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Critical Quality Attributes, Stability-Indicating Test Methods, and Cell-based Products: Untangling the Gordian Knot

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Donald Fink – Master Practice Expert

DHC client global footprint

Numerous regulatory authorities engaged

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Framing the Stability Discussion

Stability Assessment: Pulling at the Threads of the Gordian Knot

Stability Testing: Why Selection of Stability-Indicating Critical Quality Attributes Matters

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CGTP: Manufacturing, Quality and Regulatory Considerations

CMC is a key element to efficient, successful product development: Reframing the regulatory paradigm for cellular and gene therapies (CGT)

– Former FDA Commissioner Scott Gottlieb, M.D., ARM Board Meeting 22 May 2018

"In contrast to traditional drug review, where 80 percent of the review is focused on the clinical portion of that process, and maybe 20 percent is focused on the product issues, I'd say that this general principal is almost completely inverted when it comes to cell and gene therapy… The more challenging questions relate to product manufacturing and quality…"

Stability is a key element of product quality assurance

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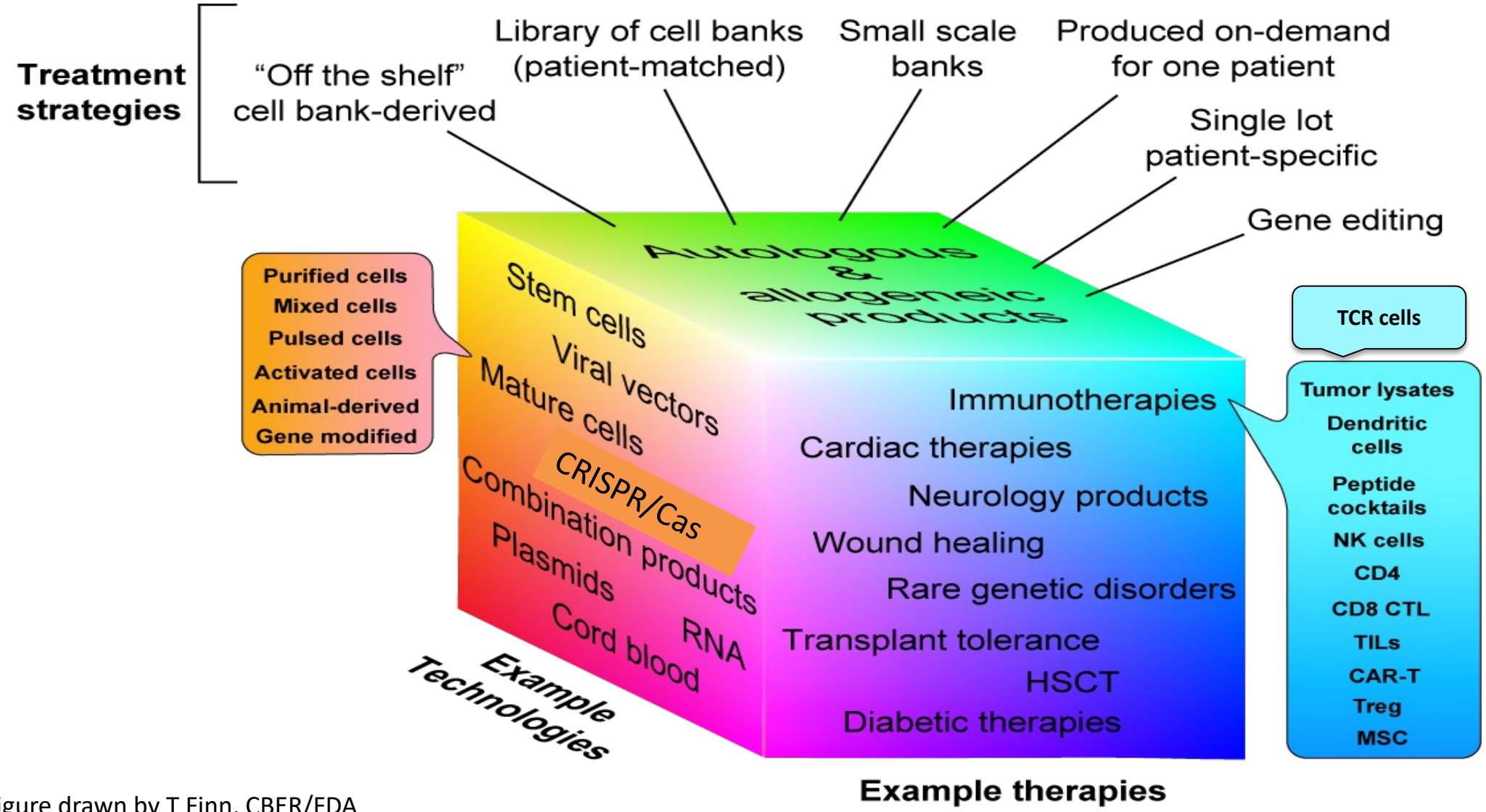
Stability Testing Confounders for Cellular-based Products



