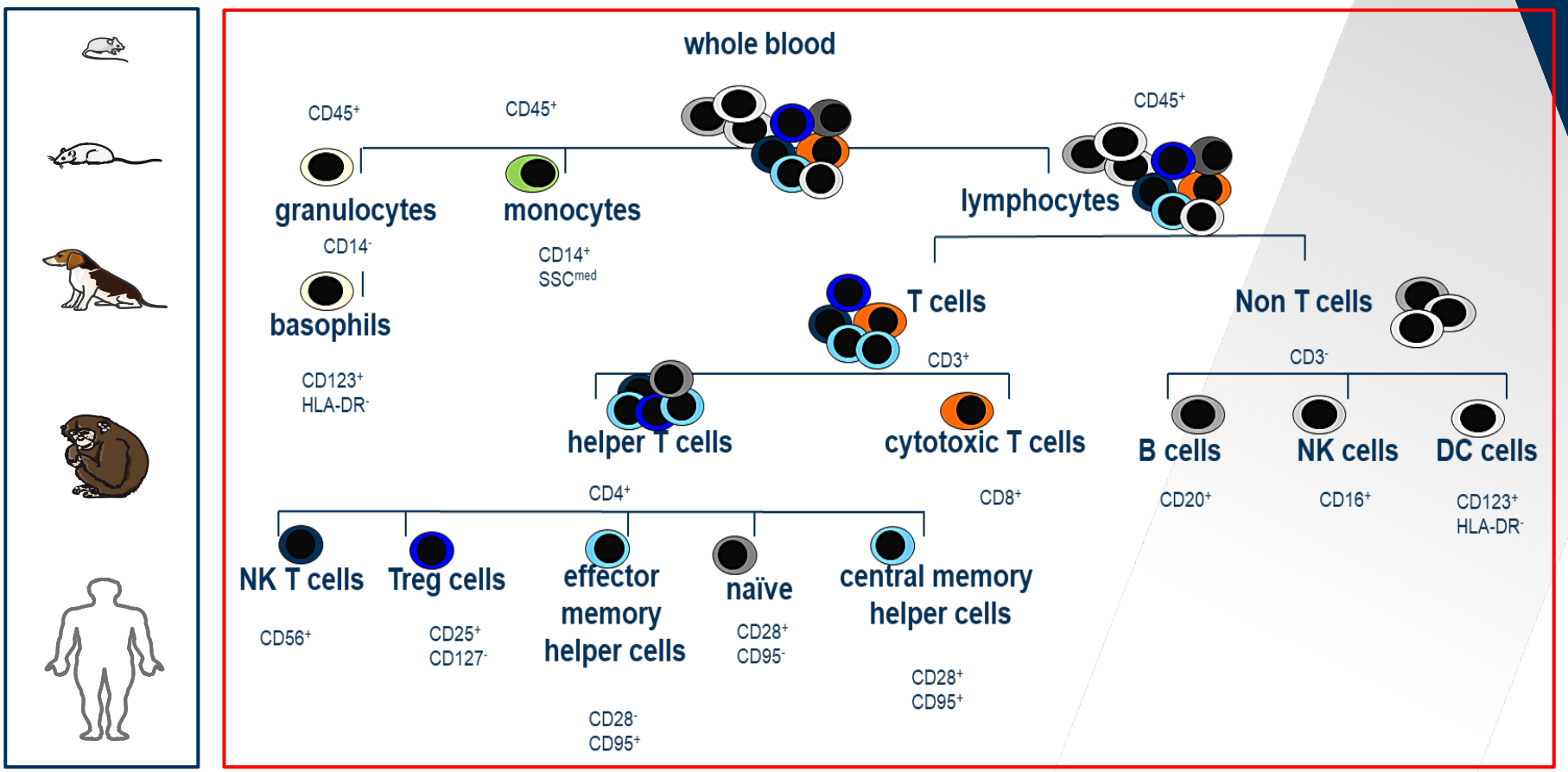
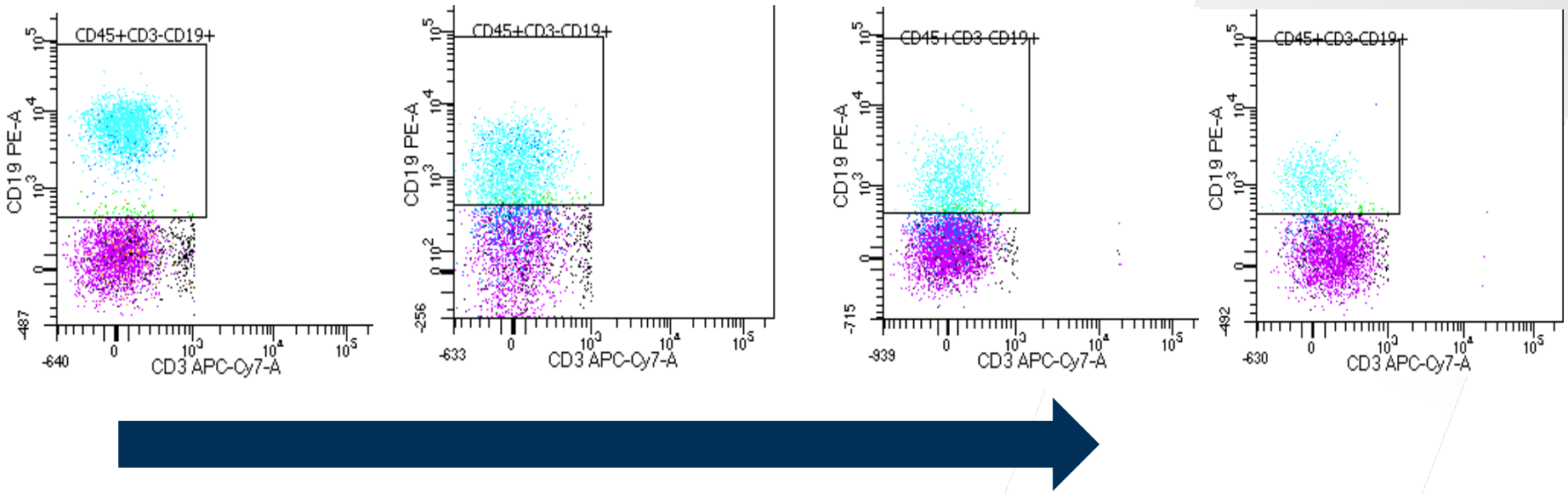
Phenotypical Characterisation of Immune Cells in a Broad Range of Animal Species



