Is it Necessary to Understand the MoA?

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Introduction

Audience survey

- Is an understanding of the MoA:
  - A. A Nice to have?
  - B. Essential?

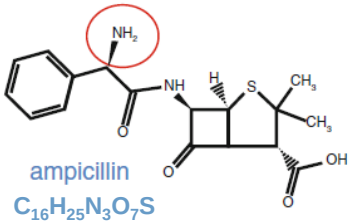
Biologicals are Complex

Grampp and Ramanan 2013 DOI 10.1007/s40259-013-0018-5

b Chemically synthesized drug

Defined process-structure-function

- Complete knowledge of chemistry and physics

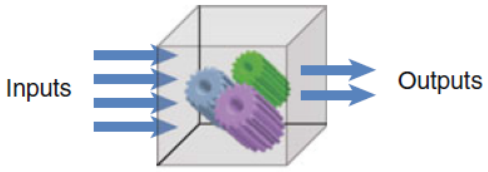


ampicillin

$C_{16}H_{25}N_3O_7S$

52 atoms

- Knowledge and measurement of all relevant inputs and outputs



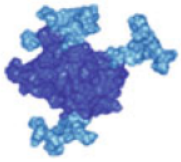
- One, defined active ingredient linked unambiguously via its identity to the safety and efficacy (S&E) profile

Chemical Process → Defined Structure → Function (Defined S&E)

c Biologic

Correlated process-structure-function

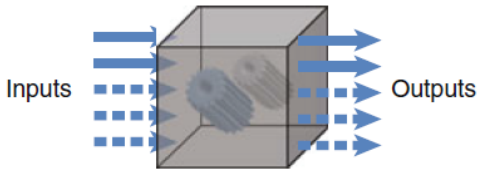
- Partial knowledge of biology and chemistry



Erythropoietin

>4000 atoms

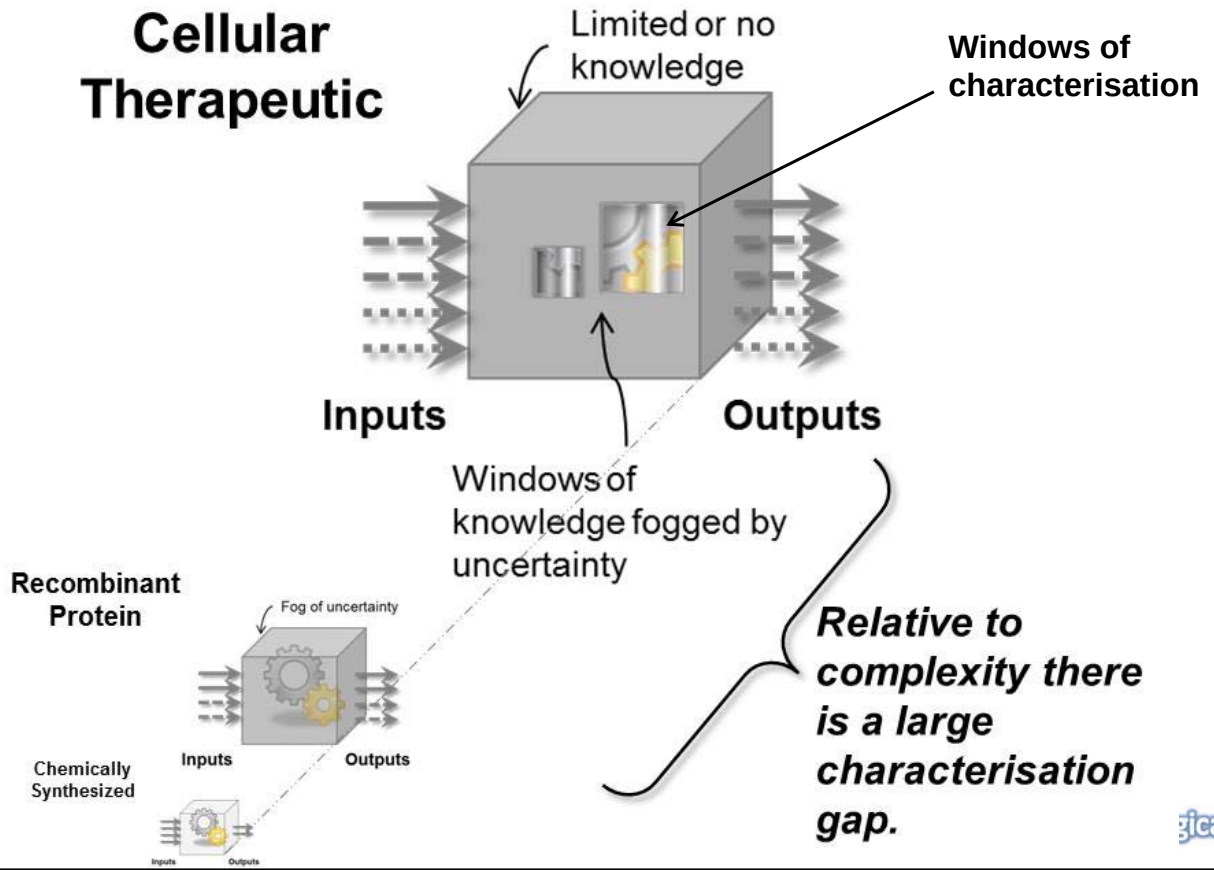
- Impossible to identify or measure all inputs and outputs



