FINDING ZEN IN STABILITY

DEVELOPING A BALANCED STABILITY PROGRAM FOR CRYOPRESERVED HEMATOPOIETIC PROGENITOR CELL PRODUCTS

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Outline

- **Background: Cryopreserved Hematopoietic Progenitor Cells (HPCs)**
- **Accreditation Requirements**
- **AABB-ISCT Joint Working Group Stability Project Team: Survey and Recommendations**
- **Cell Manipulation Core Facility (CMCF) Stability Program**

ZEN KŌAN

“A story, dialogue, question, or statement which is used to provoke great doubt and foster contemplation.”

Background

Cryopreserved Hematopoietic Progenitor Cells (HPCs)

CD34 Cells: Target Population of Concern

Hematopoietic Stem Cell Transplant (HSCT):

NOTE "HPC" = Hematopoietic Progenitor Cells

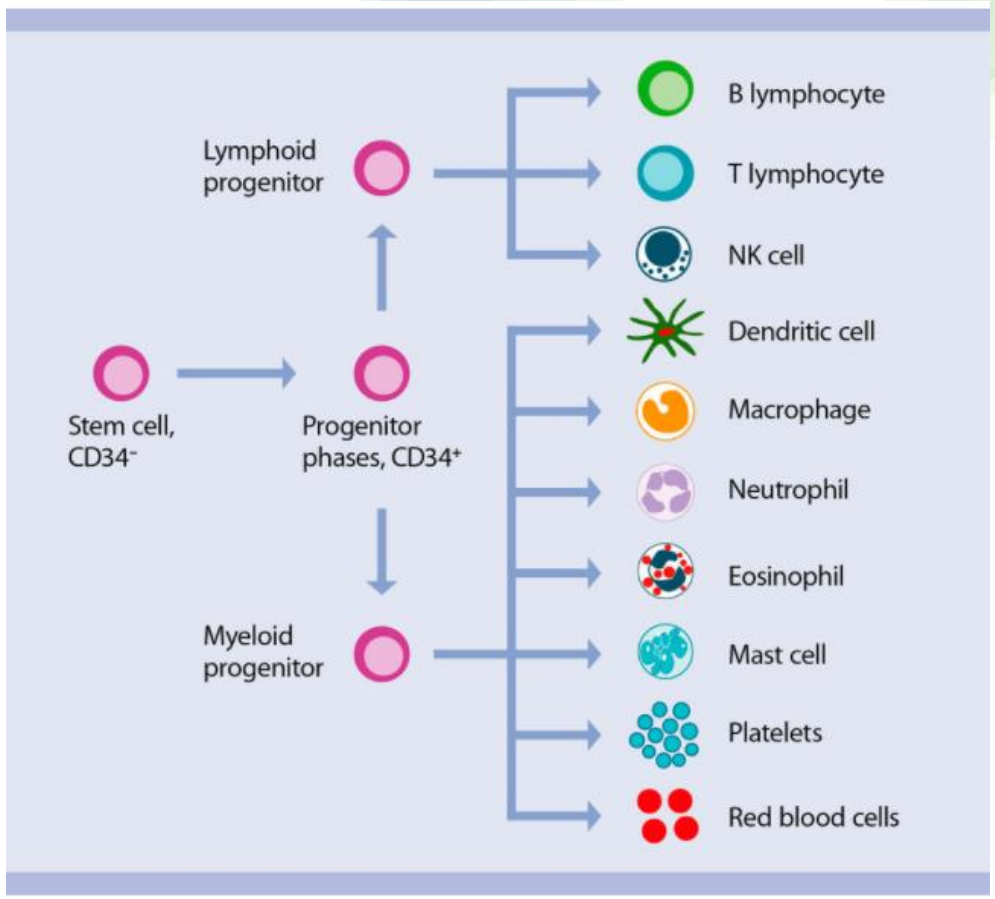
- HPC, Marrow / HPC, Apheresis / HPC, Cord Blood
- CD34+: Marker for hematopoietic stem cells
  - **<u>CD34+ cells reconstitute all hematopoietic cell lineages</u>**

Engraftment "The Gold Standard of Product Efficacy"

Stem cells engraft in the bone marrow niche and begin hematopoietic reconstitution (Typically 2-4 weeks post transplant)

NOTE:

Majority of centers base adequacy of HPC product on pre-freeze yields…



Cryopreservation of Hematopoietic Progenitor Cells (Apheresis & Marrow)

Cryoprotectant freezing solution added (10% DMSO final Concentration in product):

- Dimethyl sulfoxide (DMSO) with Human albumin & Plasm-Lyte A
- DMSO prevents extracellular & Intracellular ice crystal formation (and increase in solute concentration)

