Designing Best Practices for Stability/Biopreservation of Cells and Tissues

ISCT 2013 GRP Workshop

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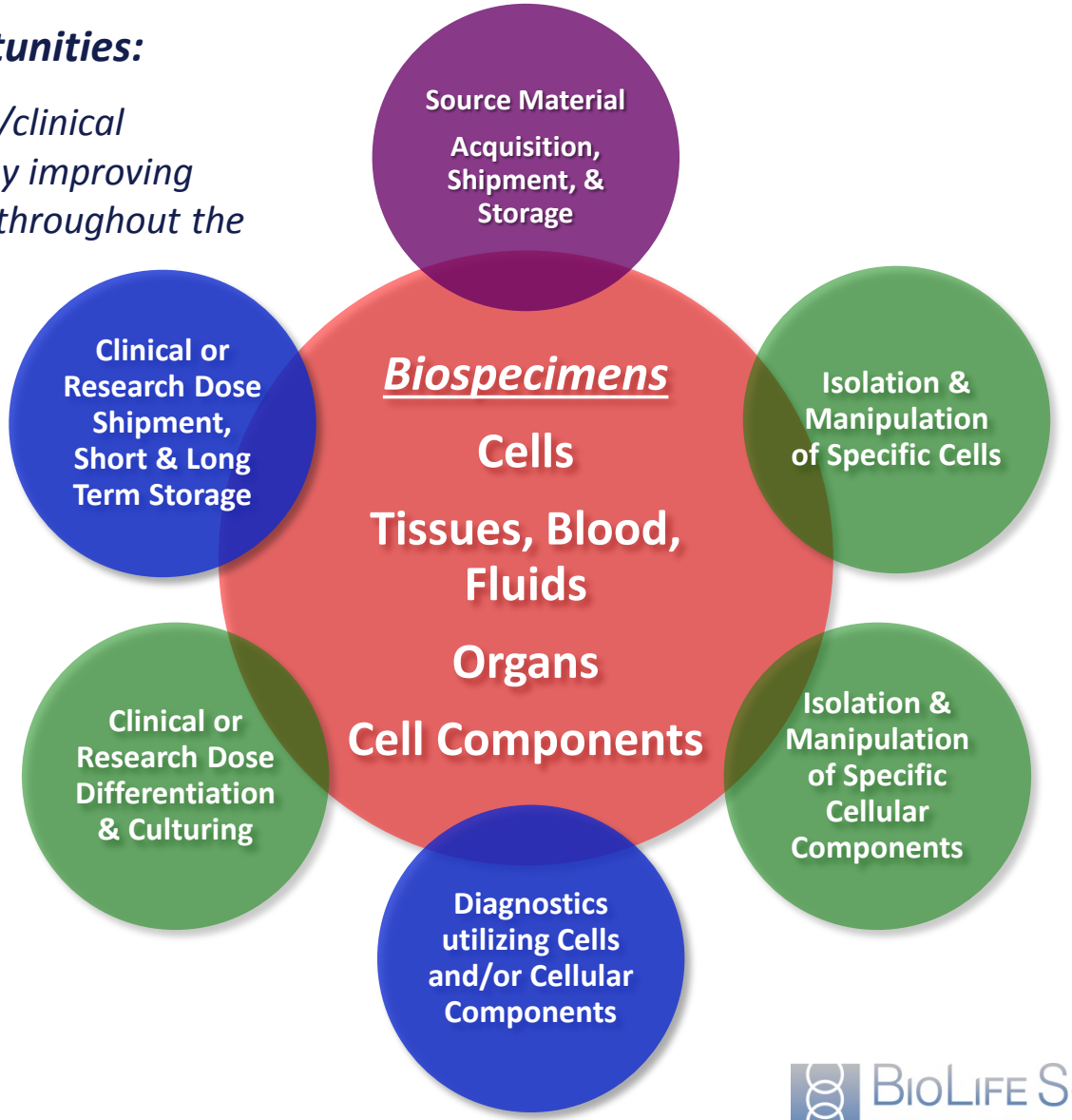
Biospecimen Life Cycle