Schematic figure drawn by T Finn, CBER/FDA

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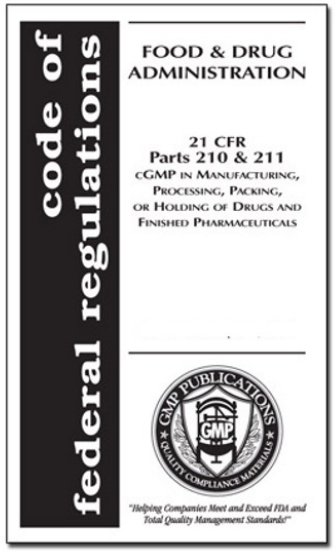
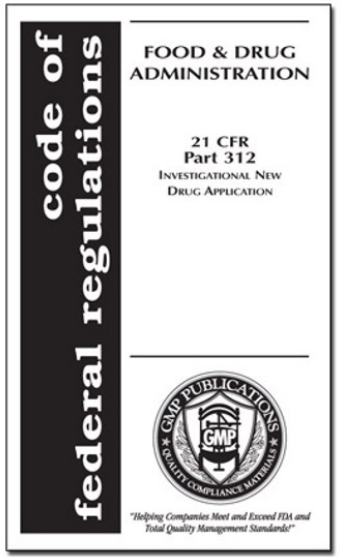
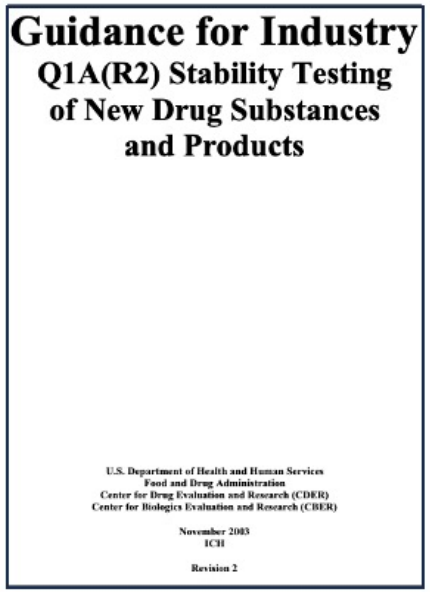
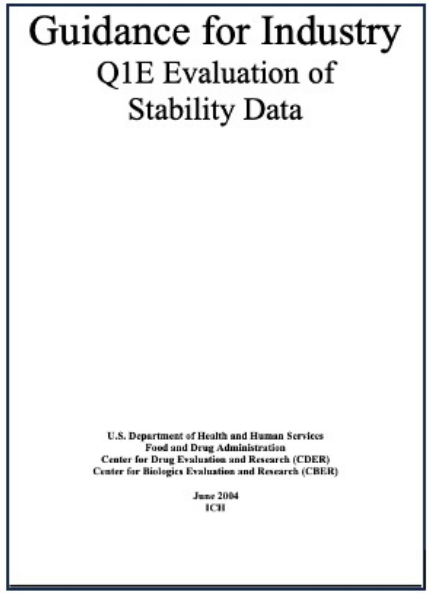
Performing Stability Testing: By rule and by recommendation









Reminder Regarding Guidance for Industry

This guidance represents the current thinking of the Food and Drug Administration (FDA). It does not establish any right for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations

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What do the regulations state regarding stability?

