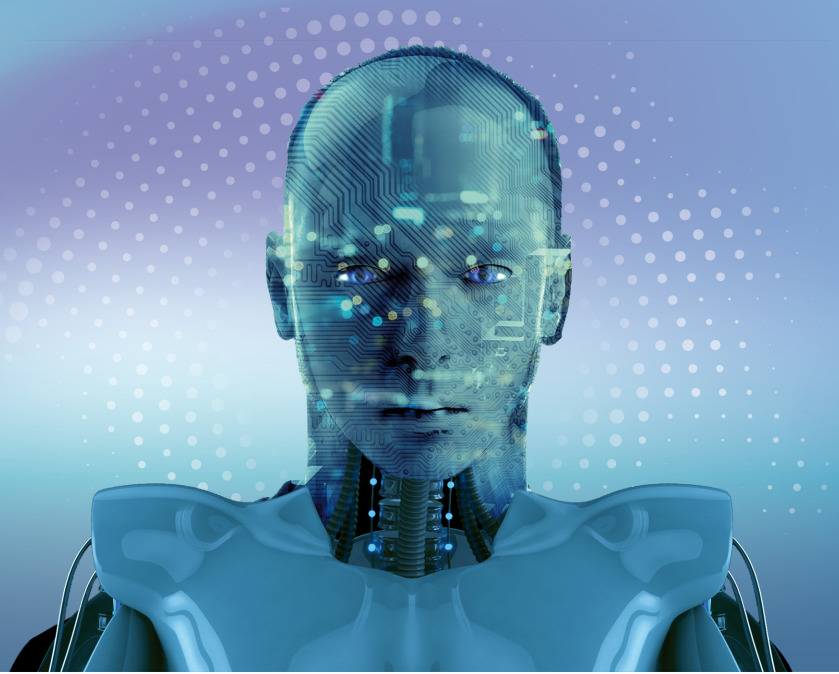
Cell therapy manufacturing process controls [presentation slides]

Cell Therapy Manufacturing Process Controls



Dr Jon Petzing

PDA Parenteral Drug Association

4th PDA Europe Annual Meeting

Global Healthcare of the Present and the Future

CONNECTING PEOPLE SCIENCE AND REGULATION®

Healthcare Engineering – Loughborough University

The Healthcare Engineering Research Group – Loughborough University
Cross disciplinary translational research group; design, manufacture and exploitation of current and next generation medical technologies.

Centre for Biological Engineering (CBE)
The University's state-of-the-art research Biomanufacturing facility.

2008/2018; DTC >>> 2014/2023 CDT in Regenerative Medicine
120+ PhD students with life science/engineering interface cutting-edge skills.

2014/2017/2022; UK Regenerative Medicine Platform 1 & 2
Pluripotent Stem Cell Platform (PSCP) - UKRMP Cell Behaviour, Differentiation and Manufacturing Hub

2005/2010; RM Grand Challenge >>> 2010/2016; EPSRC CIM in Regenerative Medicine
Defining Regenerative Medicine manufacturing technologies and scale up

2017/2023; Future Targeted Healthcare Manufacturing Hub
UCL led EPSRC Manufacturing Hub, LU contributing manufacturing tech. and scale up expertise.

2012 >>> 2018; E-TERM, UNIFY, UNIFY Plus
Fellowships and Network Development

A multidisciplinary team of:

Biologists,
Bioscientists,
Chemical Engineers,
Electronics Engineers,
Manufacturing Engineers,
Mechanical Engineers,
Pharmacologists,
Physicists…….

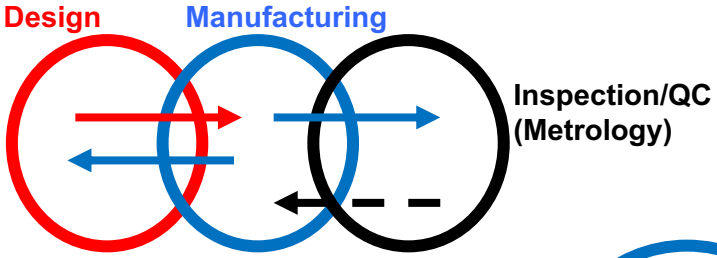
Loughborough University

Biometrology for Biomanufacturing

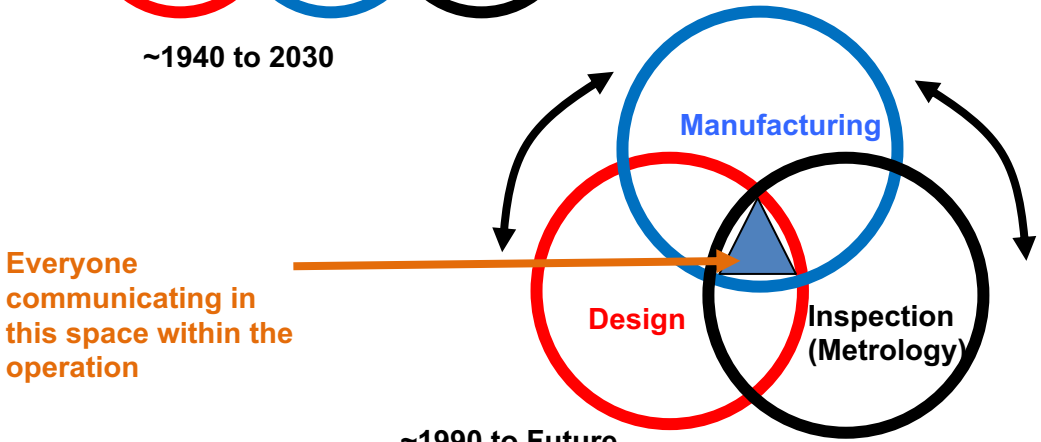
What are we trying to achieve ?