Biopreservation Opportunities:

Yield, costs, and diagnostic/clinical efficacy can be optimized by improving biopreservation outcomes throughout the cycle



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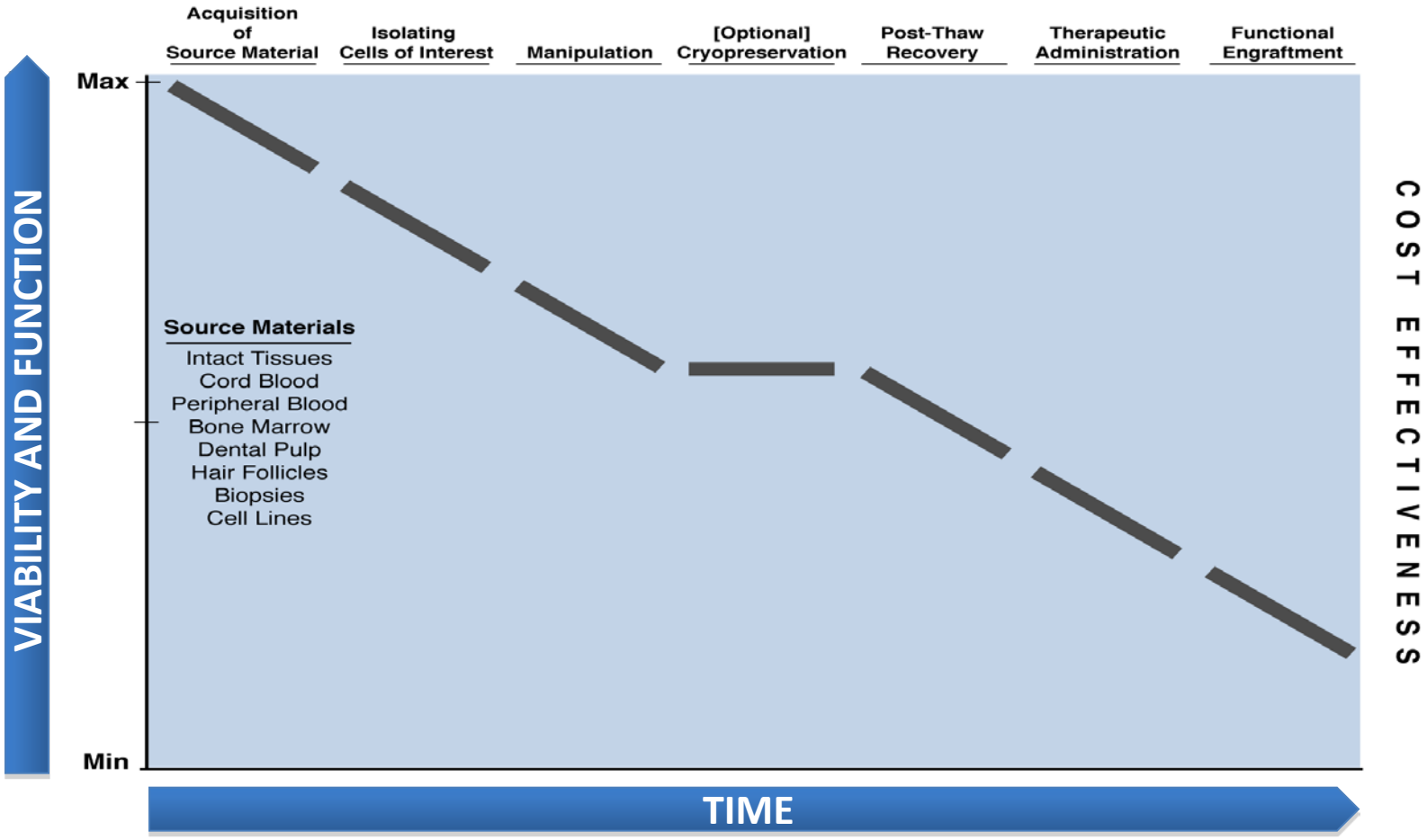
Biopreservation Yield

