CENTURY THERAPEUTICS

Case Study in Comparability for an iPSC-Derived, Genome-Edited Cell Therapy Product

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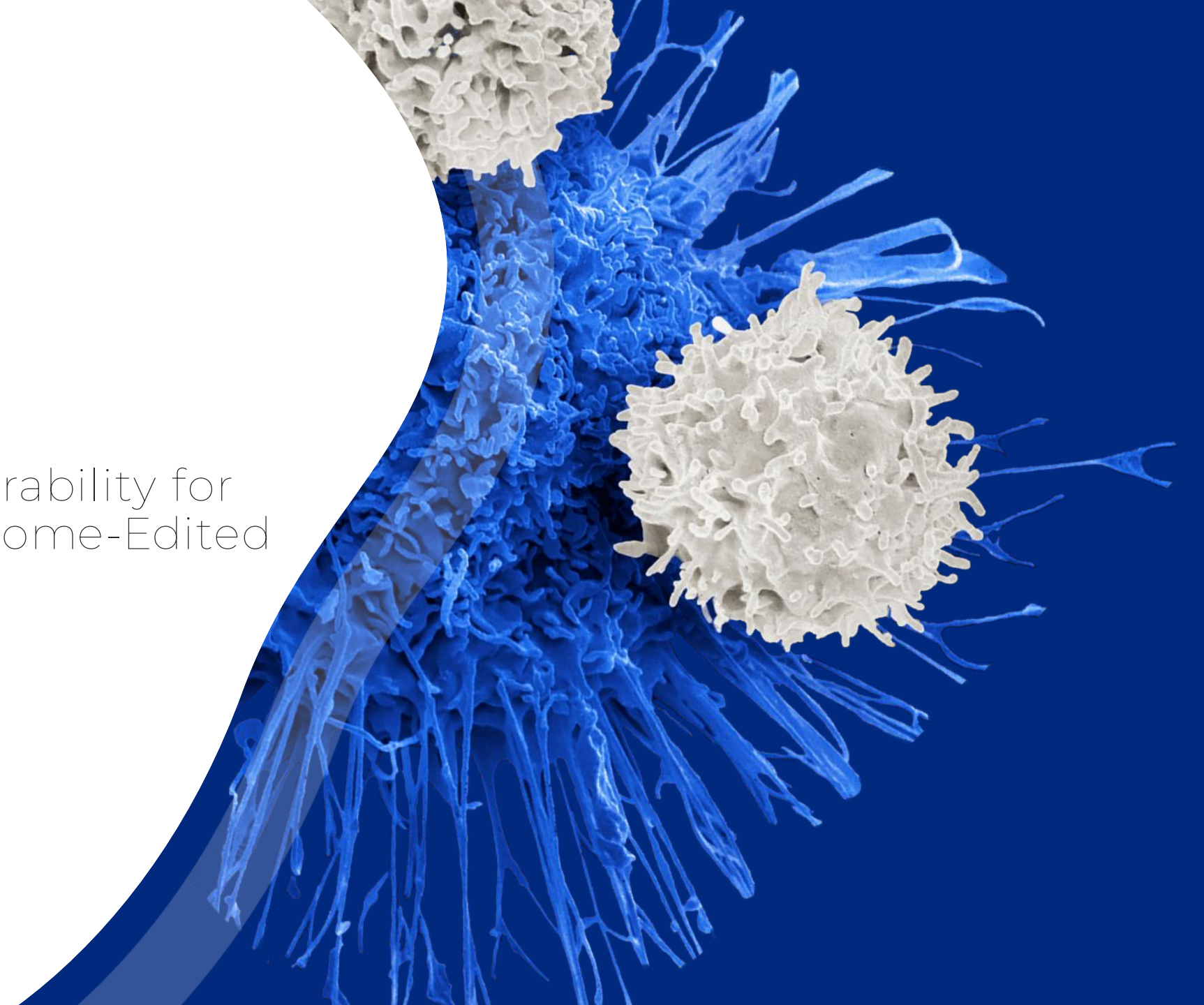
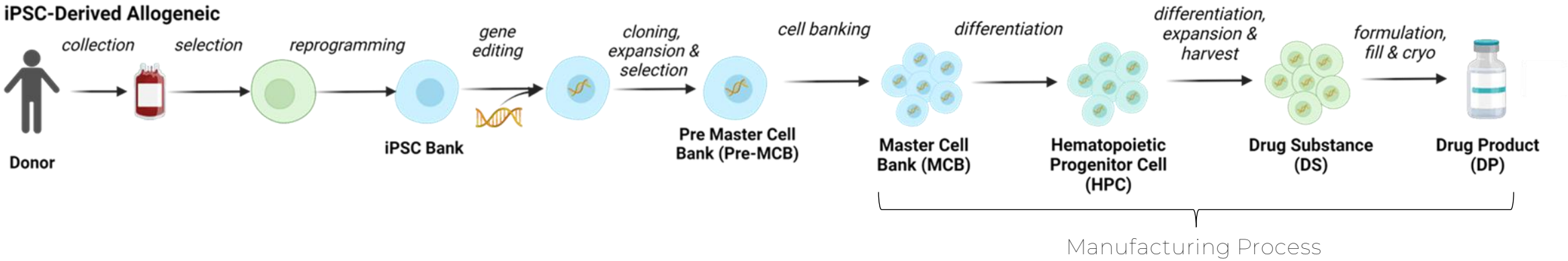
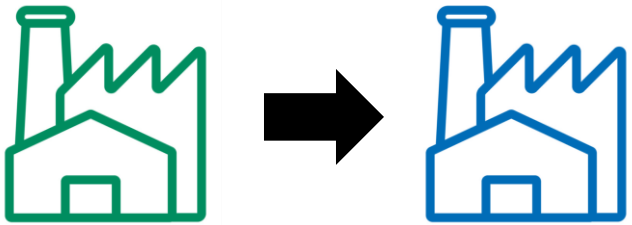