21 CFR 312.23 and 21 CFR 211.166

- Stability testing must be conducted in all phases of product development under an IND to demonstrate that the product is within acceptable chemical and physical limits for the planned duration for the proposed clinical investigation.
- There shall be a written testing program designed to assess the stability characteristics of drug products. The results of such stability testing shall be used in determining appropriate storage conditions and expiration dates.
  - ✓ Sample size/test intervals established based on statistical criteria
  - ✓ Storage conditions for samples retained for testing indicated
  - ✓ ***Reliable, meaningful, and specific test methods used***
  - ✓ Test drug product in same container-closure system as that which is used for distribution
- An adequate number of batches of each drug product shall be tested to determine an appropriate expiration date; a record of the data shall be maintained

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What statements are made in guidance regarding stability:

- Stability analysis may include measures of product sterility, identity, purity, quality and activity or potency

  NOTE: The term “quality” does not have a precise regulatory definition. Product quality typically defines the suitability of either a drug substance or a drug product for its intended use and incorporates attributes such as identity, strength (i.e., potency), and purity

- It is often helpful to demonstrate that at least one or more of the test methods in your stability analysis are **stability-indicating**
- The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors and to establish a retest period for the drug substance or a shelf life for the drug product and recommended storage conditions
- The manufacturer should propose a stability-indicating profile that provides assurance that changes in the identity, purity, and potency of the product will be detected

  CAVEAT: Regulations and guidance regarding stability were developed for small molecular entities and biotechnological products (recombinant therapeutic proteins/monoclonal antibodies); not cellular or gene therapies

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Stability-indicating test methods have a prominent role

- FDA expects procedures established for assessing stability of drug products will incorporate stability-indicating test methods that are reliable, meaningful, and specific
- Stability-indicating test methods permit monitoring of results during ongoing stability studies to provide assurance drug product safety, potency, and quality is maintained under conditions of storage
- A stability-indicating test is an analytical procedure that can detect meaningful changes in a quality attribute(s) during storage
- The CGMP regulations that are focused on stability testing offer flexibility, they do not specify what techniques or tests are to be used to ensure that test methods are stability indicating
- For cell and gene therapies, the assessment of product functionality/potency represents a stability indicating test method

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A comprehensive stability assessment program for a cell-based product engenders comparison with a Gordian knot

Gordian Knot: Metaphor for a seemingly intractable problem which may be solved by exercising an ***unexpectedly direct,*** novel, rule-bending, ***decisive and simple***, approach that dispenses with perceived constraints.

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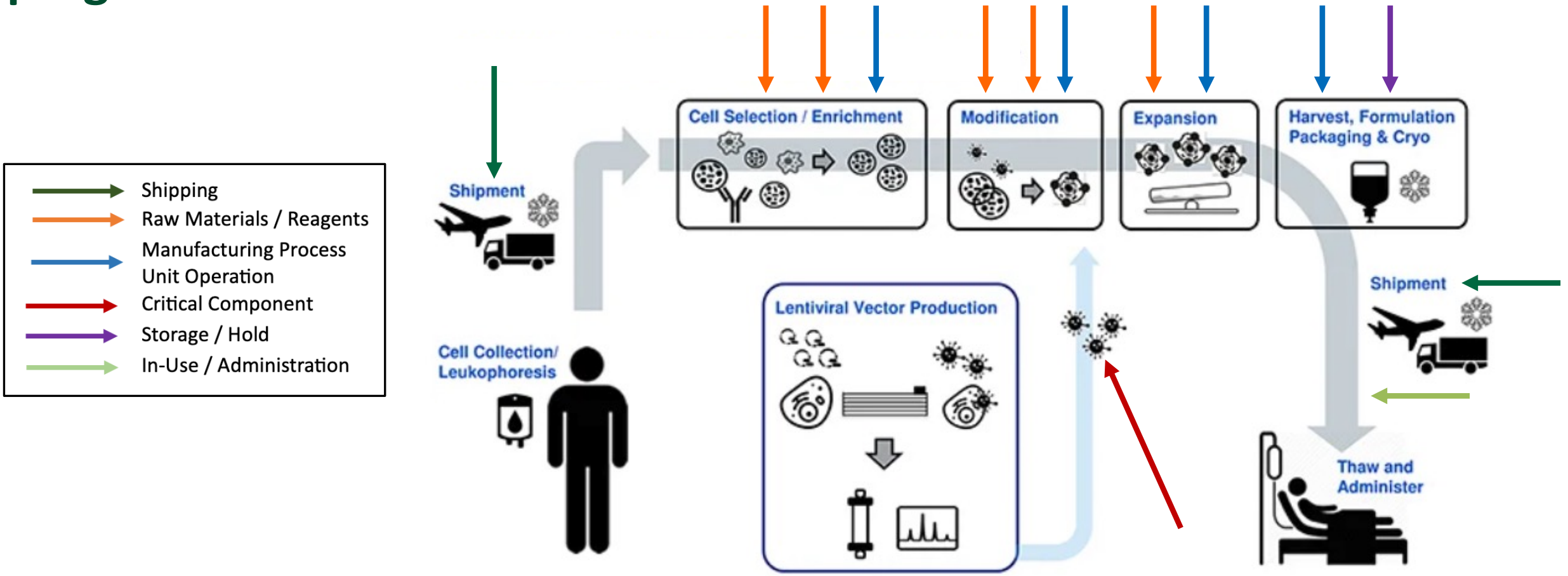
Multiple measurements contribute to a comprehensive stability program