- **Cascading negative yield impact common to nearly all biopreservation applications**
- **Outcomes heavily affected by lack of in-process efficiencies and optimization**



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Biopreservation System Objectives

Optimize commercial scale manufacturing and the broadest geographic distribution by maximizing:

- **Source material stability (transport time x recoverable yield of isolated cells of interest)**
- **Manufacturing processing time (receipt of source material, cell isolation, manipulation, culturing, packaging)**
- **Final dose stability (longest transport time that provides the highest cell viability, functional recovery, and engraftment)**

Minimize system costs:

- **Direct labor and/or contracted facility time, components and supplies**

Minimize system risks:

- **Process variability, component quality, supply continuity, and contamination exposure throughout the workflow process**

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Biopreservation Methods

- ***Ambient Storage***
  - *Extremely limited capacity for effective biopreservation for most systems*
- **Hypothermic Storage**

  Utilized for short-term storage and shipment of cells, tissues, and organs
  - Refrigerated temperatures around 2-8^{o}C
  - Reduced cellular metabolism
  - Allows for an effective and user friendly transport method
- **Cryopreservation**

  Long-term suppression of biological function
  - Cessation of cellular metabolism
  - Restoration of structure and function is possible after decades of liquid nitrogen storage (-196^{o}C)

Efficacy can vary depending on whether tissue or cells, cell/tissue type, and methods.

- ***Vitrification***
  - *"Ice-free" conversion to glassy state*
  - *Generally requires high concentrations of cryoprotectants, often multiple steps for addition and washing*
  - *Positive reports in the literature in some cell and tissue systems*
- **Anhydrobiosis**
  - Dry state preservation
  - Promising research in some cell systems such as red blood cells

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Biopreservation Impacts COGS