- Manufacturing and Biomanufacturing processes (centralised or distributed manufacture) require Process Control – and this Process Control requires measurement data input.
- Metrology is the Science of Measurement – it is about providing measurement data with high levels of confidence.
- By providing better measurement data, with definitions of measurement confidence, then the following benefits will be enabled:
    - Better biomanufacturing Process Control
    - Better enable GMP compliance
    - Better success rate of cell therapy batch biomanufacture
    - Better therapeutic outcome for the patient

Conventional Manufacturing Organization Models

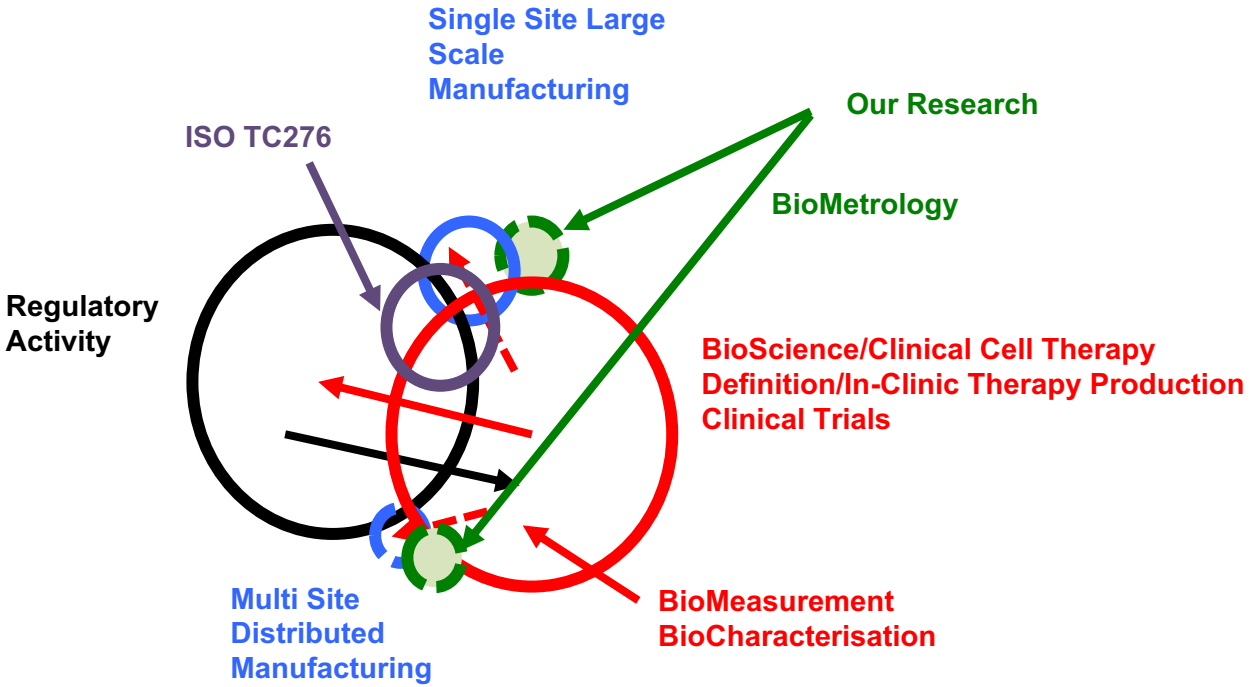


~1940 to 2030

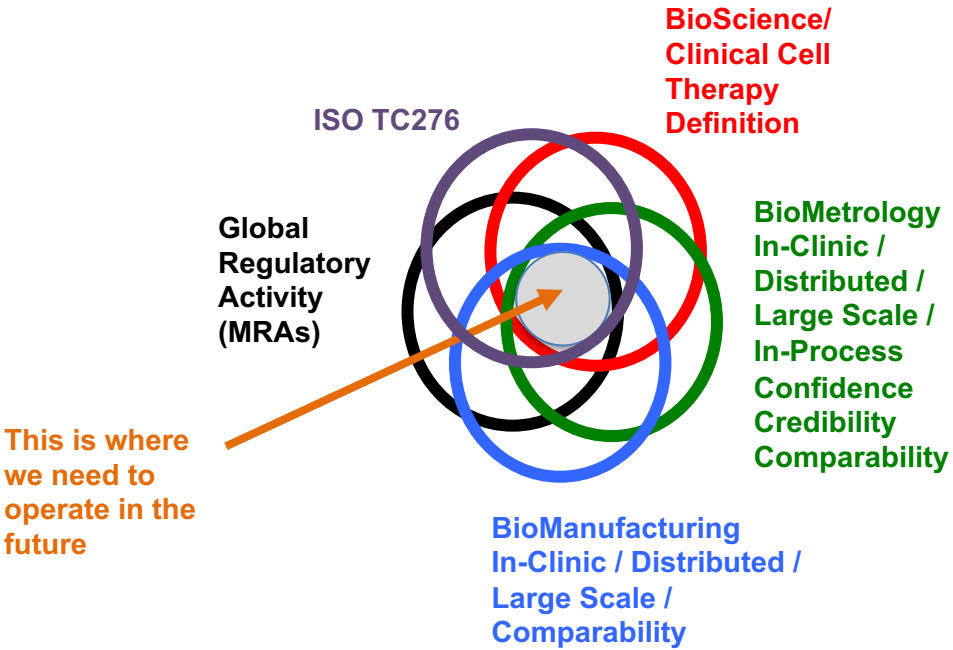


~1990 to Future

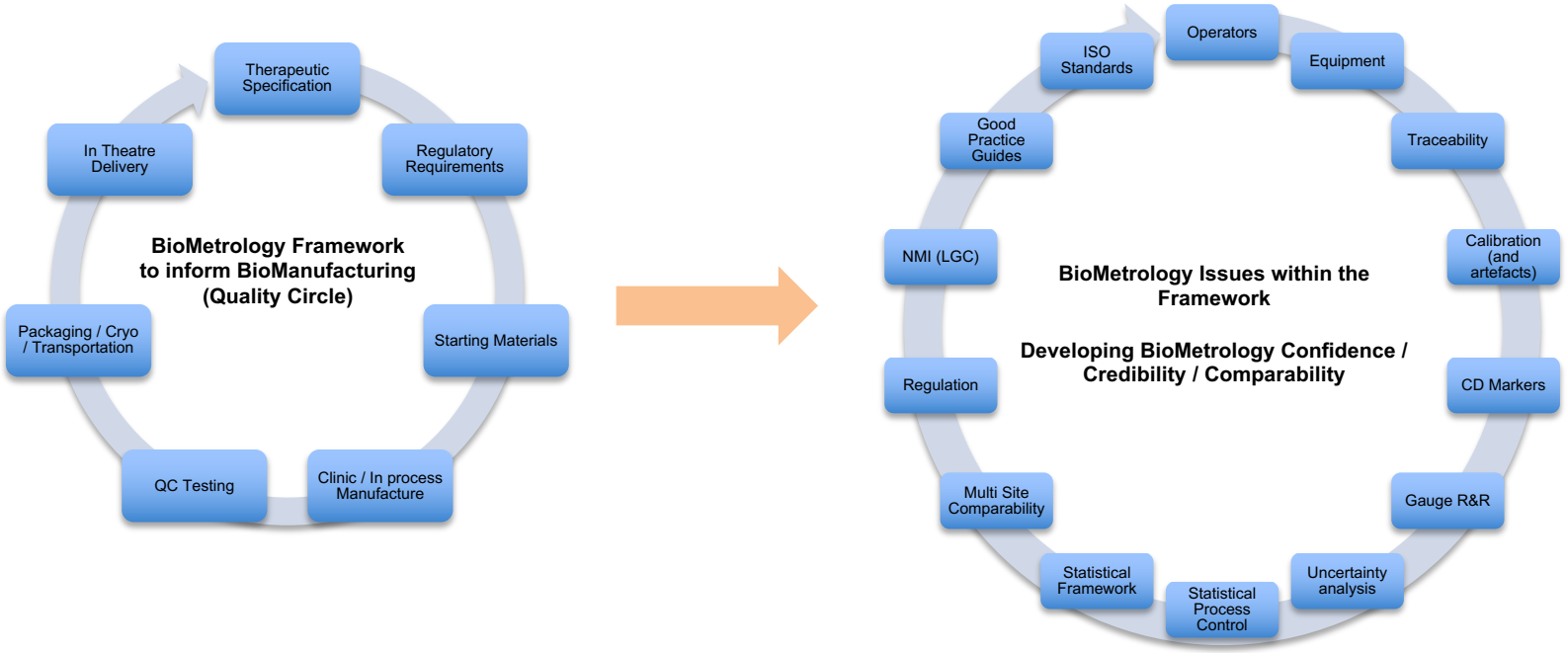
Biomanufacturing Organization Model – Current ?



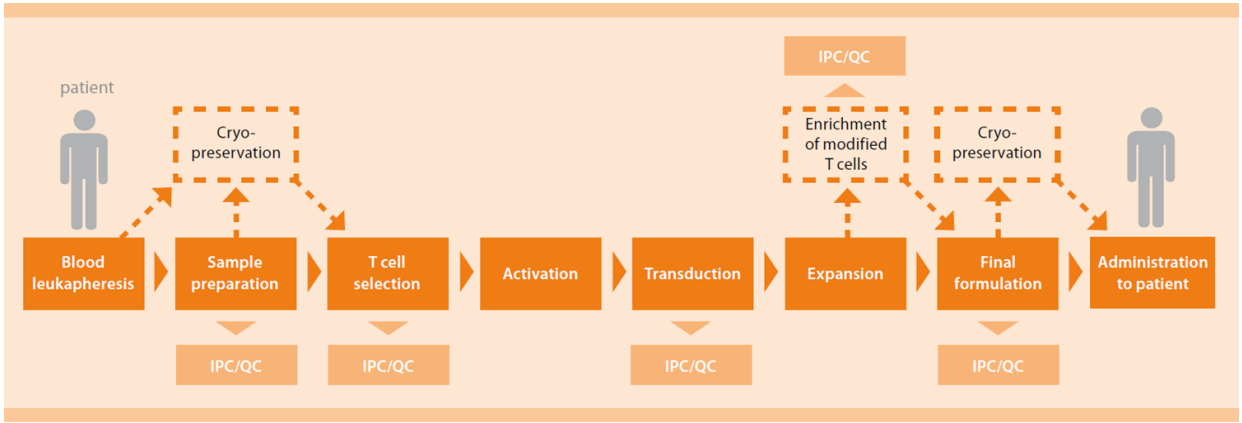
Biomanufacturing Organization Model – Future?