SOURCE: https://insights.globalspec.com/article/11287/cancer-curing-gene-therapies-present-unique-manufacturing-challenges **(Accessed: 16Jun2023)**

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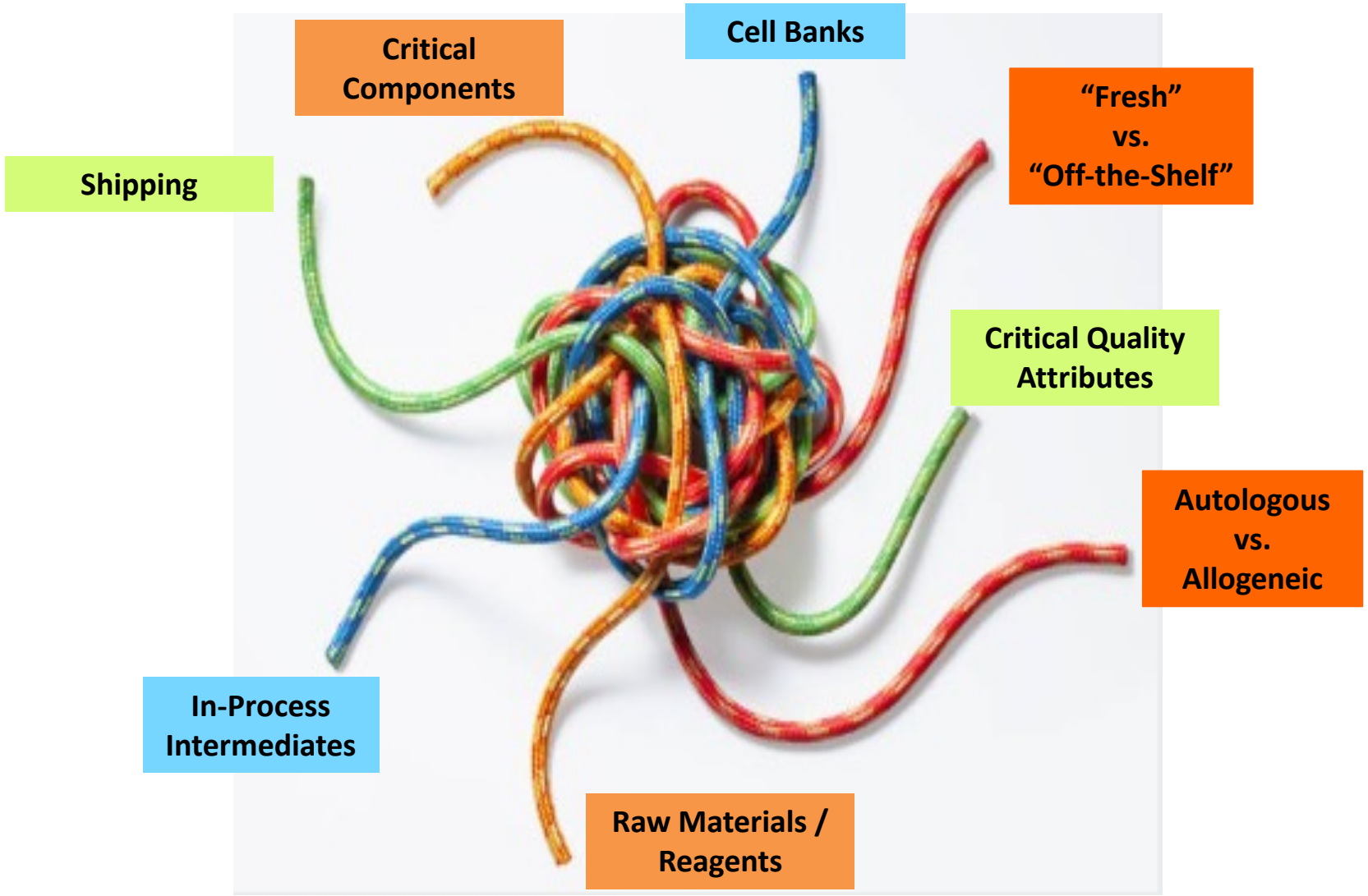
Stability Assessment: Pulling at the threads of the Gordian Knot