For cell based therapies flow cytometry can be used to monitor both exposure of dosed cells but also monitor biological effects > E.g. CAR-T efficacy involves complex interactions of multiple cell types

Example data for monitoring B-Cells (% Distribution)

CD19 expression used to define pre-B cell until the terminal differentiation to plasma cells



With time CD19 population depleted from peripheral blood (qualitative measurement)

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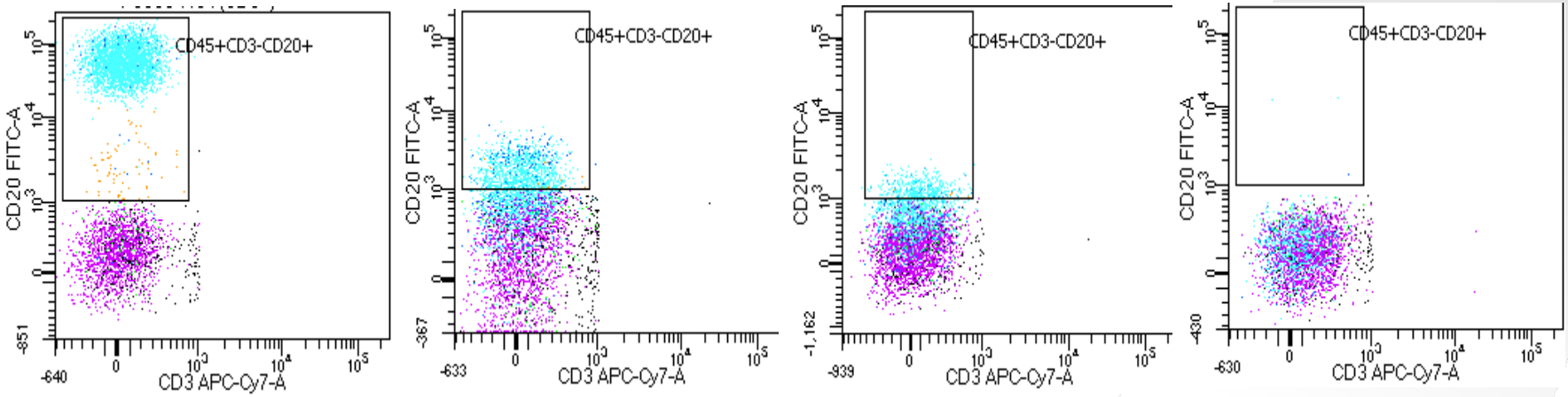
Example data for monitoring B-Cells (% Distribution)