Biometrology Framework – informing Biomanufacture



Biometrology within the Biomanufacture process



Biometrology is applied at multiple points

Case Studies to investigate Biometrology / Biomanufacture

Case Study 1: Understanding variation in Cell Therapy Starting Materials
(Dr Jamie Thurman-Newell, Prof David Williams)

Case Study 2: Defining a healthy population starting material benchmark
(Dr Jamie Thurman-Newell, Rebecca Grant, Prof David Williams)

Case Study 3: Investigating Flow Cytometer Operator variation
(Rebecca Grant, Dr Karen Coopman)

Case Study 4: Investigating Flow Cytometer automated software platforms
(Melissa Cheung, Prof Rob Thomas)

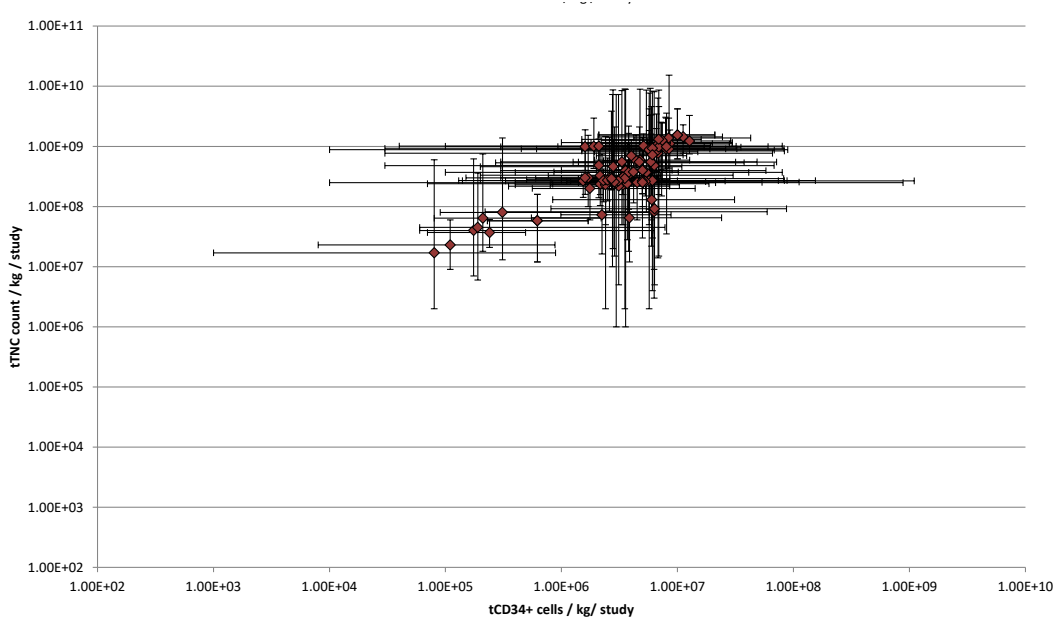
Case Study 1: Understanding variation in Cell Therapy Starting Materials

Justification for Case Study 1 (Dr Jamie Thurman-Newell – 2012/2017):

- Community awareness that Starting Material volumes may vary in cell count
- Recognition that manufacturing processes need to manage variation
- Existing Process Control paradigms and algorithms assume Normal Distribution of data
- No independent measure of variation in Starting Materials
- Requirement to map Starting Material variation to inform Process Control design
- Minimally manipulated Haematopoietic Stem Cell Transplantation (HSCT) used as the exemplar

(Dr Jamie Thurman-Newell, Dr Jon Petzing, Prof David Williams)

Case Study 1: Variation Meta-Analysis of Literature

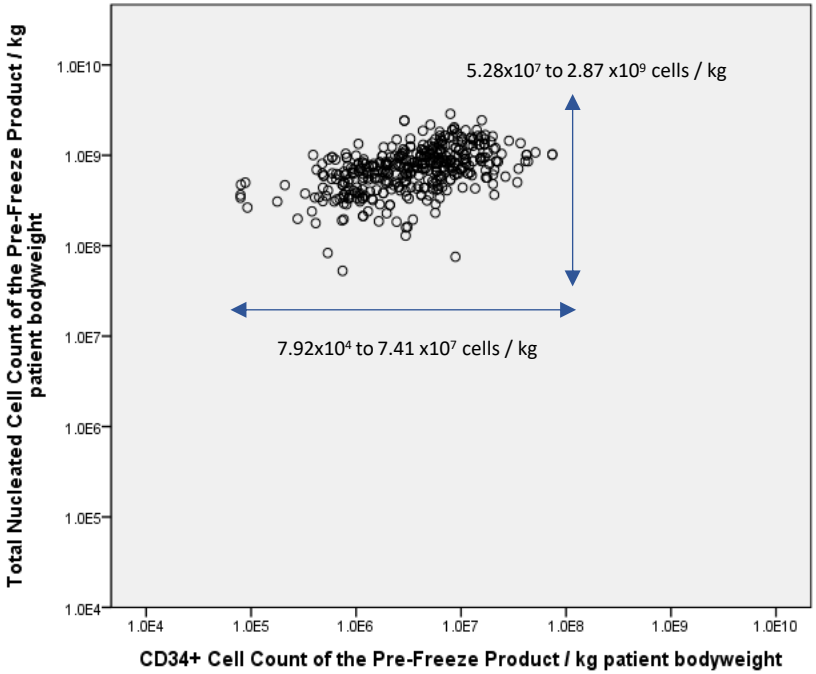


- Analysis of HSCT published studies from 1992 to 2016
- 269 studies with published data sufficient to map variation
- Autologous trials: 6.00×10^4 to 3.00×10^8 CD34+ cells / kg
- Allogeniec trials: 1.00×10^3 to 1.21×10^9 CD34+ cells / kg
- Between 4 and 6 orders of magnitude variation

J A Thurman-Newell, J Petzing, & D Williams, Quantification of biological variation in blood-based therapies: A summary of a meta-analysis to inform manufacturing in the clinic, Vox Sanguinis, 109(4), 394-402, 2015