New facility introduction during Phase 1 is considered a change requiring comparability evaluation



Manufacturing Change



Facility 1 → Facility 2

Facility fit and risk assessment performed to describe change and determine risk:

- New facility, same single-use process
- Scope included HPC to DP (MCB to HPC not included)
- Limited changes:
  - Minor process choreography differences
  - Instrument model differences (new vs discontinued model)
  - Bioreactor design update (generation 2 vs 1)
  - Raw material changes (vendor changes)

Risk to product quality, safety, and efficacy considered low

Prospective comparability study structured as comparison of new facility batches to historical batches

Master Cell Bank (MCB)

Hematopoietic Progenitor Cell (HPC)

Drug Substance (DS)

Drug Product (DP)

MCB 1	*HPC Demo 1*	*DS Demo 1*	*DP Demo 1*
		DS Demo 2	*DP Demo 2*
		DS Shakedown	DP 1 Shakedown
	HPC Engineering	DS Engineering	DP Engineering
	HPC 1	DS 1	DP 1
		DS 2	DP 2
		DS 3	DP 3
		DS Demo 1	*DP Demo 1*
		DS Demo 2	*DP Demo 2*
		DS Engineering	DP Engineering

Facility 1

Facility 2

Prospective Comparability Study Design

Historical (Facility 1) batches:
- Seven (7) batches
- Mix of full-scale batches:
  - Demo (non-GMP, lab)
  - Shakedown (non-GMP, mfg)
  - Engineering and Clinical (GMP, mfg)

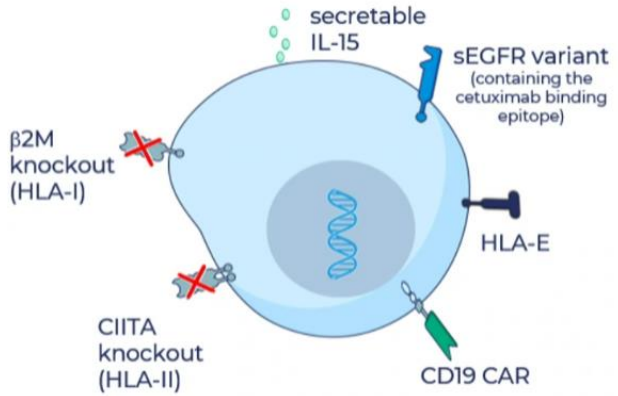
Comparability (Facility 2) batches:
- Three (3) batches
- Matched starting material to Facility 1 (MCB1, HPC1)
- Mix of half and full-scale batches:
  - Demo (non-GMP, lab, half-scale)
  - Engineering (GMP, mfg, full-scale)

Italics: Lab-generated; Non-Italics: Manufacturing-generated; Bold: Clinical
Note: Half-scale is not a scale-down, rather half the number of parallel processing trains