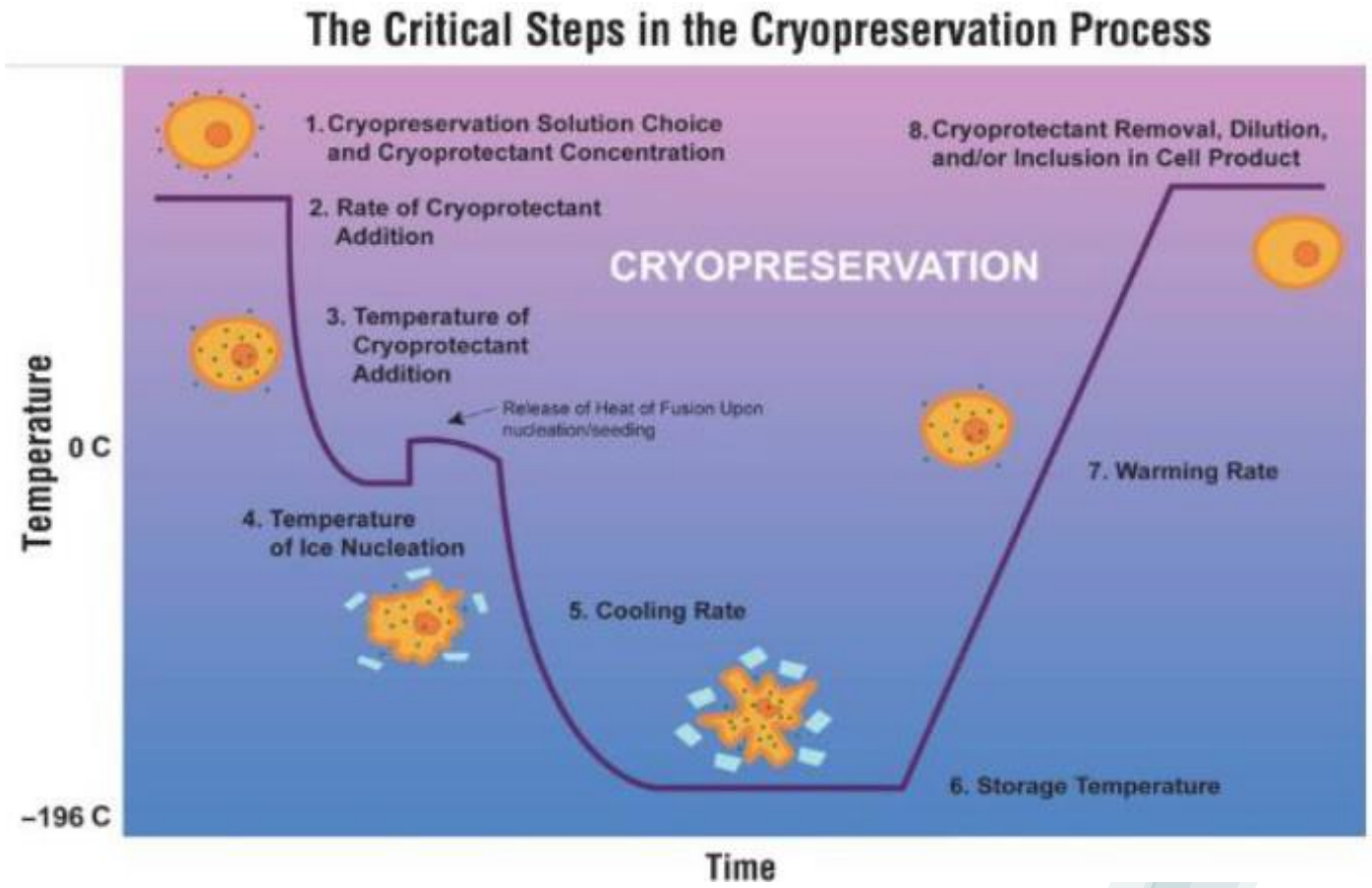
Controlled freezing Process:

- Products placed in a control rate freezer and frozen stepwise to -80C (≈1°C / min)

Long term Storage (LN2 tank)

- Cells stored at < -150 °C where they enter “glass phase”

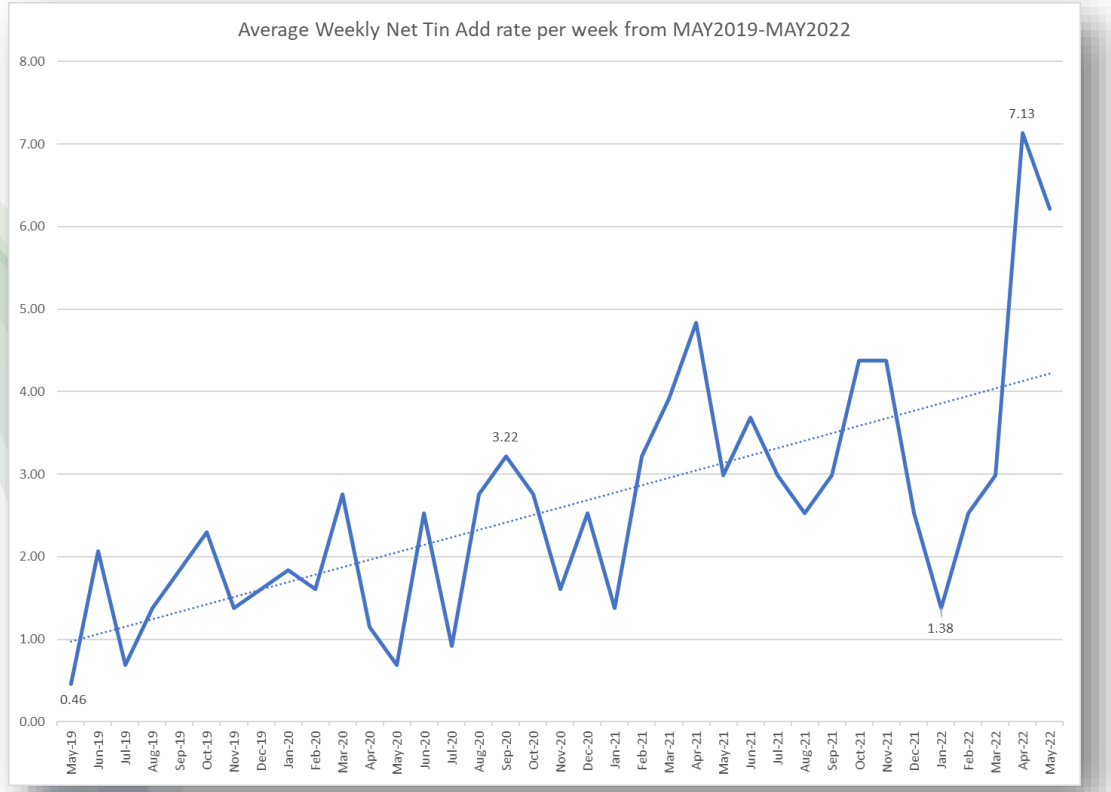
Biochemical processes are halted and cryopreserved cells may likely be stable indefinitely…



Expiration: More Than A Matter of Time…

Starting in 2003 cryopreserved hematopoietic progenitor cell products were assigned a "**10-year expiration date**" by the Cell Manipulation Core Facility.