CD20 expression used to define late pro-B cells through memory cells, but not on either early pro-B cells or plasma blasts



With time CD20 population depleted from peripheral blood

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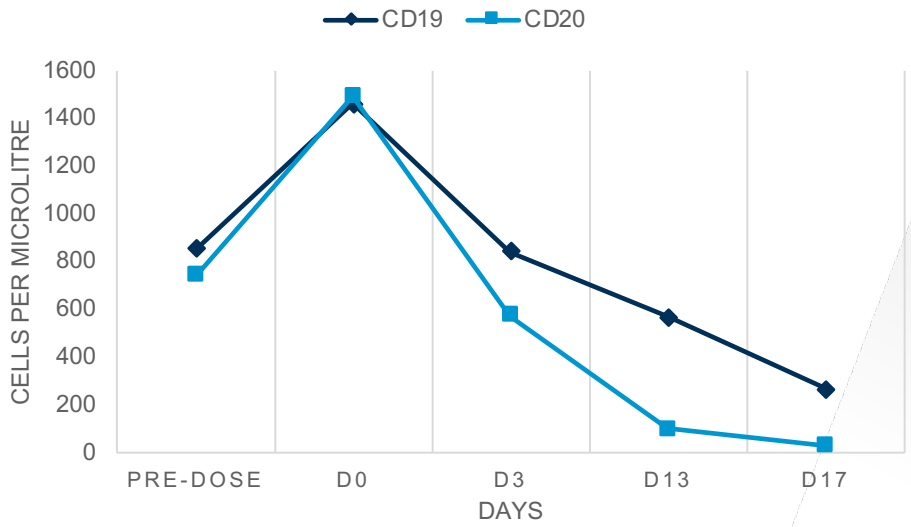
Example data for monitoring B-Cells (Concentration Cells/µL)



Absolute cell counting by flow cytometry

- Absolute counting is an application that allows flow cytometry scientists to quantify the total number of cells within tissue (e.g. can measure tumour infiltration by CAR-T cells)
- Use of distribution measurement as a sole read out versus absolute counts can lead to miss interpretation of mechanistic activity > CASE study (David Draper)

Methods for absolute cell counting

1. Dual platform method

   - Combine flow population data with a separate cell concentration determination on a hematology analyser (E.g. Advia)

2. Single platform method

   - Most common > Add internal microsphere counting standard to the flow cytometric sample
   - Volumetric cytometry platform
   - Microfluorimetry (image analysis)