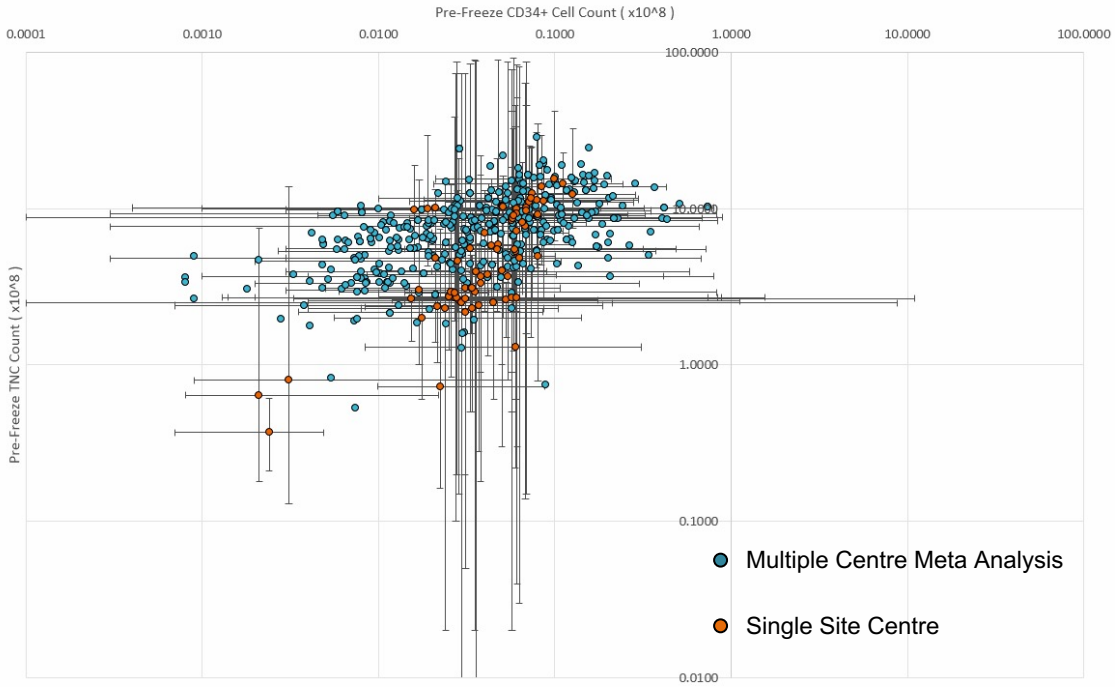
J A Thurman-Newell, J Petzing, & D Williams, A Meta-analysis of biological variation in blood-based therapy as a precursor to biomanufacturing, Cytotherapy, 18, 686-694, 2016

Case Study 1: Variation within a Single Site Clinical Centre



- Single site Clinical Centre patient data for 2015
- 440 patient records with data sufficient to map variation
- Ethical clearance obtained
- Autologous procedures: $4.8x10^6$ to $4.14x10^9$ CD34+ cells / kg
- Allogeneic procedures: $4.0x10^7$ to $1.82x10^9$ CD34+ cells / kg
- Between 2 and 3 orders of magnitude variation

Case Study 1: Comparison of Variation for Sick Populations



- Analysis of HSCT published studies from 1992 to 2016
- Between 4 and 6 orders of magnitude variation
- Single site Clinical Centre patient data for 2015
- Between 2 and 3 orders of magnitude variation
- Positively skewed non-parametric data distributions
- Autologous and allogeneic analysis

Case Study 1: Conclusions

- Orders of magnitude variation in HSCT Starting Materials
- Can Biomanufacturing processes accommodate this variation ?
- Should Biomanufacturing processes control this variation ?
- Positively skewed distributions do not fit traditional manufacturing Process Control paradigms
- Outliers are patients – and cannot be ignored
- Limited (or no) biomanufacturing specifications to relate to from a Process Control viewpoint
- Biometrology is a cause of variation in Starting Materials (and other elements of the process)
- A healthy population variation benchmark is required

Case Study 2: Defining a healthy population starting material benchmarks

Justification for Case Study 2 (Dr Jamie Thurman-Newell – 2012/2017):

- Clear evidence of variation of HSCT Starting Materials in the public body of knowledge
- Variation seen for Autologous and Allogeneic sources
- Single site clinical centre with improved variation of Starting Materials – but still 2 to 3 orders
- Significantly positively skewed data distributions
- Requirement to benchmark Starting Material variation from a nominally healthy population

(Dr Jamie Thurman-Newell, Dr Jon Petzing, Prof David Williams)

Case Study 2: Biobank Variation – Core Metrics

		Valid N	Minimum	Maximum	Mean	Median
Unique ID		502,664	-	-	-	-
Gender	Male	229,182	-	-	-	-
	Female	273,467	-	-	-	-
Birth Year		502,649	1934	1971	1952	1950
Age		502,649	45	82	64.46	66.00
Weight (kg)		499,874	30.0	197.7	78.1	76.4
BMI		499,543	12.12	74.68	27.43	26.74

- >500,000 recruited individuals
- Donors sampled between 2006 and 2010
- 22 UK collection centres
- Nominally healthy population
- TNC and CD34+ count not available
- White Blood Cell (WBC) count used as an analogue

Case Study 2: Conclusions

- White Blood Cell (WBC) count chosen as an analogue to CD34+ with a statistically robust data set
- WBC count can be between 7.00 x 10^7 cells/litre and 3.90 x 10^{11} cells/litre
- Variation of up to 4 orders of magnitude
- Skewed distributions with significant data ‘outliers’
- Gender appears to impact the amount of variation
- Increasing age shows a trend towards increasing variation
- Increasing weight shows a trend towards decreasing variation
- UK Biobank Assessment Centre (analogous of geographical area) appears to have a statistically significant effect on WBC counts