Multiple orthogonal methods selected to evaluate structure and function

Structure

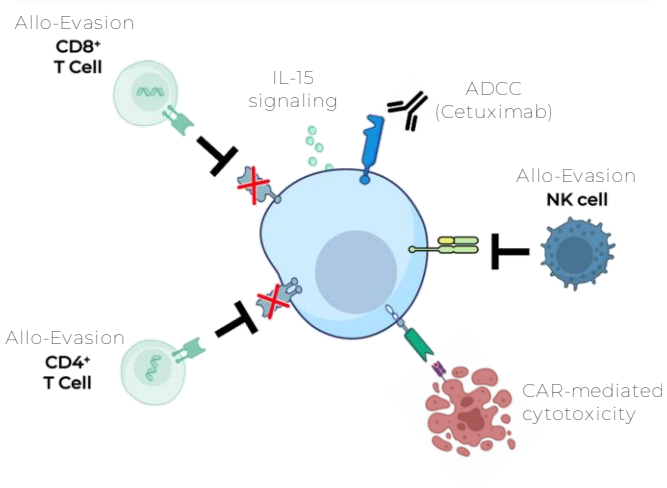
What attributes are required for a product to affect a certain function?



- Gene knockout
- Transgene on-target insertion
- Transgene sequence
- Protein expression
- Cell phenotype

Function

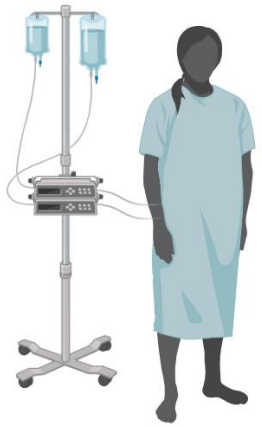
What functions (i.e., mechanisms of action) are required for biological effect?



- CAR-mediated cytotoxicity
- NK cell persistence
- Allo-evasion
- ADCC

Potency

What amount of a product (i.e., strength) is required to produce an effect?



- Dose (amount, function)
- Extrinsic factors (tumor burden, distribution, antigen expression, microenvironment)

Based on scope of the change, a mix of in-process metrics, release, and characterization data also considered

In-Process Metrics

Drug Substance Process
- Seeding density
- Cells harvested/vessel
- Lactate

Drug Product Process
- Formulation hold time
- Pre-cryo cell count
- Pre-cryo viability
- Visual inspection pass rate*

Release (select)

Identity
- Donor identity
- Engineering identity
- Cell identity

Purity / Function
- Transgene expression (flow)
- Cell phenotype (flow)

Quantity
- Cell count and viability

Impurities / Safety
- Residual iPSCs
- Karyotype
- Genome variants
- Microbial and viral testing*

Structure / Function Relationship

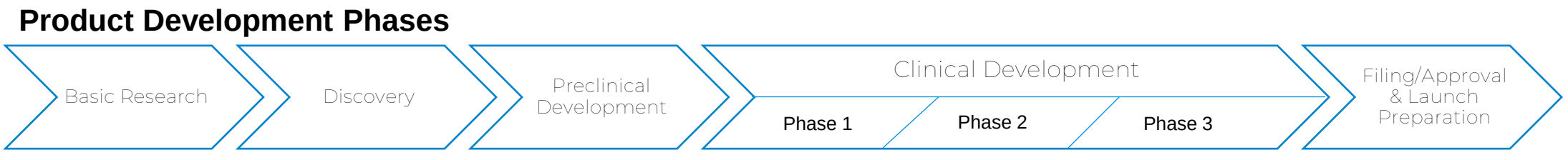
Characterization

Purity / Function
- Extended cell phenotype (flow)
- Cytotoxicity
- Degranulation
- IFN-gamma
- ADCC

Quantity
- Cell health / apoptosis

Not included for comparability:
- HPC in-process metrics, release, and stability (upstream of change)
- Some testing (*) not performed on comparability demo batches (NA – lab produced)
- DP stability not performed for comparability (but included 1 batch for annual stability)

Limited historical data during early development considered when establishing comparability criteria



Early development batches

- Frequent process changes make establishing representative dataset difficult

Clinical batches

- Clinical batches establish link to clinical performance
- Limited manufacturing campaigns underrepresent process and analytical variability