- Products cryopreserved prior to 2003 have no assigned expiration date
- Inventory includes units 30 years+ in age
- Glut of Multiple Myeloma double transplant products & CART backups



Expiration Paradox

“Expired HPC products remain viable and functionally potent after 10 years”

- **Are these HPC products expired?**

YES…AND NO

Expiration should be based on validated data supporting the efficacy of the cryopreserved products.

- Support use of product without additional potency testing prior to infusion
- Can serve as justification for discard.

<u>Remember</u> theoretically products should remain stable indefinitely!

Anecdotally …

CMCF has issued 3 expired products (up to 13 years old) without issue (product viability, sterility, patient engraftment)

AABB-ISCT Joint Working Group Survey

(based on 32 of 62 respondents)

54% store products indefinitely

Expiration Determination:

25% published literature –CMCF?

15% internal validation

11% arbitrary –CMCF?

3% stability program

<u>Reality: Most centers (including CMCF) will not discard SOC HPC products unless the patient is deceased</u>

“If a tree falls in a forest and no one is around to hear it, does it make a sound?”

- Popular Zen Koan

“If our patients engraft, is there any relevance in creating and maintaining a stability program?”

-Karl Stasko et al.

Accreditation Requirements

Accreditation Requirements

AABB: Association for the Advancement of Blood & Biotherapies

ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)

FACT: Foundation for the Accreditation of Cellular Therapy

Multiple voluntary accreditation agencies require or recommend that facilities implement a stability program but are generally non-prescriptive regarding:

- Specific assays used
- Calculations
- Acceptable criteria

Currently no single assay which is accepted by the cell therapy field by consensus to accurately predict engraftment potential of cryopreserved hematopoietic progenitor cells (HPCs).

FACT STANDARDS

STANDARD:

D9.2.3 *There shall be a written stability program that annually evaluates the viability and potency of cryopreserved cellular therapy products.*

D9.2.3.1 *Samples should include those representative of all processing methods and those representative of maximum storage duration.*

"The purpose of a stability program is to assess cellular therapy products over time in <u>storage</u> for potency and viability"

MUST (FACT Definition: <u>Complied with at all times</u>)

- Annual testing
- Assess oldest stored products (based on expiration – 10 years @ Cell Manipulation Core Facility)
- Pre-determined acceptance criteria for potency and viability

SHOULD (FACT Definition: Highly recommended, but which there may be effective alternatives)

- Assess characteristics that affect safety and efficacy (label & container integrity, sterility)
- Define actions to take if products fail to meet specifications

MAY

- Assess products at various time periods

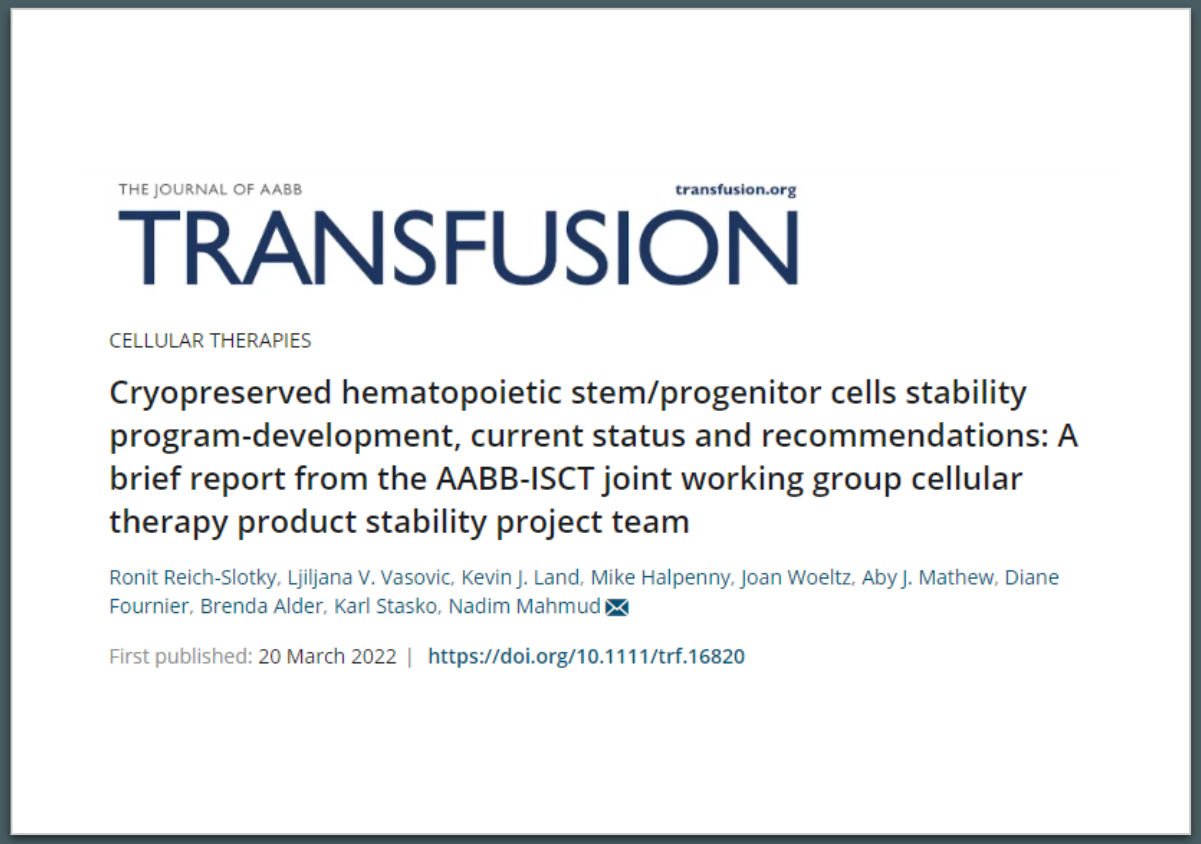
RECOMMEND

- Annually test a minimum of 3 cellular therapy products to ensure potency

AABB-ISCT Joint Working Group Stability Project Team

Survey and Recommendations

Recognition: AABB-Joint Working Group Cellular Therapy Product Stability Team



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Cryopreserved hematopoietic stem/progenitor cells stability program-development, current status and recommendations: A brief report from the AABB-ISCT joint working group cellular therapy product stability project team