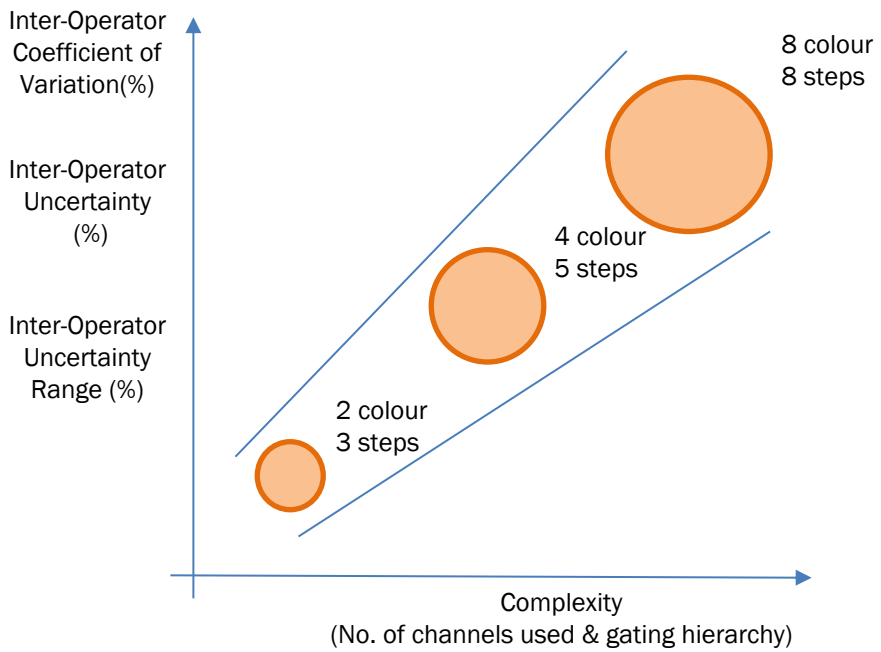
Case Study 3: Investigating Flow Cytometer Operator variation

Justification for Case Study 3 (Rebecca Grant– 2015/2019):

- Biometrology (Flow Cytometry) variation is a key component of Starting Material variation
- Community awareness that Flow Cytometer manual analysis may vary in cell count
- Single site clinical centre with observed Flow Cytometer data analysis variation
- Recognition that biomanufacturing processes need measurement data with high confidence
- Requirement to map Flow Cytometer operator subjectivity and manual data analysis variation
- Define operator Uncertainty budgets for Flow Cytometer gating studies

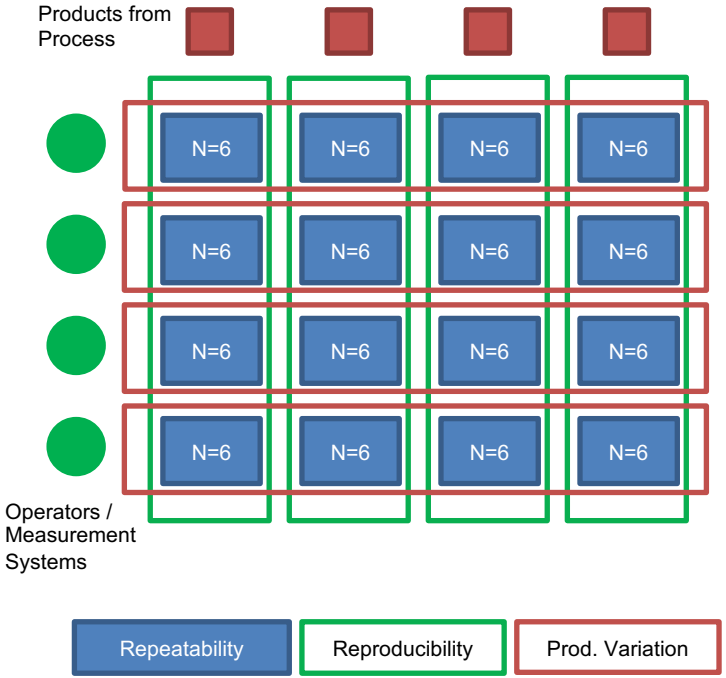
(Rebecca Grant, Dr Karen Coopman, Dr Jon Petzing)

Case Study 3: Working Hypothesis



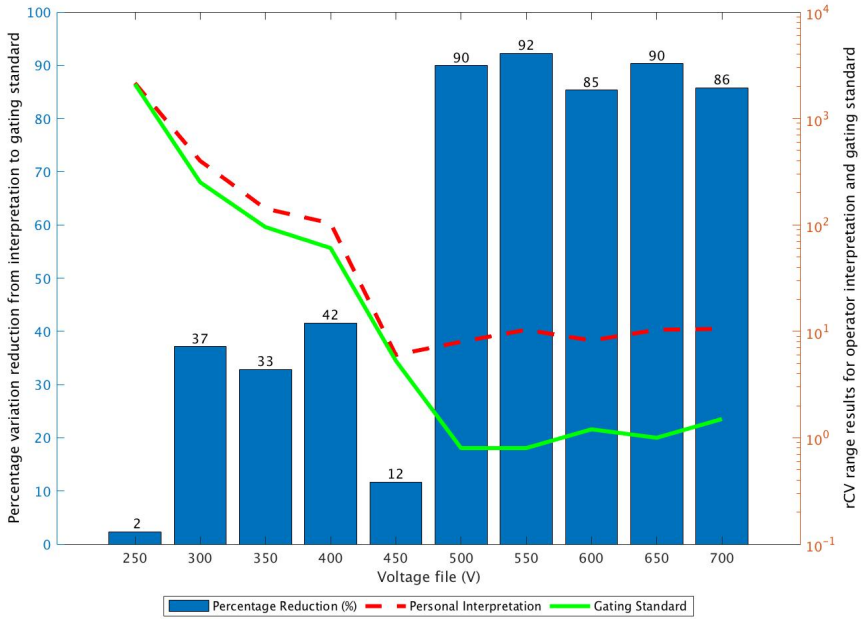
- Working hypothesis: Data analysis variation will increase, with increasing analysis complexity
- Coefficient of Variation to be measured
- Uncertainty budgets to be developed across gating steps
- Multiple operators across 3 sites (GSK, LGC, LU)
- Qualitative and quantitative evaluation of operator performance
- Ethical clearance obtained