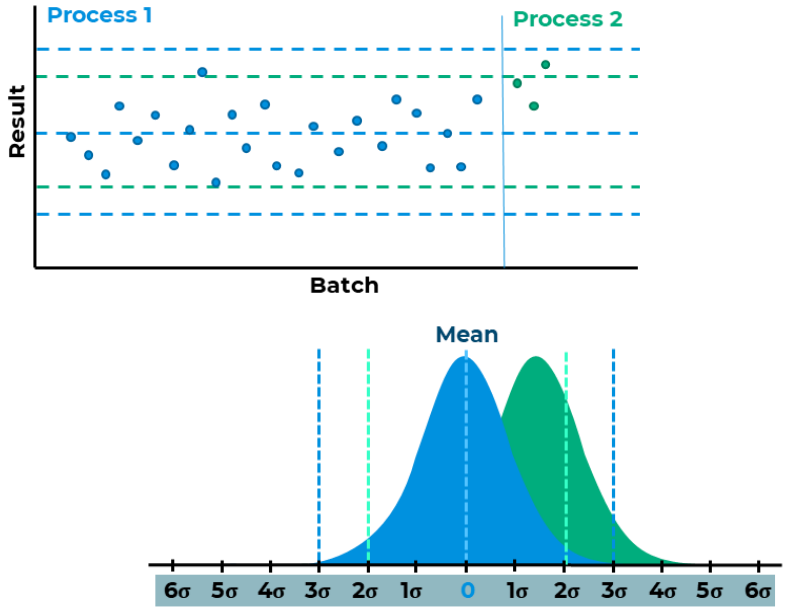
Commercial batches

- Frequent manufacturing provides larger dataset
- Process drift may occur over time

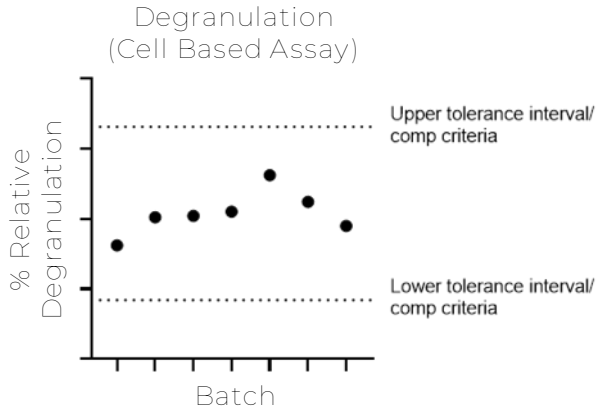
Comparability criteria include tighter alert levels in addition to specifications

Specifications
- Evaluates safety & efficacy (lot disposition)
- Based on technical justification or statistics (e.g., 3σ or 99/99 tolerance)

Comparability criteria
- Detects process shifts (investigation)
- Based on technical justification or statistics (e.g., 2σ or 95/95 tolerance)

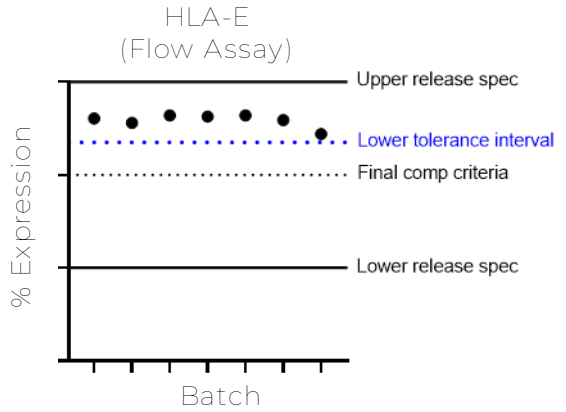


Example 1: Statistical basis



Comparability criteria set based on 95/95 tolerance limit (n=7)

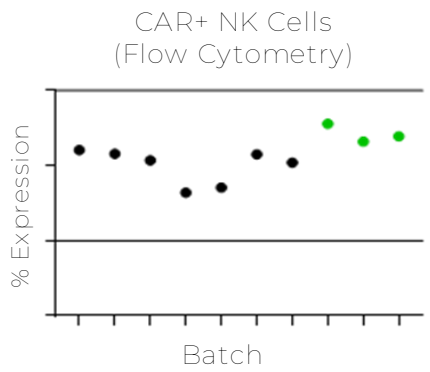
Example 2: Technical basis



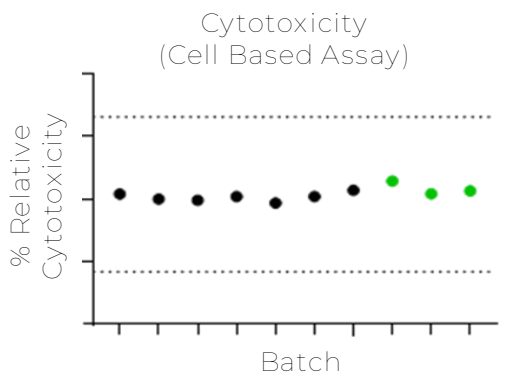
Comparability criteria set at 50% specification range based on technical justification