Ronit Reich-Slotky, Ljiljana V. Vasovic, Kevin J. Land, Mike Halpenny, Joan Woeltz, Aby J. Mathew, Diane Fournier, Brenda Alder, Karl Stasko, Nadim Mahmud

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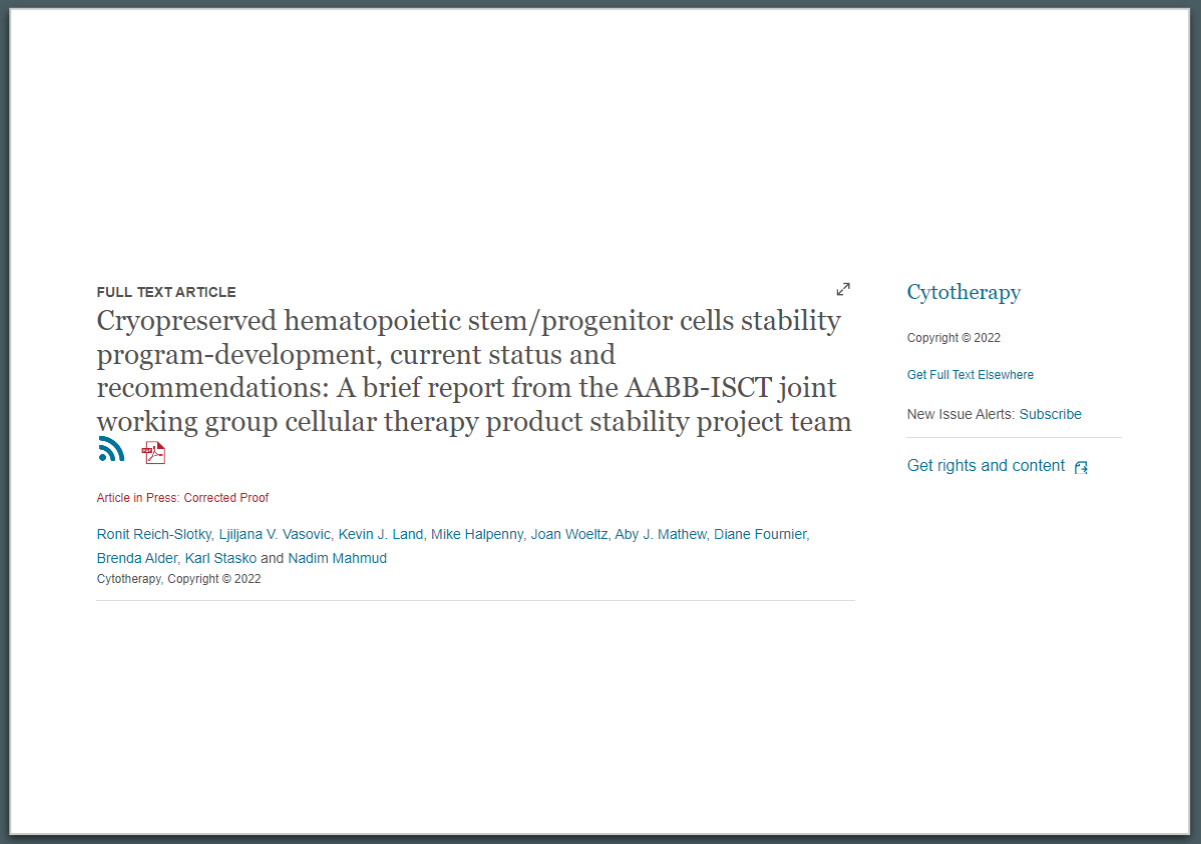

FULL TEXT ARTICLE

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Article in Press: Corrected Proof

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Common Assays for HPC Stability Testing

Cell counts (hematology analyzer)
- Susceptible TNC loss post thaw (RBC lysis and granulocytes)

Flow Cytometry
- Short turn around time
- No standard methodology (post thaw)