Case Study 3: Application of GR&R Techniques



- Gauge Repeatability & Reproducibility (GR&R) framework used to define analysis
- Flow Panels defined by GSK and LGC
- Quadrant Gating strategies defined by GSK and LGC
- EC 2102 Ep, PBMC, and CAR T cells used for cell models of increasing complexity

Case Study 3: Simple Histogram Gating variation

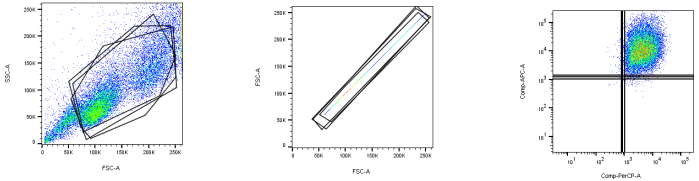


- Initial simple histogram based gating study completed
- Calibration beads used for data source
- Phase 1 (operator initiative) showed significant variation
- Phase 2 (operator protocols) showed significantly reduced variation
- Defined procedures for quadrant gating of cell models

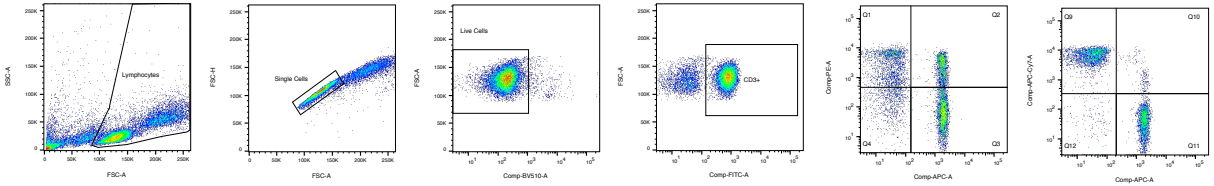
R Grant, K Coopman, N Medcalf, S Silva-Gomes, J J Campbell, B Kara, J Braybrook, J Petzing, Understanding the contribution of operator variability within flow cytometry data analysis for quality control of cell and gene therapy manufacturing, In-Review – Journal of Measurement, May 2019

Case Study 3: Simple to complex cell model quadrant gating

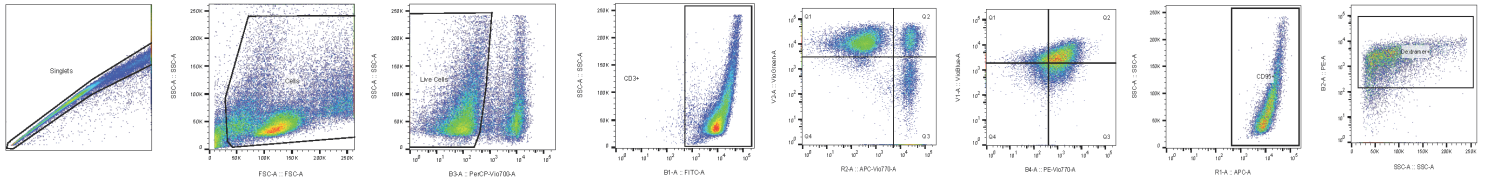
Embryonal Carcinoma (EC) 2102Ep cell line (2017) analysis – in publication review



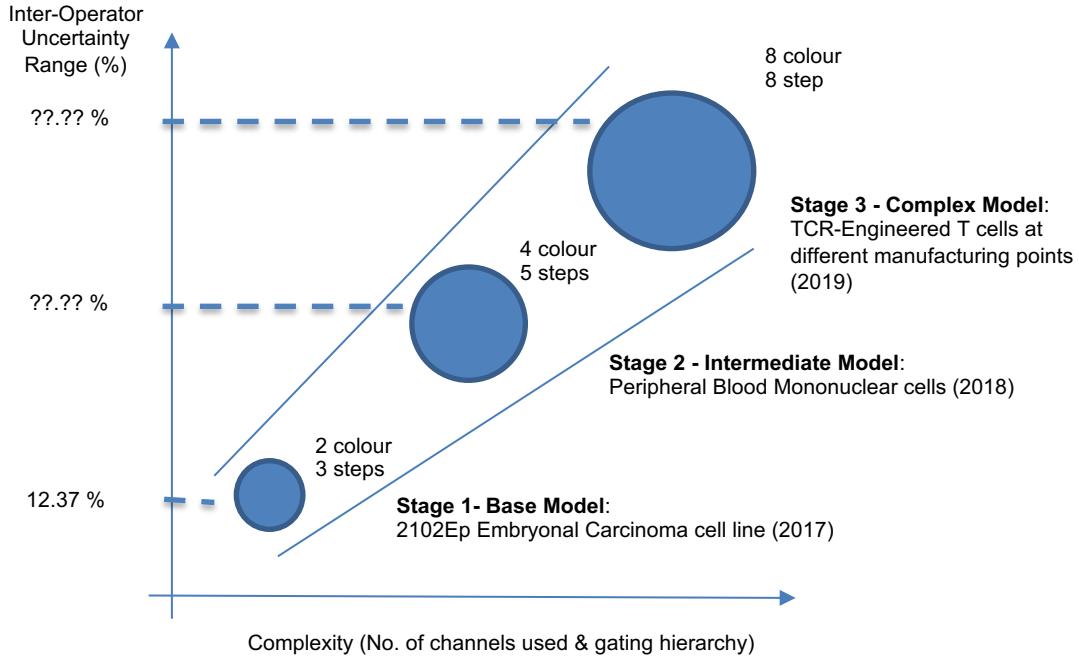
Peripheral Blood Mononuclear Cell (PBMC) cells (2018) – in data integrity checking and publication formulation