Advantages/Disadvantages of different methods

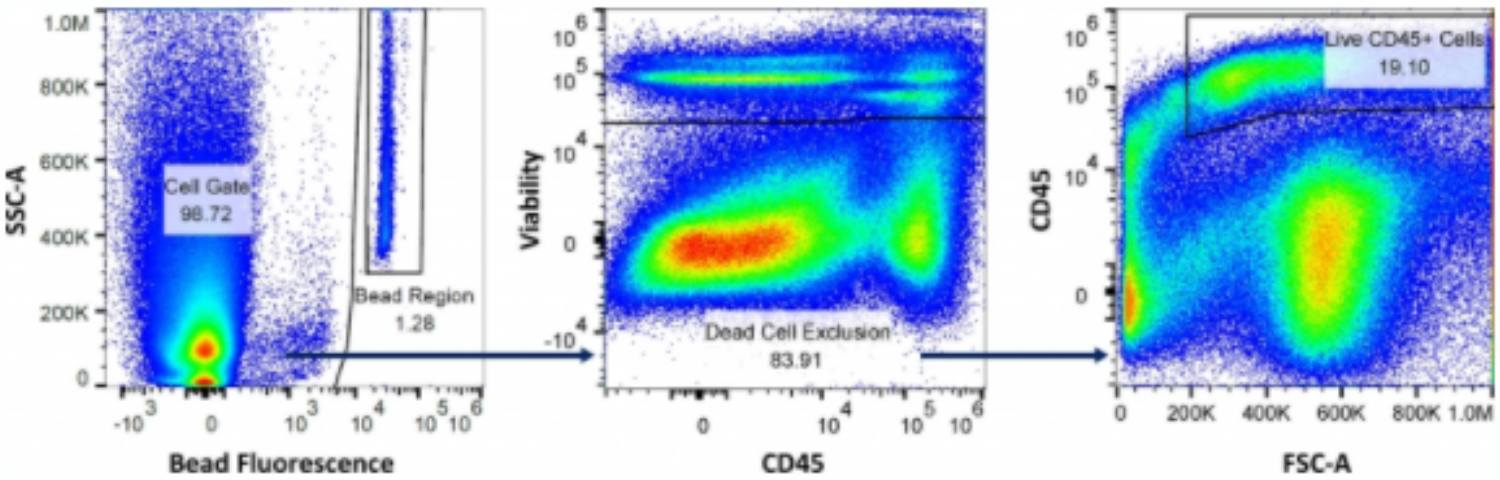
Advantage of Single assay Methods

- Reduced sample volume required
- Not reliant on two instruments to maintain and can perform everything required all on one system
- Haematology instruments are generally less sensitive
- Haematology system uses different method of counting compared to bead system on flow

Advantage of Dual assay Methods

- Count aligns with other Haematology parameters tested on a study

Absolute counting by beads



$$\frac{\text{Live CD45}^{+}\text{ Cells count}}{\text{Bead Region count}} \times \frac{\text{\# of beads/sample}}{\text{Volume of sample (µL)}} = \text{Absolute cell count/µL}$$

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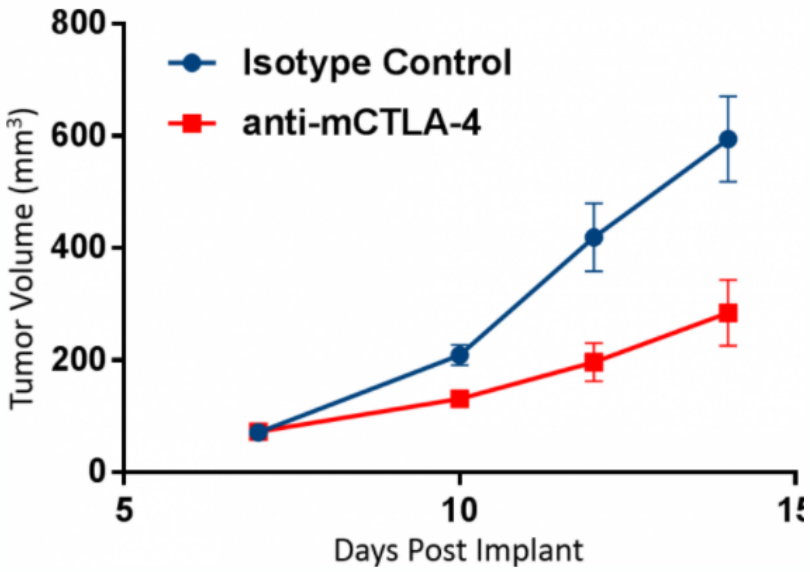
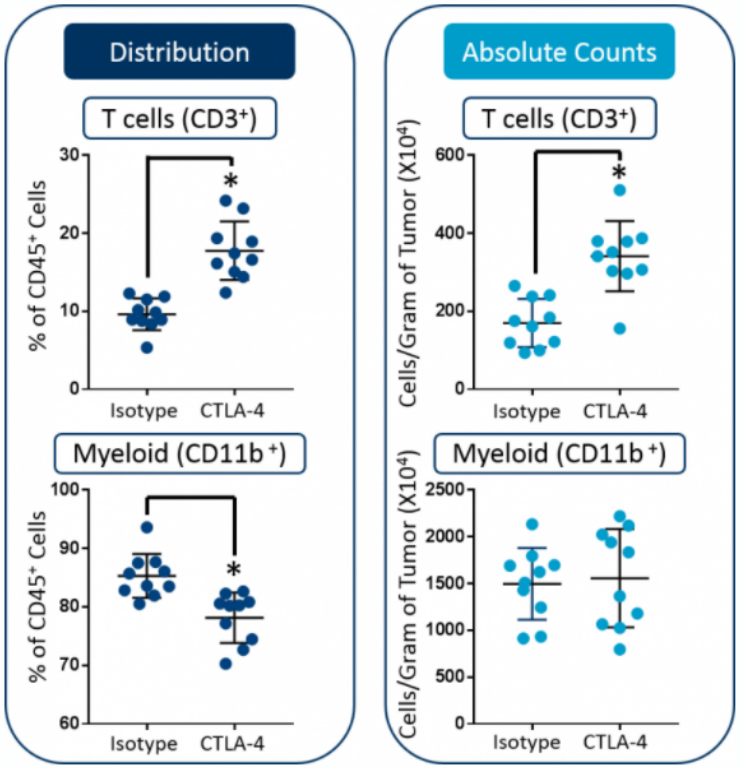
CT26 tumour bearing mice treated with anti-CTLA-4





- Using distribution analysis the interpreter might conclude from this data that CTLA-4 blockade triggered both an increase in the number of T cells and a simultaneous decrease in myeloid cells.
- From absolute counting this conclusion would be incorrect.