- Heterogeneous, partially defined active ingredient correlated to the safety and efficacy profile – contingent on process consistency

Biological Process → Complex Structure → Function

Cell Therapy Products even more so



Without a MoA how can you….?

Have a manufacturing strategy?

Have a characterisation strategy?

Other ramifications

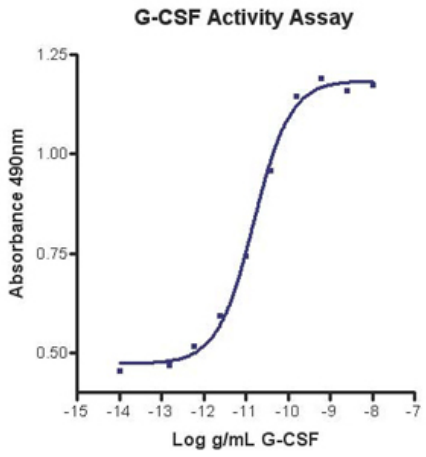
Comparability

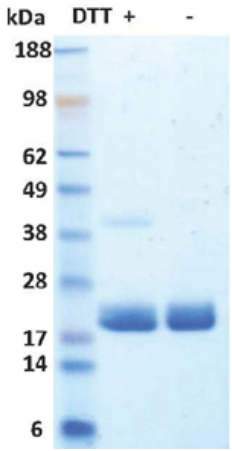
- How do you know the tests you are using to establish comparability are relevant?
- More so than other biologics, comparability is determined based on the totality off data (which is a limited data set)

Example of a recombinant protein

DS Specifcation tests for G-CSF (INN filgrastim)		
Test		**Analytical procedure**
Appearance		Visual inspection
pH		Potentiometry
Identity	Molecular size	Size exclusion chromatography (SEC)
	Hydrophobicity	Reversed phase chromatography
	Isoelectric Point	Isoelectric focusing
Purity		Size exclusion chromatography
Product-related Impurties	High MW variants	
	Product related substances and impurities	Reversed phase chromatography (RP-HPLC)
	Charged variants	Isoelectric focusing
Process-related Impurities	Bacterial endotoxins	LAL, kinetic chromogenic
	E. coli host cell proteins	ELISA
	Bioburden	Microbial limit test
	Residual DNA	Threshold method
Content	Assay	Reversed phase chromatography
Potency	Bioactivity	In vitro assay