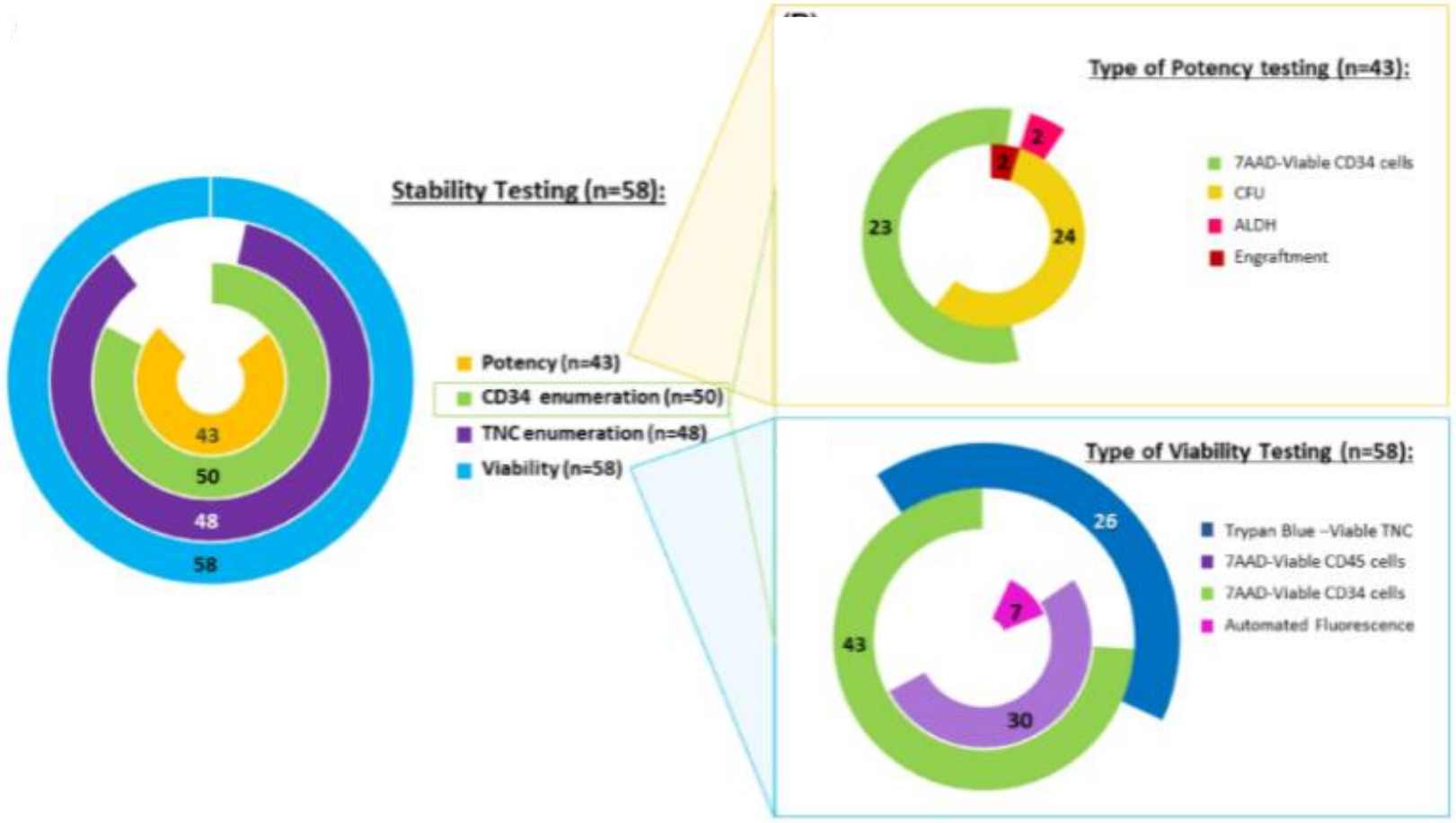
Trypan Blue
- Limited in scope (target population)
- Only capture compromised cell membrane

Colony forming units (CFU)
(Current "gold standard" for function)
- Progenitor cell viability, hematopoietic differentiation.
- Challenges with methodology & time

Platform	Quantification	Viability	Function
Hematology Analyzer	Total Nucleated Cells	No	No
Flow Cytometry	CD45+ and CD34+	Yes (7-AAD)	Yes (surrogate)
Microscope	Total Nucleated Cells	Trypan blue	No
Colony Forming Unit (CFU)	CFU	Yes, CFU	Yes, specific to Hematopoietic stem and Progenitor cells

AABB-ISCT Joint Working Group Stability Project Team: Survey and Stability Program Recommendations for Cryopreserved HPC Products

- 82 Survey Respondents
- 67 have a stability program
- 58 answered testing questions
  - 52 of 58 perform multiple assays
  - 37 of 58 combo of CD34 & TNC Testing
  - 4 of 58 only perform CD34 testing