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Importance of defining the appropriate dose

Tegenero clinical trial Incident

Cytokine Release Syndrome is the most frequent serious adverse event after CAR-T cell therapy

News of incident that occurred in the UK in 2006 – Public Press

- Withdrawn from development after inducing severe inflammatory reactions in the first-in-human study
- 4 healthy volunteer patients suffered from severe cytokine storm response
- Drug acted in a different fashion in humans as compared with the laboratory animals that were tested and effect related to dose

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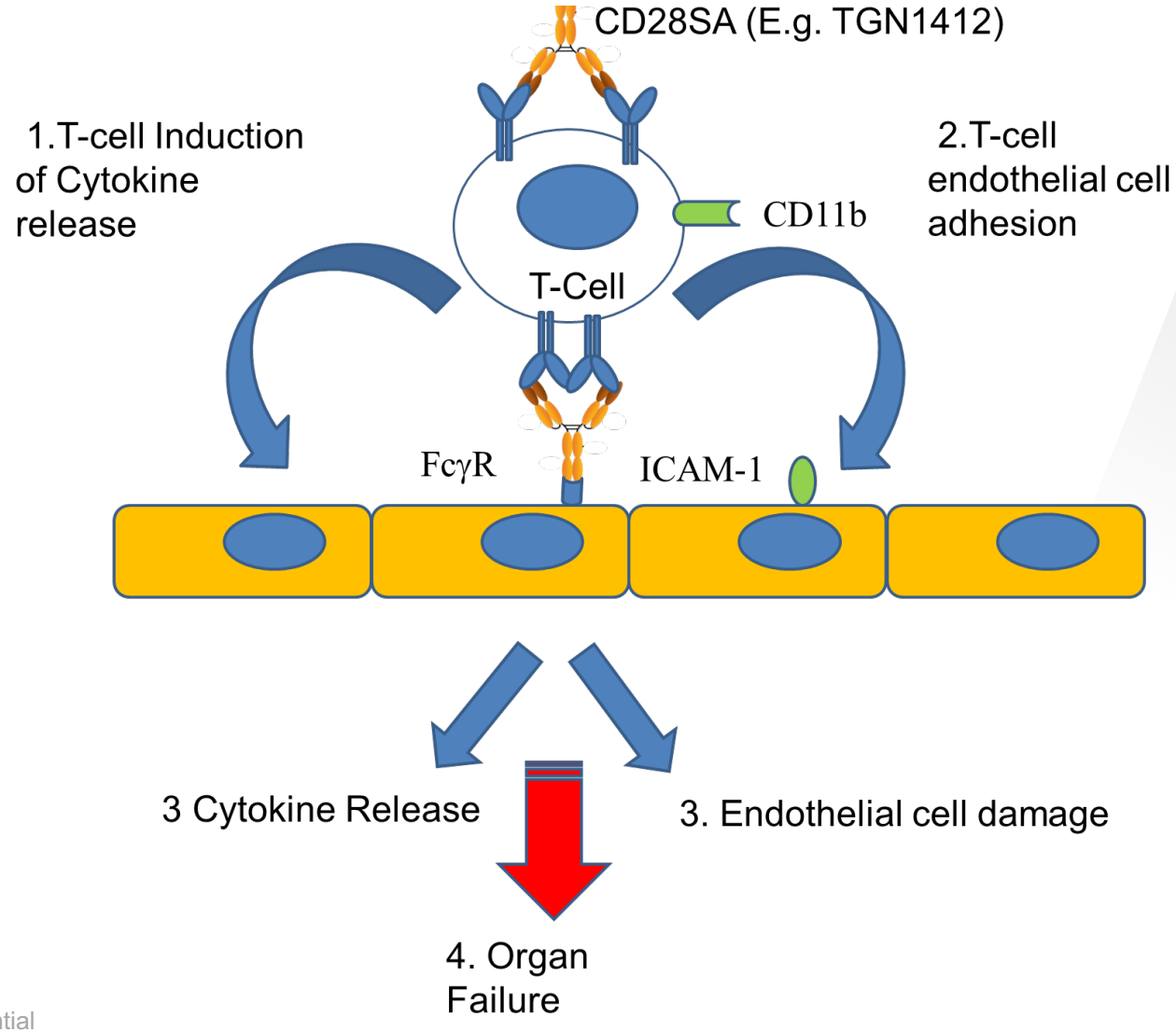
Cytokine release mediated by activation of immune cell receptor (E.g. CD28)