Potency assays for proteins are usually quantitative



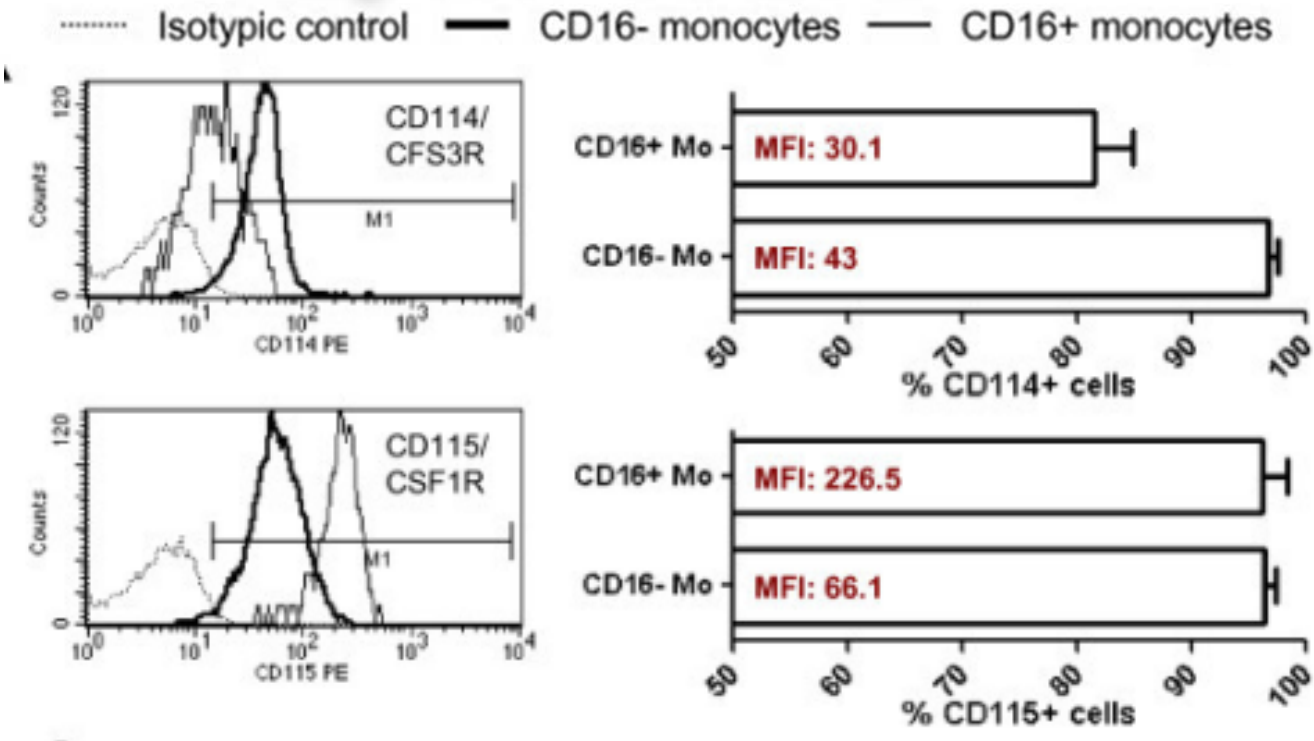


Cell therapeutics potency assays are generally not quantitative and even where the assay is quantitative/ semi-quantitative it is unclear whether the quantitation means anything.

Methods with sensitivity to change

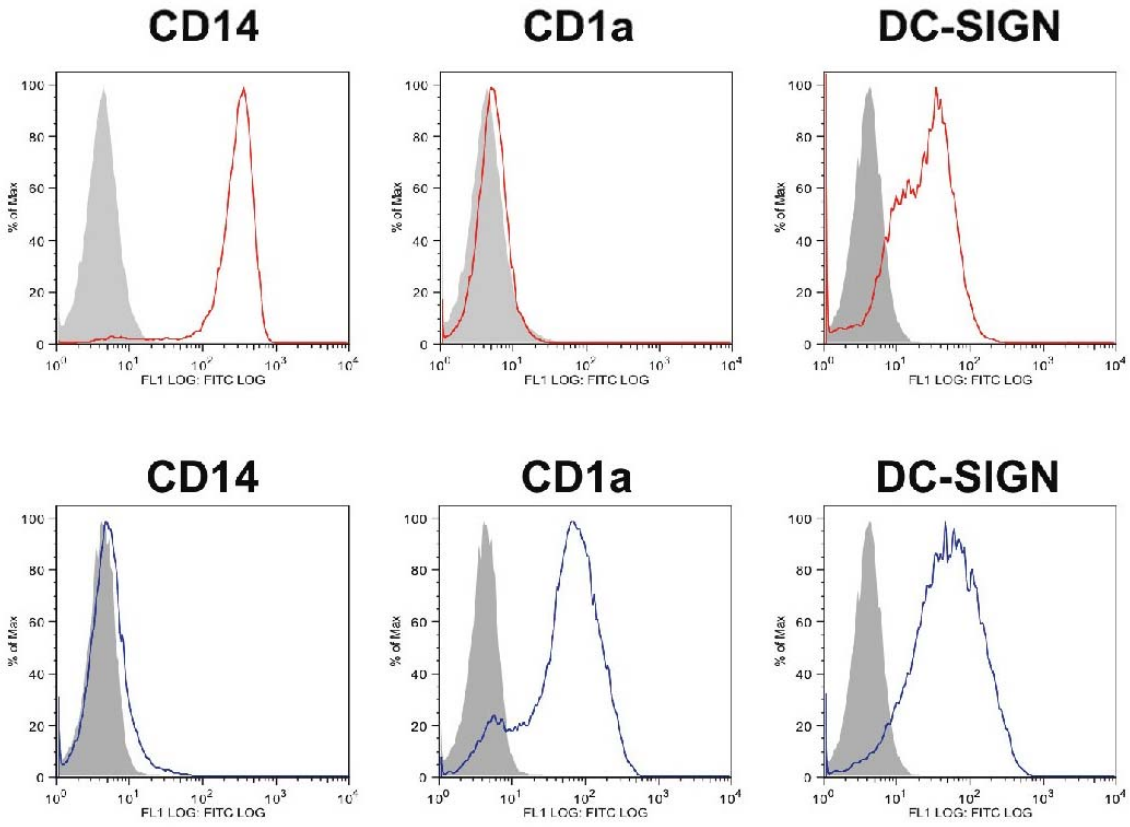
- Obviously need to be determined case-by-case
- Possible considerations
  - Fluorescent intensity (modulated proteins)
    - Maturation markers
    - Secreted factors
  - Early markers of cell death
    - Apoptosis (PI, annexin etc)