Acquisition and transportation of source material

In-process storing or freezing isolated or manipulated cells

Culturing up to a clinical dose

Storage and shipment of final cell product dose to patient

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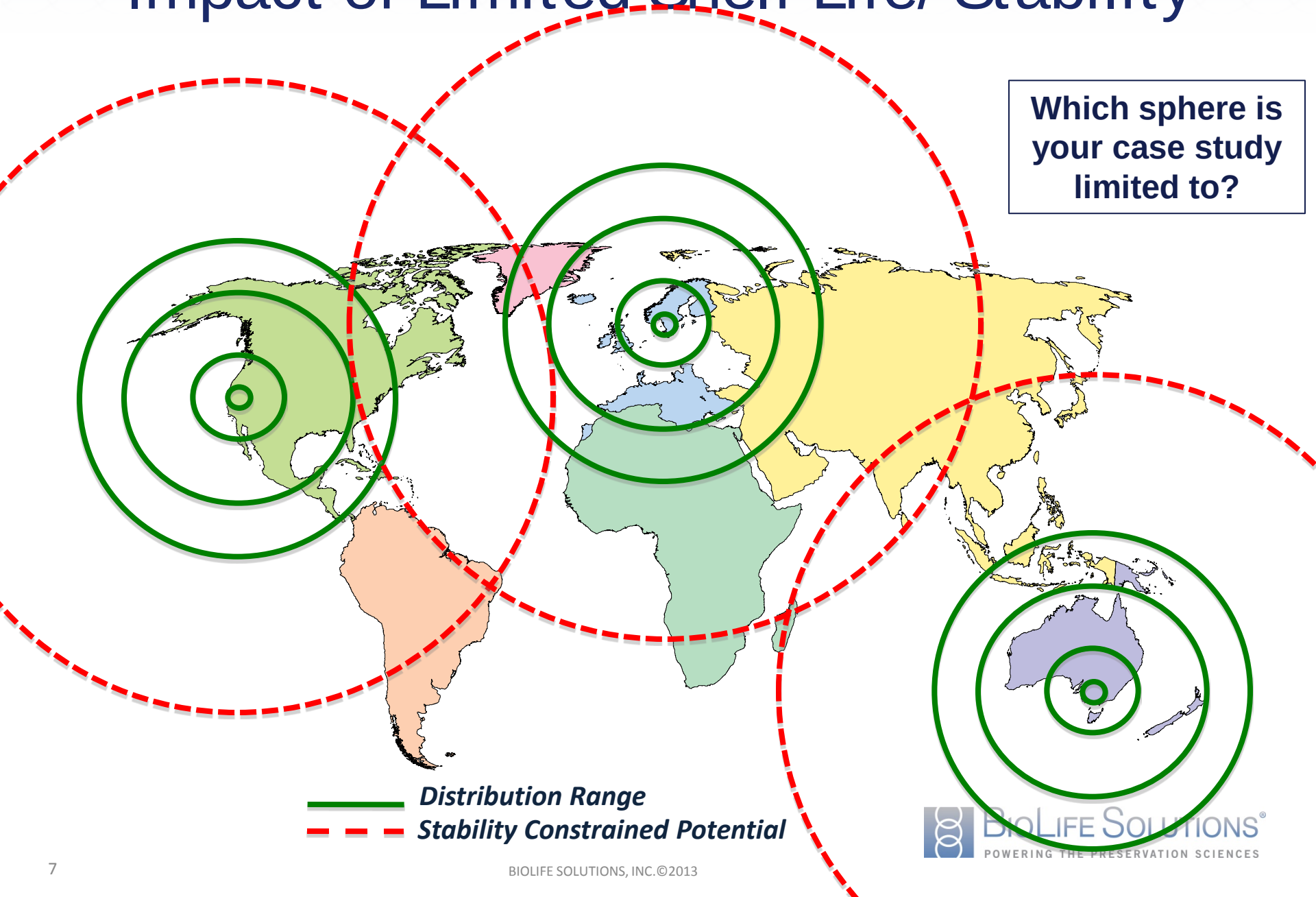
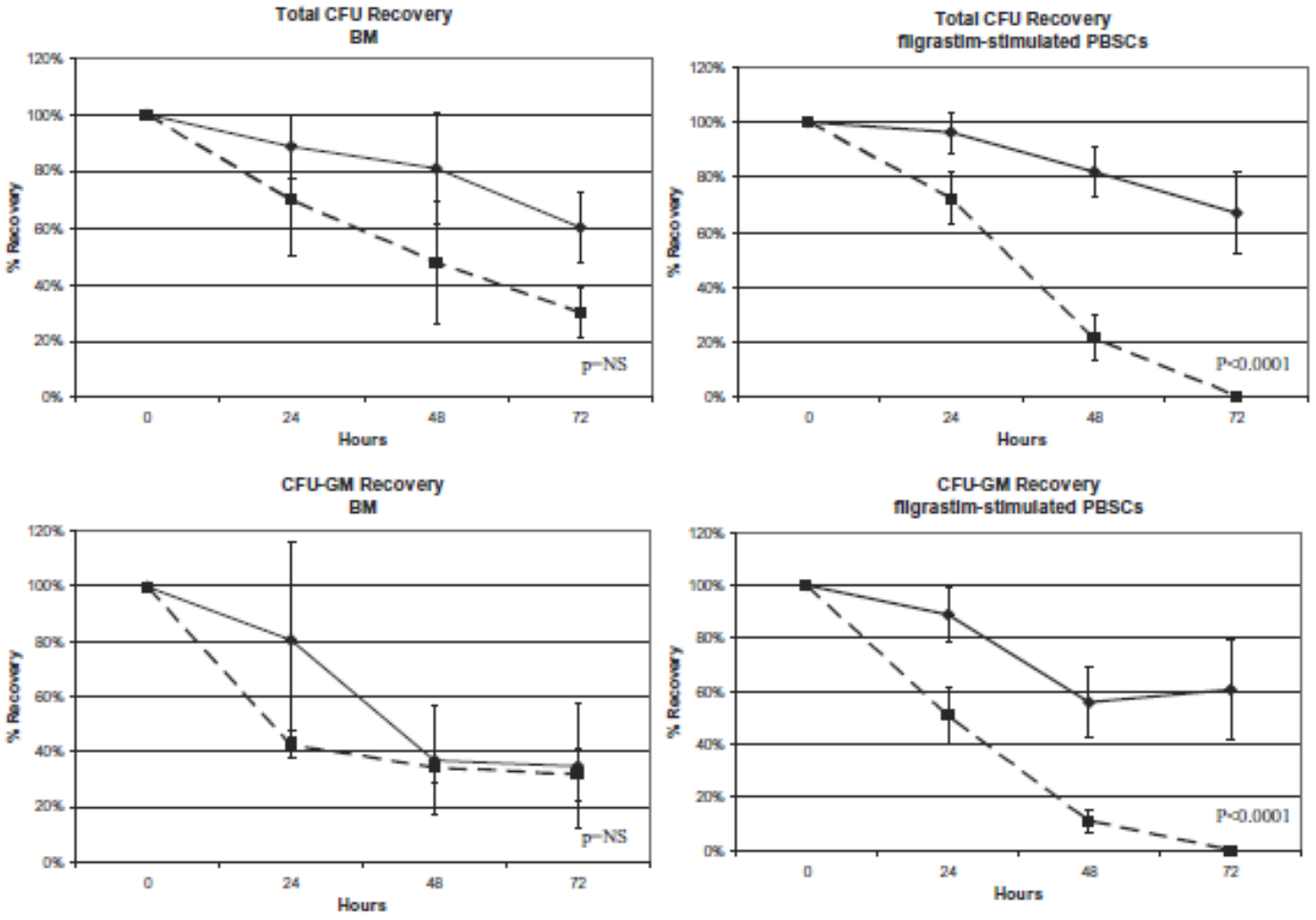
Impact of Limited Shelf Life/ Stability