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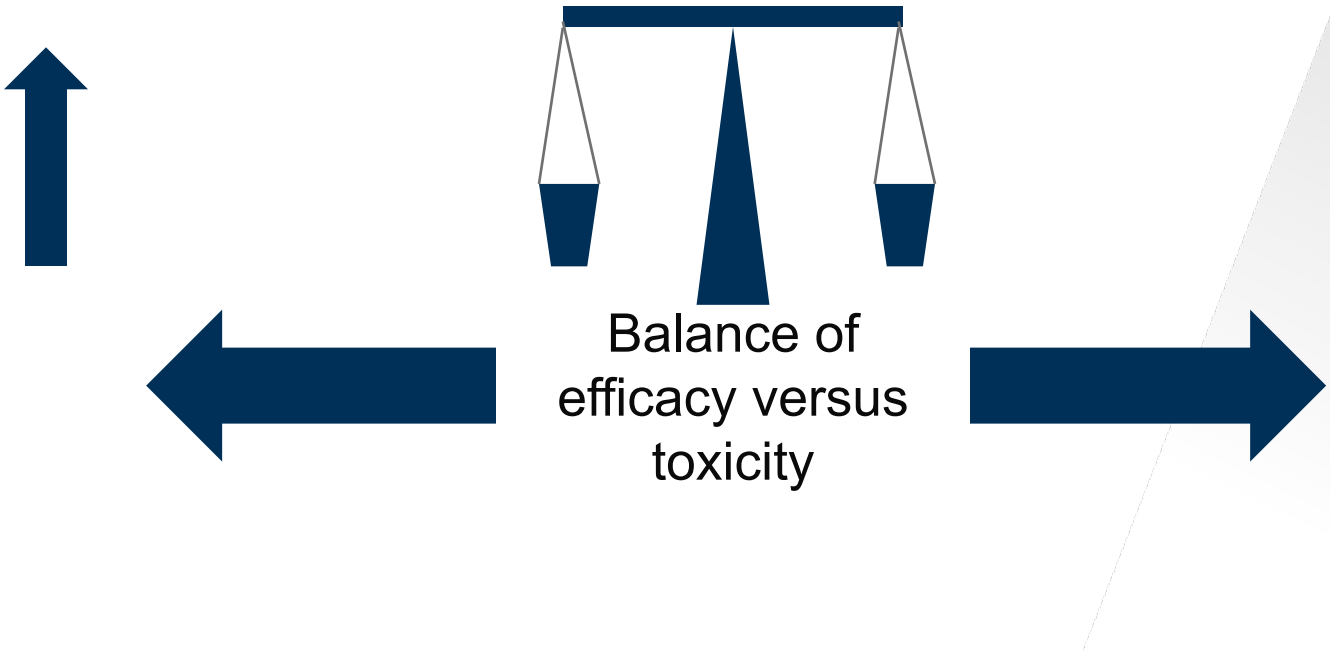
Appropriate Balance of dose for CAR-T

Highest cause for failure of CAR-T clinical trials is cytokine storm response

Too many CAR-T cells
Causing adverse effects



Too few CAR-T cells
Causing loss of efficacy

- Same challenge for CAR-T as was observed for Tegenero
- Flow cytometry and other bioanalytical techniques are powerful tools to address this

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Conclusions

- PK/PD is complex for cell and gene therapies and not performed using traditional bioanalytical techniques
- Industry is adapting to new challenges but limited regulatory guidance available due to complexity and range of possible approaches
- Flow Cytometry is a key bioanalytical tool that can be used to understand PK/PD when developing
- Percent distribution versus absolute cell count can lead to different interpretation of exposure results
- Defining the appropriate dose by understanding of PK/PD relationships for cell therapies is still a fundamental requirement for our industry but the means by which we do this have evolved

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Acknowledgements

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  - Andy Head
  - Matt Mckechnie

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