TCR-Engineered T cells (2019) – in data analysis stage



Case Study 3: Flow Cytometer Operator variation confirmed



- Absolute cell count, CV, and combined Uncertainty evaluated for all 3 Stages
- Variation observed at all Stages
- Variation observed to increase with cell model complexity
- Introduction of protocols seen to reduce variation
- Stage 2 data undergoing data integrity processes and pre-publication
- Stage 3 data being analyzed

Case Study 3: Conclusions

- 3 Stages of increasing cell model complexity considered
- 30+ operators involved at 3 sites completing manual analysis of Flow Cytometer data
- Increasing variation (cell count, CV, Uncertainty) seen as cell model complexity increases
- Application of protocols seen to reduce analysis variation
- Gauge Repeatability & Reproducibility (GR&R) framework shown to work
- Absolute cell count, and, Coefficient of Variation defined for each Stage / operator
- Individual Gate Uncertainty, and combined Uncertainty (*k=1*) defined for each Stage / operator

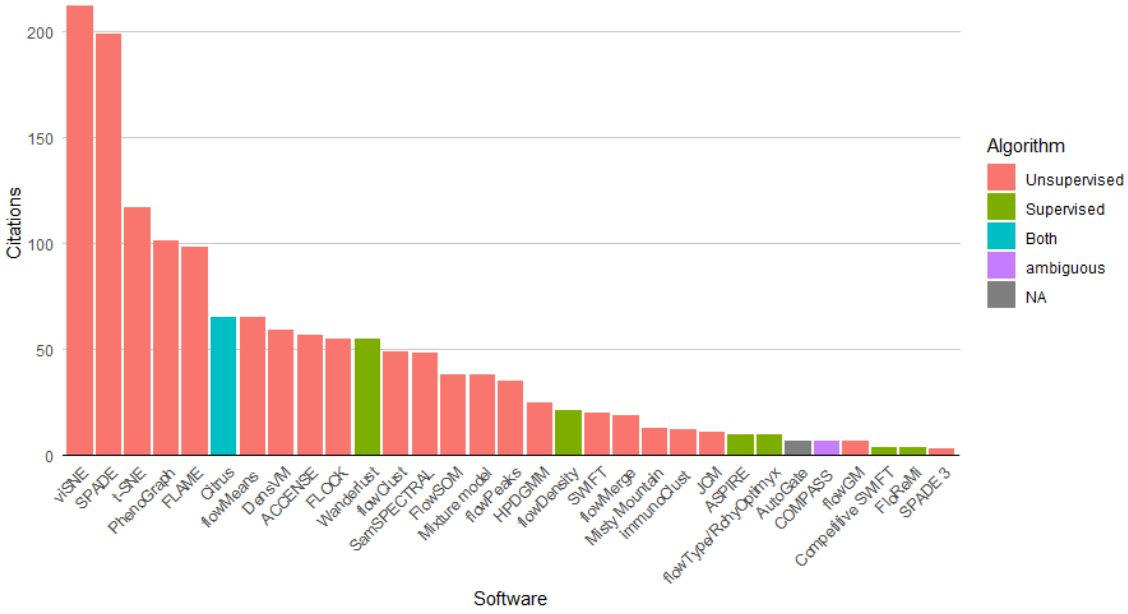
Case Study 4: Investigating FC automated software platforms

Justification for Case Study 4 (Melissa Cheung– 2017/2021):

- Community belief that automated platforms may be more reliable than manual FC analysis
- Desire to integrate automated FC platforms into biomanufacturing Process Control
- Multiple Flow Cytometer automated software platforms available
- Limited standardisation of algorithms and performance factors
- Limited independent review of biomanufacturing integrity of platforms
- Need for definitions of data/platform confidence to inform biomanufacturing Process Control

(Melissa Cheung, Prof Rob Thomas, Dr Jon Petzing)

Case Study 4: Investigating FC automated software platforms



- Multiple platforms to be assessed
- Different platform algorithms
- Synthetic data testing
- Synthetic data design
- Cell model data testing
- Multiple cluster testing
- Rare cell event testing
- Accuracy and repeatability analysis
- Work in progress to be reported during 2019/2020/2021

Take Home Messages

- This work is investigating and developing biometrology in the context of biomanufacturing
- Specifically aiming to define levels of confidence in biometrology processes and data analysis
- Allowing biomanufacturing Process Control to benefit from better quality input
- HSCT cell therapy Starting Materials have previously varied by upto 6 orders of magnitude
- Single site therapy centres have reduced Starting Material variation to 3 orders of magnitude
- Flow Cytometer operator variation for manual data analysis varies as a function of complexity
- Flow Cytometer operator variation can be improved through careful protocol definitions
- Flow Cytometer automated platforms are currently the focus of our attention – work in progress

Acknowledgements

Loughborough University: Rebecca Grant, Melissa Cheung, Dr Jamie Thurman-Newell, Dr Karen Coopman, Prof Rob Thomas, Prof David Williams

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- EPSRC/MRC Doctoral Training Centre for Regenerative Medicine at Loughborough University (EP/L105072/1)
- EPSRC Centre for Innovative Manufacturing in Regenerative Medicine (EP/H028277/1)
- LGC
- GlaxoSmithKline
- Dana Farber Cancer Institute (Boston, USA)
- UK Biobank (Application No. 4047)
- MIT (Cambridge, USA)

The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents from the supplier and Loughborough University human participant Ethical Sub-Committee.

Thank you for listening

Questions ?

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