Consider whether Fluorescent Intensity might be more useful



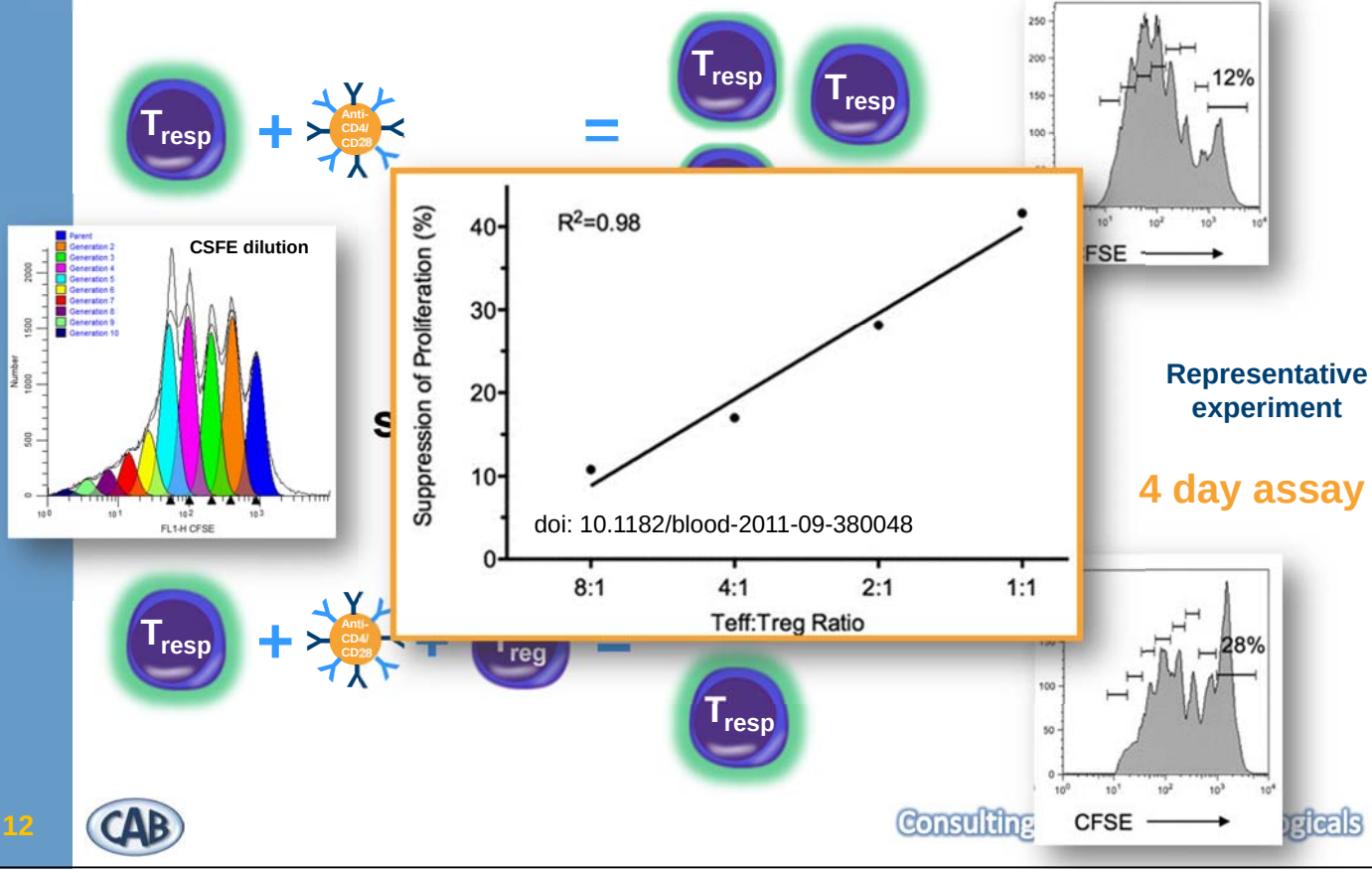
Will require appropriate reference materials to calibrate fluorescence intensity