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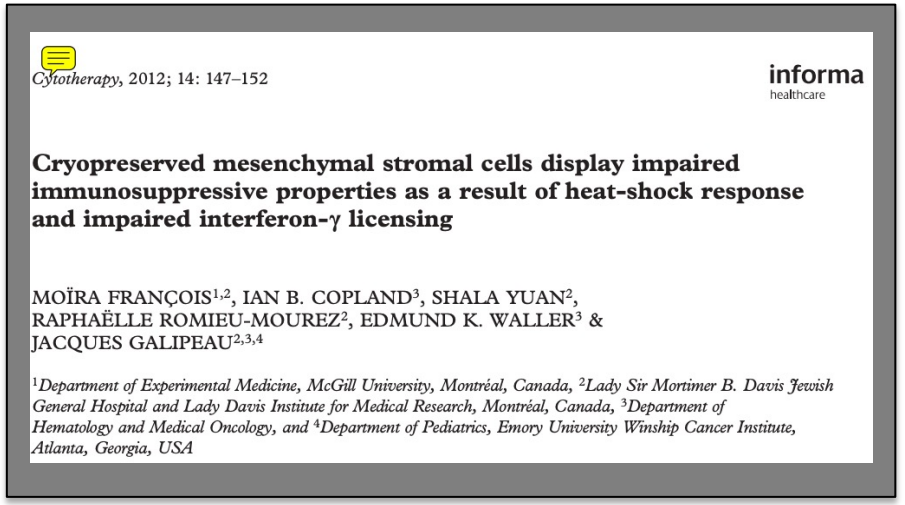
Selection of appropriate critical quality attributes is key for a successful stability testing strategy

Scenario

- **PRODUCT:** Ex vivo expanded, bone marrow-derived mesenchymal stromal cells (MSCs). Manufacture begins with thawing of a cryopreserved vial from Working Cell Bank
- **INDICATION:** Acute Graft-versus-Host Disease (GVHD)
- **PHASE OF STUDY:** Phase 3
- **ROUTE OF ADMINISTRATION:** Intravenous
- **FORMULATION:** Ready-to-Infuse, cryopreserved MSCs post-thaw. Change from "fresh" product administered during small, single site Phase 2 trial to accommodate logistics of multiple clinical sites and central manufacturing of the product.
- **CLINICAL OUTCOME:** No evidence of clinical benefit in the Phase 3 trial in GVHD despite positive Phase 2 clinical findings.