Effect of Non-Frozen Storage on Blood and Marrow



Kao - 2010

Fig. 3. Comparison of the CFU recovery over 72 hours of storage at 4 (◆) and 20°C (■). Samples containing 1×10^5 cells were taken from BM and PBSC products and plated for CFU-GM, BFU, and CFU-GEMM counts. Total CFU is the sum of all three types of colonies. Total CFU and CFU-GM recovery is calculated based on the comparison of result of each time point to t = 0 hour. These graphs summarize the result (mean and standard error) from five BM and four PBSC products.

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Effect of Non-Frozen Storage on Isolated Cells

International Society for Cellular Therapy ISCT

Cytotherapy (2009) Vol. 11, No. 1, 79–85

informa healthcare

Transportation of peripheral blood progenitor cell products: effects of time, temperature and cell concentration

Jan Jansen, Pamela L. Nolan, Margaret I. Reeves, Luke P. Akard, James M. Thompson, Michael J. Dugan and Susan G. Hanks

Indiana Blood and Marrow Transplantation and St Francis Hospital and Health Centers, Beech Grove, Indianapolis, USA

Table II. Effect of duration of storage on survival of PBPC at 200×10^9/L and 4°C or 22°C ($n = 3$).

Time (h) and temperature (°C)	Trypan blue	Viable CD34 cells	CFU-GM
24, 4	94.1 ± 1.1* (0.05)	94.6 ± 2.3* NS	62.1 ± 21.7* NS
48, 4	93.2 ± 3.2 NS	92.5 ± 3.8 NS	64.1 ± 9.6 NS
72, 4	87.2 ± 0.4 (0.001)	86.5 ± 4.4 NS	57.3 ± 11.7 (0.01)
96, 4	81.6 ± 4.4 (0.05)	82.4 ± 5.8 NS	56.8 ± 14.5 NS
24, 22	86.2 ± 1.9 (0.02)	74.3 ± 7.5 NS	22.1 ± 9.0 (0.01)
48, 22	72.8 ± 1.9 (0.005)	19.1 ± 15.9 (0.03)	7.3 ± 6.7 (0.005)
72, 22	63.5 ± 8.1 (0.05)	7.5 ± 5.7 (0.004)	6.0 ± 2.7(0.001)
96, 22	39.2 ± 8.4 (0.02)	3.0 ± 2.5 (0.002)	13.0 ± 6.6 (0.006)

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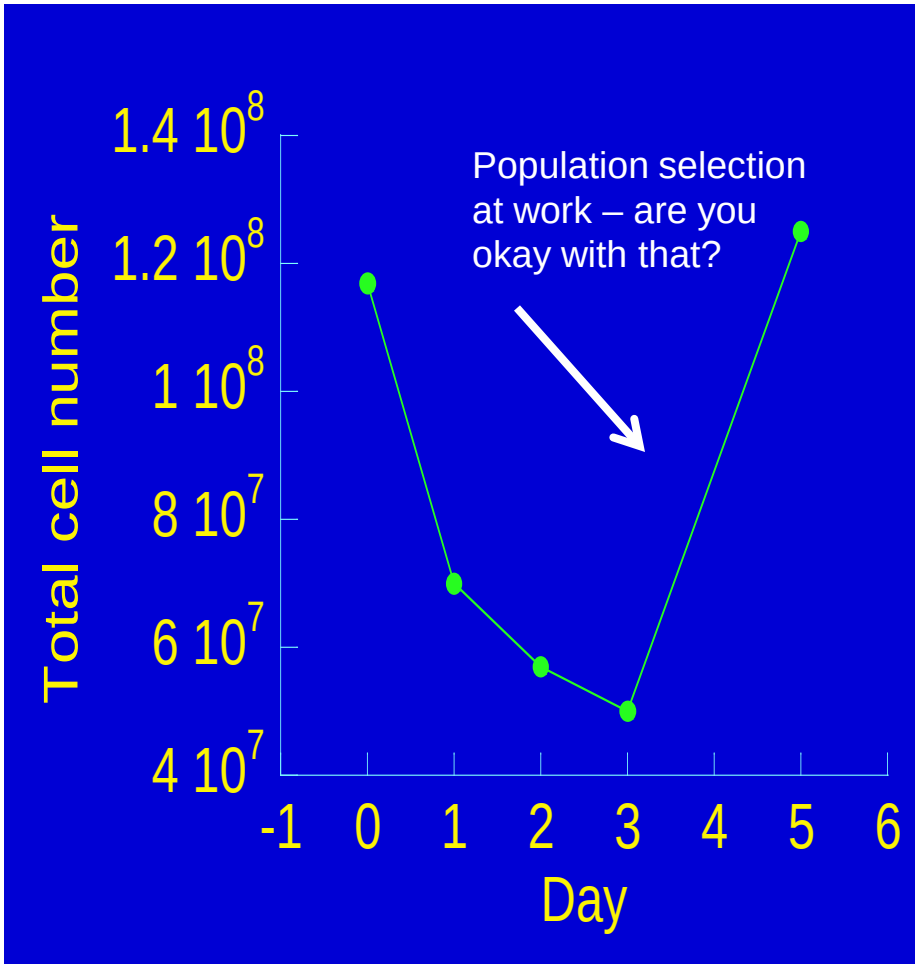
Delayed Onset Cell Death Curve

- Transduced Lymphocytes were frozen using conventional methods (isotonic media, DMSO).
- Cell counts and viability were performed at different times post-thaw.
- Variation in viability with time implies:
  - Timing of post-thaw assessment is critical
  - Other mechanisms of damage may be at play post-thaw than initially understood