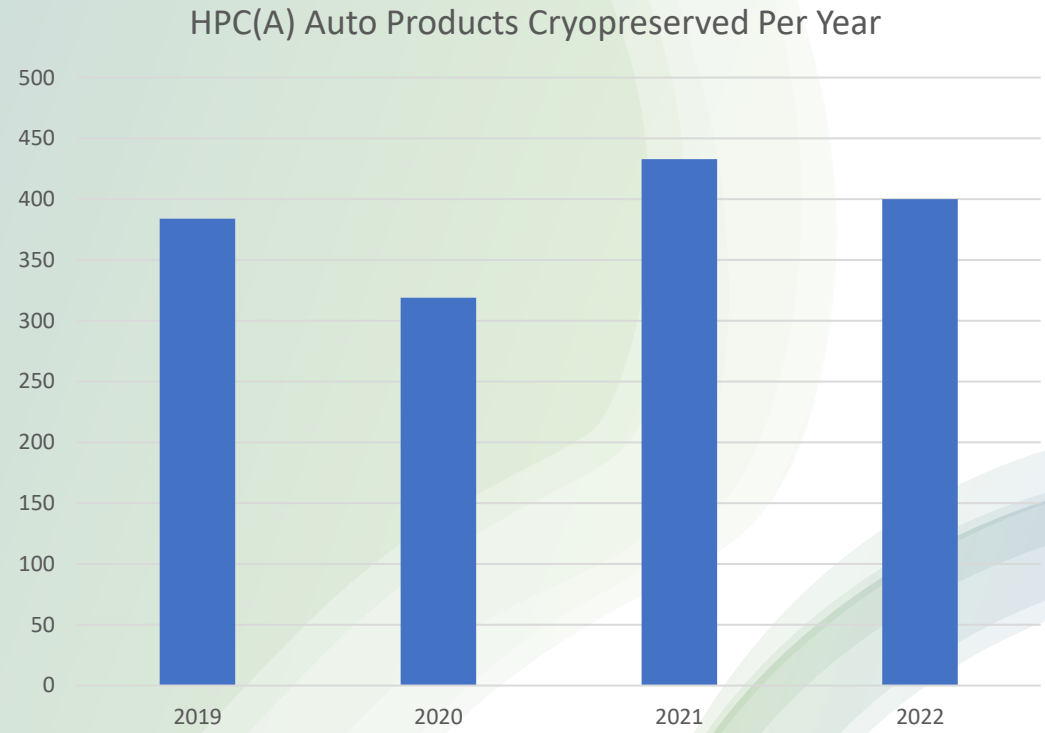


Stability Project Team Recommendations

- Develop and implement a formal (written) HSPC Stability Program whose key components are validated and regularly verified
- Expiration times for each product based on internally validated data
- Regular verification of representative HSPC product aliquots and if feasible more frequent than annual verification
- Minimum of three sentinel samples (or data points such as one product per 5 products cryopreserved) annually per process or product type
- Does not recommend using engraftment data as the sole metric for stability
- Utilize the same assay(s) pre-freeze and post-thaw to determine the viability of the target cell product and allow for true comparison analysis within their stability program
- Validate critical steps during post-thaw HSPC sample testing by flow cytometry (or other methodologies) to reduce intra- and inter-laboratory variation
- Utilize a validated functional potency assay if available to determine the potency of the target cell product
- Container and label integrity and maintenance of microbial sterility be part of monitoring of HSPC Stability Program
- Use of consensus terminology and associated calculations when available within a formal Stability Program

Cell Manipulation Core Facility (CMCF) Stability Program

Balancing resources / Meaningful Data



Average 384 HPC(A) Autologous Products / Year

CMCF Stability Program

- **Retrospective Quarterly Review - Summary Report**

“Relatively easy data retrieval from electronic systems”

- 100% thawed HPC(A)/(M) products (≈100/quarter)
  - Trypan blue viability
  - Sterility
  - Engraftment
  - Label Integrity
  - Bag Integrity

4th Quarter 2020 Stability Data: HPC(A) and T Cells(A) Products Thawed For Infusion

Data Set 10/1/2020 – 12/31/2020

Products Thawed	Trypan Blue Viability Percentage: ≥ 60%	Final Sterility (Negative)	Engraftment (Yes)	Label Integrity (passed)	Bag Integrity (passed)
HPC(A) N = 86	86 products met the specified criteria. -**Median** = 89 -**Mean** = 87.6 (standard deviation ± 5.8%)	All products were resulted “Negative” .	84 of 86 patients engrafted in the 4th quarter of 2020.	All product labels were acceptable.	All product bags were intact without issue at thaw.
T Cells(A) N = 3	All products met the specified criteria. -**Median** = 89 -**Mean** = 86 (standard deviation ± 7.9 %)	All products were resulted “Negative”		All product labels were acceptable.	All product bags were intact without issue at thaw.

CONCLUSION: 84 of 86 HPC, Apheresis products, and all thawed T Cells, Apheresis products passed established criteria for the 4th quarter of 2020. Viability results post thaw exceeded criteria (≥ 60%) for each product type without exception, with no notable negative trends.

Engraftment Criteria Exceptions: Due to NMDP guidance during the COVID-19 pandemic, the number of cryopreserved HPC(A) allogeneic products (delivered through the NMDP) rose sharply in 2020. Please note that the 2 thawed HPC(A) allogeneic products associated with engraftment exceptions passed all other criteria. Given that the remaining 84 thawed HPC products passed all criteria including engraftment, there is no indication that there was an issue with product stability in the 4th quarter of 2020.

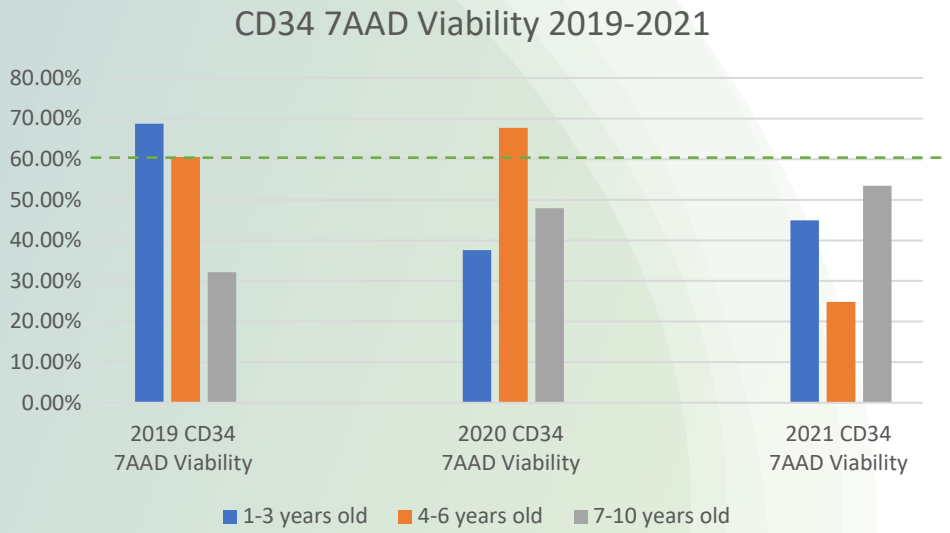
Collection / Infusion Timeframe: For the 4th quarter of 2020, 10 products were over 6 months old (1 at 7 months+, 3 at 8 months+, 1 at 12 months+, 1 at 15 months+, 1 at 26 months+, 1 at 44 months+, 1 at 44 months+, 1 at 81 months+) at the time of thaw.