Cytotherapy, 2012; 14: 147–152

informa healthcare

Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon-γ licensing

MOÏRA FRANÇOIS[1,2], IAN B. COPLAND[3], SHALA YUAN[2], RAPHAËLLE ROMIEU-MOUREZ[2], EDMUND K. WALLER[3] & JACQUES GALIPEAU[2,3,4]

[1]*Department of Experimental Medicine, McGill University, Montréal, Canada,* [2]*Lady Sir Mortimer B. Davis Jewish General Hospital and Lady Davis Institute for Medical Research, Montréal, Canada,* [3]*Department of Hematology and Medical Oncology, and* [4]*Department of Pediatrics, Emory University Winship Cancer Institute, Atlanta, Georgia, USA*

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3279133/

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Not immediately apparent that stability of manufacturing material / working cell bank is a contributing factor

Parameter		Acceptance Criteria	Working Cell Bank Stability[1]	Final Product Release Test[2]
Viability		≥ 80%	PASS	PASS
Identity	CD105	≥ 95%-pos	PASS	PASS
	CD73		PASS	PASS
	CD90		PASS	PASS
Impurities Profile	CD45	≤ 2%-pos	PASS	PASS
	CD34		PASS	PASS
	CD14		PASS	PASS
	CD19		PASS	PASS
	HLA-DR		PASS	PASS
Adherence to Plastic		Demonstrates Adherence to Plastic	PASS	Not Tested
Multipotent Differentiation Potential	adipogenic	Visual: lipid vesicle accumulation	PASS	Not Tested
	osteogenic	Visual: Alizar Red S staining	PASS	
	chondrogenic	Visual: Alcian Blue straining	PASS	
Self-Renewal Proliferation		< 30-hour/doubling	PASS	Not Tested

[1]Working Cell Bank stability testing performed on post-thaw cryopreserved vials
[2]Drug Product release testing performed on pre-cryopreservation samples

Comparison: Stability Testing of Working Cell Bank and Product Release Testing

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Insights from Process Development Team

- **Immune suppressing Mechanism of Action (MOA)**
  - ✓ The in vitro suppression of T-cell activation by human MSCs occurs in an indoleamine 2.3.-dioxygenase (IDO)-dependent manner
  - ✓ IDO activity in MSCs is upregulated by interferon (IFN)-γ
- **In vitro MSC-mediated immunosuppressing activity is compromised in freshly thawed cryo-preserved MSCs**
  - ✓ Post-thaw MSCs are refractory to IFN-γ-induced upregulation of IDO in vitro
  - ✓ Post-thaw MSCs are unable to suppress in vitro CD3/CD28-driven T-cell proliferation

MITIGATION: Immune suppressor activity is fully restored following 24 hours of MSC tissue culture post-thaw.

CONCLUSION: Stability testing performed for the WCB failed to anticipate a negative impact of cryo-preservation storage on the the Drug Product as a result of not assessing a key critical quality attribute: MOA-based bioactivity/potency.

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TAKEAWAYS

- Cell-based products are biologically dynamic constructs that may be pleiotropic with respect to their mechanism of action which poses a challenge to standardizing the best approaches to take when evaluating stability
- CGMPs are prescriptive with respect to expectation that stability assessment be performed during all phases of product development but allow for flexibility in determining what techniques or tests are to be used to ensure that test methods developed are stability indicating
- Stability of a cell-based therapy is more that just assessment of final product shelf-life and should consider all elements of the manufacturing process that may impact product quality
- A successful stability program is premised on the relationship between establishment of critical quality attributes and their assessment using stability-indicating test methods
- Assays based on an emerging understanding of mechanism of action and that assess a relevant biological activity should be incorporated into stability testing protocols as soon as feasible

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Thank you!

DFink@darkhorseconsultinggroup.com

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References

- François M, Copland IB, Yuan S, Romieu-Mourez R, Waller EK, and Galipeau J. Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon-γ licensing. *Cytotherapy* 2011, 14(2):147-152. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3279133/pdf/mcyt14-147.pdf
- Q5C Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products (July 1996): https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q5c-quality-biotechnological-products-stability-testing-biotechnologicalbiological-products
- Guidance for Industry Q1A(R2) Stability Testing of New Drug Substances and Products (November 2003): https://www.fda.gov/media/71707/download
- Guidance for Industry Q1E Evaluation of Stability Data (June 2004): https://www.fda.gov/media/71722/download

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