Consider changes in Fluorescent Intensity



Example of Treg Potency Assay

doi: 10.1182/blood-2011-09-380048

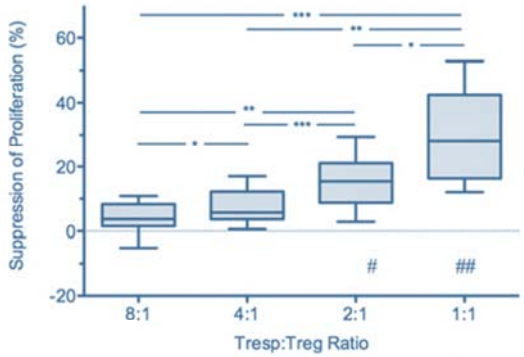


Rapid Treg Potency Assay

doi: 10.1182/blood-2011-09-380048

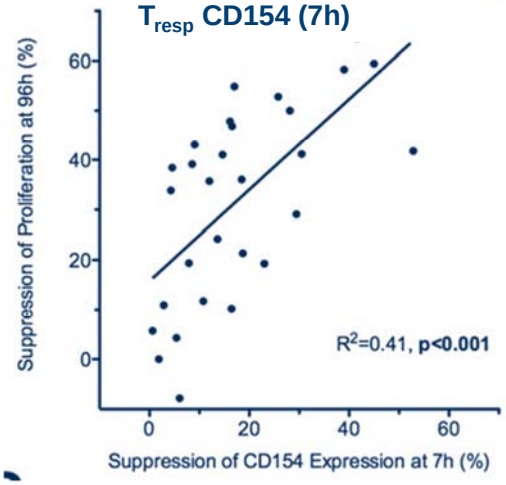
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

N=10

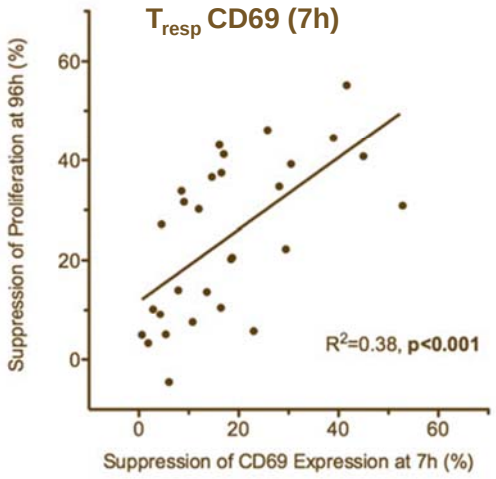


Suppressor Assay (96h)

T_{resp} CD154 (7h)



T_{resp} CD69 (7h)



Advantages of a more rapid method

- Earlier/more sensitive to change
- Useful for:
- Compatibility studies
- Stability studies
  - Shipping studies
  - In-use stability
  - Manufacturing hold step stability
  - Fill/freeze steps

Conclusions

- A working MoA is essential to direct characterisation and manufacturing strategy
- Important to remember selection of control criteria is based on the assumed MoA – therefore it might be wrong.
- You will likely NOT have identified all (or perhaps any) critical quality attributes (cell too complex); or they may not be as critical as you assume.
- Measures of biological function (e.g. potency) are more likely to be meaningful than phenotypic markers
- Potency assays even where quantitative are typically not sensitive (high variability)
- Comparability is therefore highly dependent on

Conclusions

- Comparability determination is therefore highly dependent on whether your understanding of the MoA is correct
- Many methods typically employed for comparability and stability are not sensitive to change
  - There is a need to consider this and look for methods sensitive to change.

Consulting on Advanced Biologicals