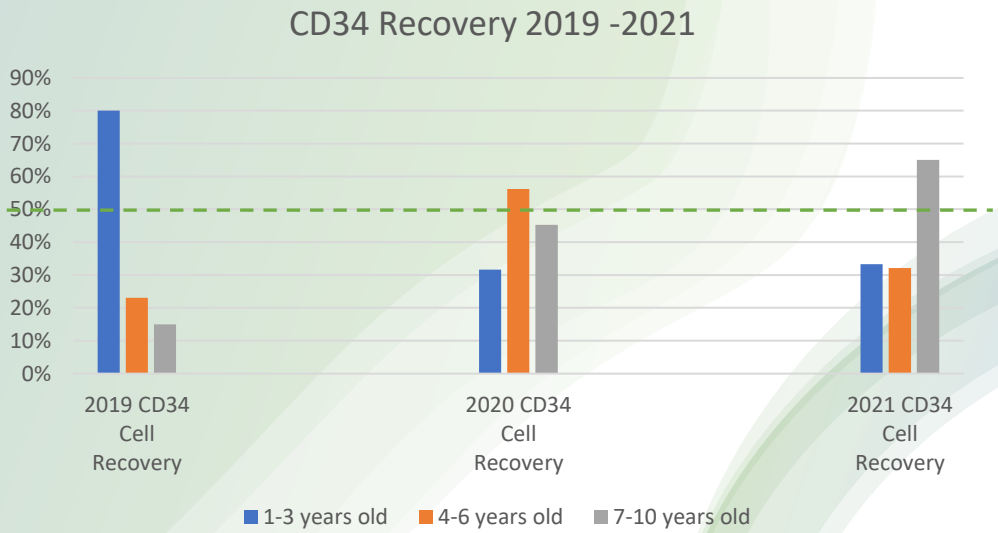
Potency and Viability: Not Stable!

- Original Plan (Circa 2018)

Test Samples: Representative QC vials or product discards

- CD34 7AAD Viability (potency surrogate)
  - Criteria ≥ 60%
- CD34 Recovery (post thaw to pre- freeze)
  - Criteria ≥ 50%
- Thaw one sample (total of 3 annually from the following time periods):
  - 1-3 years
  - 4-6 years
  - 7-10 years old (in reality all are @ 10 years)





Determining Criteria

Establishing predefined criteria is a FACT standard must, but what values make sense when all of your patients engraft?

- Very limited data available on post thaw samples
- Potency testing performed on thawed products with trypan blue viabilities < 60%
  - Resulting product CD34 7AAD viabilities < 20% have engrafted!!

Come to a consensus on criteria and adjust values as results mature

Maybe we should lower our criteria??

But…

Anecdotal Intuition and Data

The Great Doubt:

"Our patients engraft, yet in conversations with colleagues from other cell processing facilities our post thaw CD34 results are exceedingly low."

Experts agree that there is no fool proof method for post thaw CD34 enumeration:

- Transient warming
- DMSO toxicity
- Fragile state of cells post thaw

Turning Doubt into Data

"Are there opportunities to improve our post thaw CD34 enumeration workflow?"

Best practices via AABB-ISCT Joint Project Team and cell therapy peers:

- Control for temperature impacts ✖
- Thaw quickly and dilute with isotonic solution containing a protein source to prevent ice crystal formation & membrane destabilization ✔?
- Use ISHAGE Protocol / single platform ✔

Consult Your Experts

Preintervention Workflow

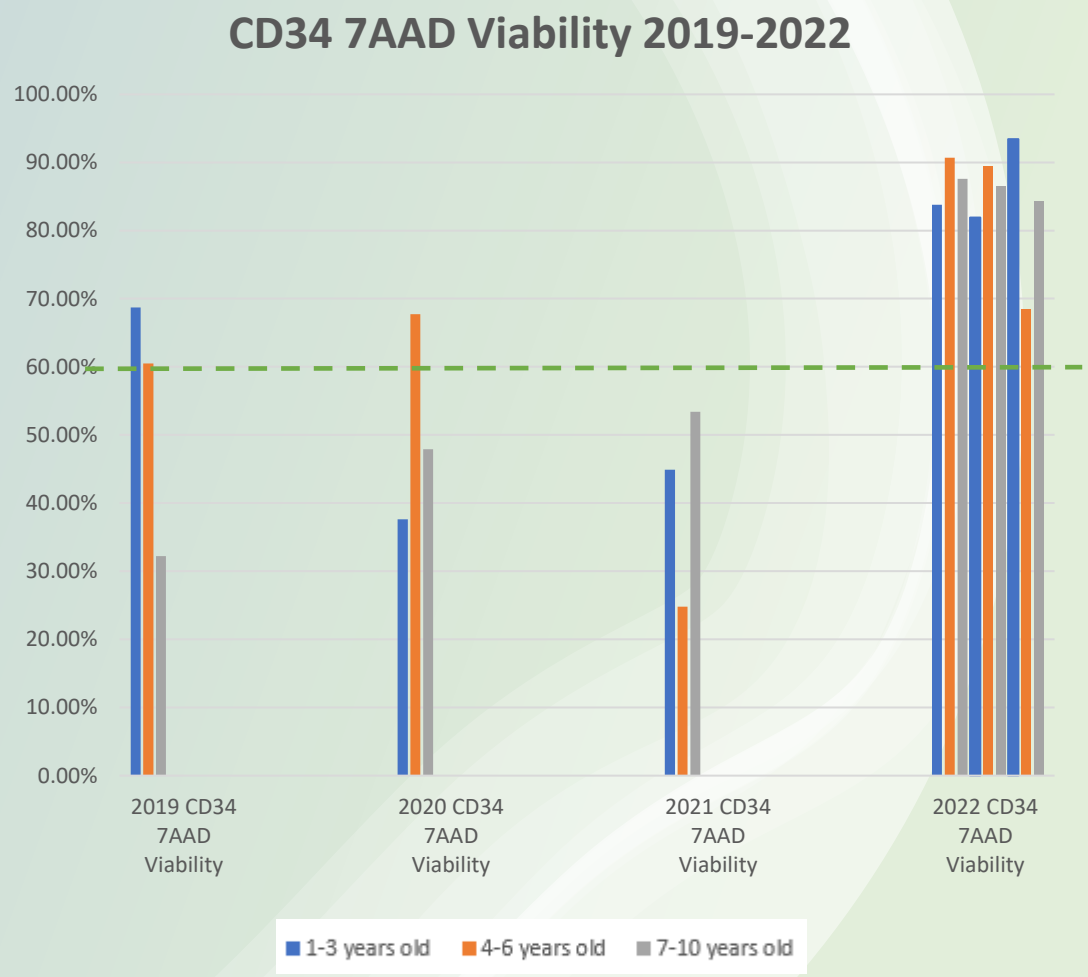
- Deliver frozen vial sample on dry ice or take deliver directly to QC for immediate setup = Transient Warming Risk
- Thaw frozen vial sample in 37°C water bath
- Perform cell count = DMSO Toxicity Risk (no dilution)
- Prepare sample dilution for flow with HBSS = No Protein
- Proceed with normal CD34 enumeration workflow