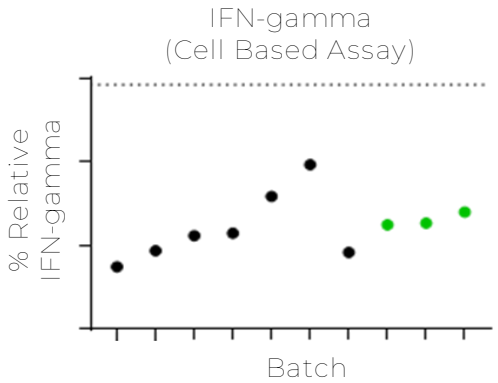
Assessment includes evaluation against specifications, alerts, and for shifts & trends

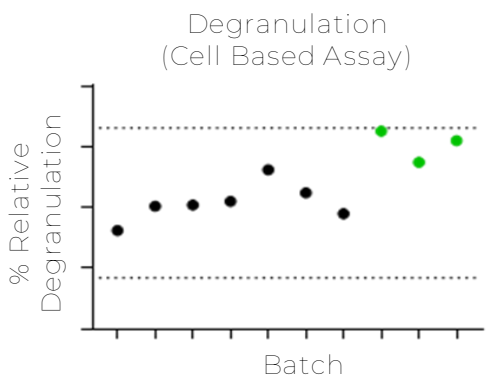
Release Assay



Orthogonal Characterization Assays





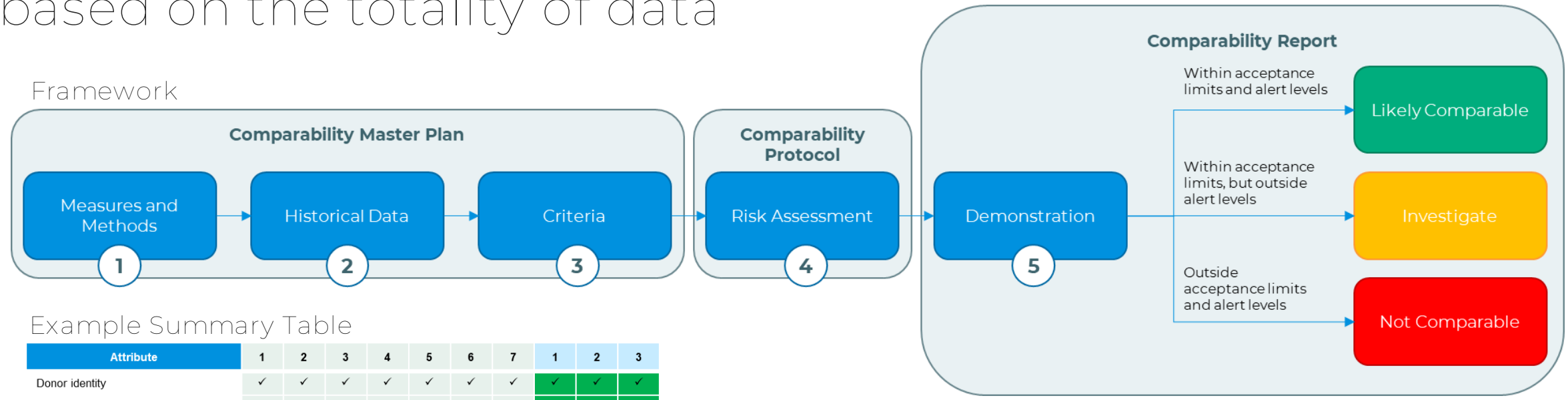


Comparability batch results (green):

- Within specifications (solid lines)
- Within comparability alerts (dashed lines)
- Potential trend noted (degranulation):
  - No observed trends in orthogonal assays (CAR+NK Cells, Cytotoxicity, IFN-gamma)
  - Change in analytical reagents may explain observed shift (control sample – not shown – also trended higher)

Comparability conclusions are based on the totality of data

Framework



Example Summary Table

Attribute	1	2	3	4	5	6	7	1	2	3
Donor identity	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Engineering identity	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Cell identity	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Transgene expression (flow)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Cell phenotype (flow)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Extended cell phenotype (flow)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Cytotoxicity	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Degranulation	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
IFN-gamma	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Cell count and viability	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Cell health and apoptosis	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Residual iPSC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Karyotype	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Genome variants	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Microbial and viral safety	✓	✓	✓	✓	✓	✓	✓			✓

Facility 2 batches deemed comparable to historical Facility 1 batches:

- Comparability results generated for all attributes with evaluation against historical data, specifications, and alerts
- Conclusion based on totality of results
  - All results within specification and comparability alerts
  - Trend in one attribute identified, but not supported by orthogonal data
  - No impact to quality, safety, and efficacy
  - Non-clinical or clinical studies not required for this study