Improved Workflow

- Deliver frozen vial sample in Cryopod shipper @ ≤ -150°C = Mitigate Transient Warming Risk
- Thaw frozen vial sample in 37°C water bath
- Dilute sample (slow drip) 1:1 in isotonic solution with protein = Protein Added & Reduction of DMSO Concentration & Toxicity
- Perform cell count
- Prepare sample dilution for flow with isotonic solution with protein
- Proceed with normal CD34 enumeration workflow

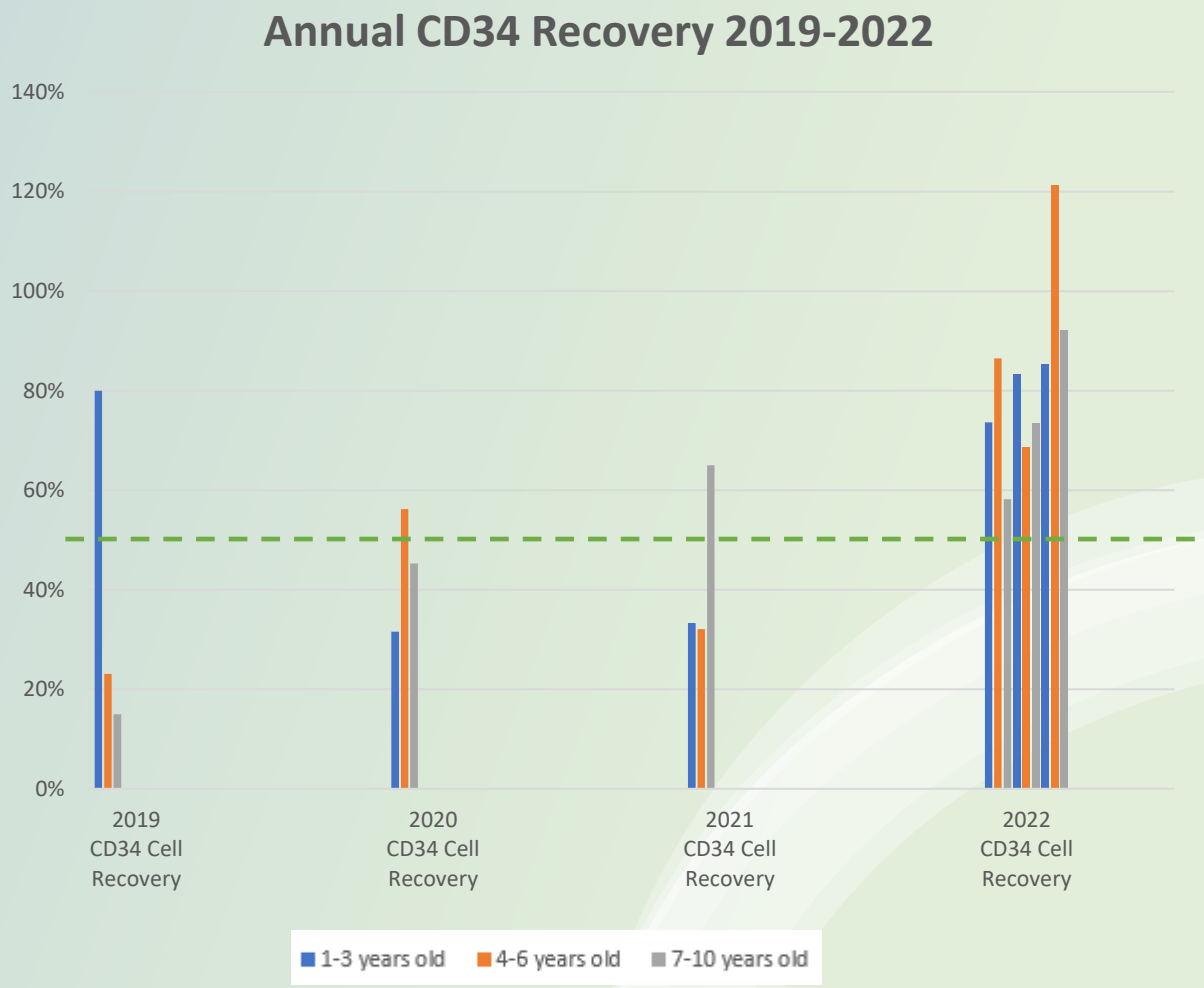
Improvement: CD34 7AAD Viability

Quick Stats	Old Method 2019-2021	New Method 2022 (3 quarters)
Mean	48.6%	85.1%
Median	48.3%	85.8%
Standard Dev.	15.4%	7.2%



Improvement: CD34 Recovery

Quick Stats	Old Method 2019-2021	New Method 2022 (3 quarters)	New method adjusted for Outlier 121%
Mean	42.4%	82.5%	77.6%
Median	37.9%	82.8%	77.6%
Standard Dev.	21.1%	17.9%	**11.2%**



Final Thoughts in Finding Balance

Simplifying Framework

Define product and population	• Specify by HPC product Type (Apheresis, Marrow, Cord Blood) if cryopreservation technique is not identical • HPC products and sample aliquot should have identical storage conditions
Define testing and other attributes	• Specify tests for determining target cell product recovery, viability and potency • Additional considerations: Container and label integrity and sterility
Define sample number and frequency	• Specify the minimum number of samples to include in the stability program • Specify the frequency of testing

Thoughts

- **Balance data needs and resources**
- **Speak to peers in the cell therapy community**
- **If you experience "Great Doubt", look at the data.**
- **Be open to change as data matures**

Thanks!

CMCF

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