Modified from Stroncek,Hubel,Burger 1999

Understand the difference between Perceived Viability and True Viability

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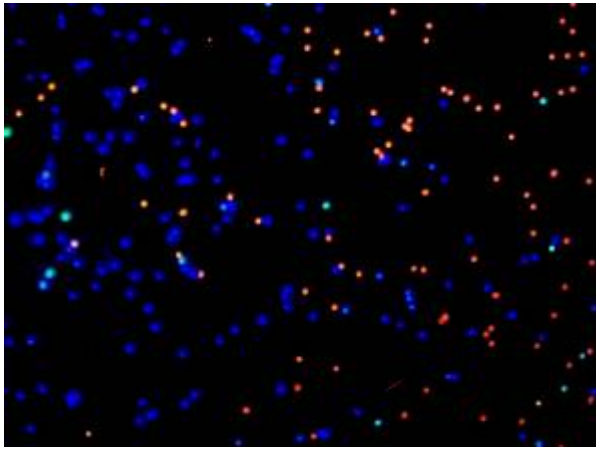
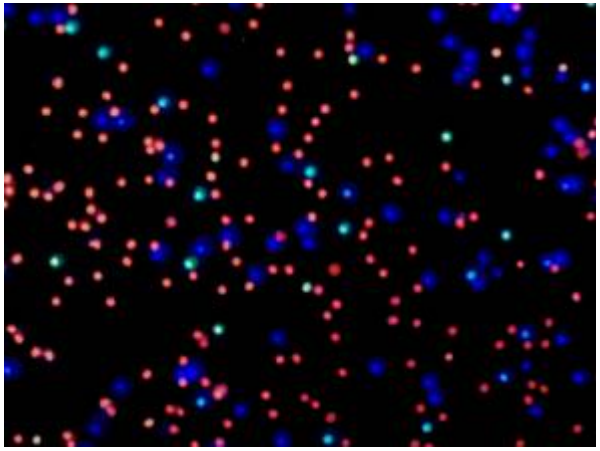
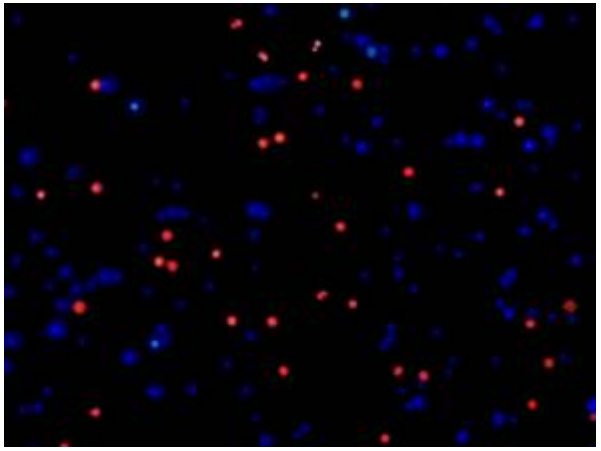
Designing the Assessment Methods – Consideration to Delayed Onset Cell Death



0 Hour



8 Hour



24 Hour

Traditional survival assays do not detect the latent effects of biopreservation

Need to examine samples over time to truly evaluate success (~24 hours post-preservation for recovery of viable cells; later timepoints for potency)

Traditional biopreservation methods (extracellular-like media + serum + DMSO) cannot completely protect cells

PBMC (Peripheral Blood Mononuclear Cells) Cryopreserved traditionally in Standard Culture Media + Serum (7%) + DMSO (10%)

Green = Apoptotic Cells
Red = Necrotic Cells
Blue = Live Cells

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Stability Stresses Occur at Multiple Levels

Genomic

Mitochondrial

Proteomic

Heat Shock Proteins

Thermal Cycling (Transient Warming Events)

Unfolded Proteins

Enzymatic – Caspases, Calpains, Liposomal

Ionic Dysregulation

Etc., etc…..

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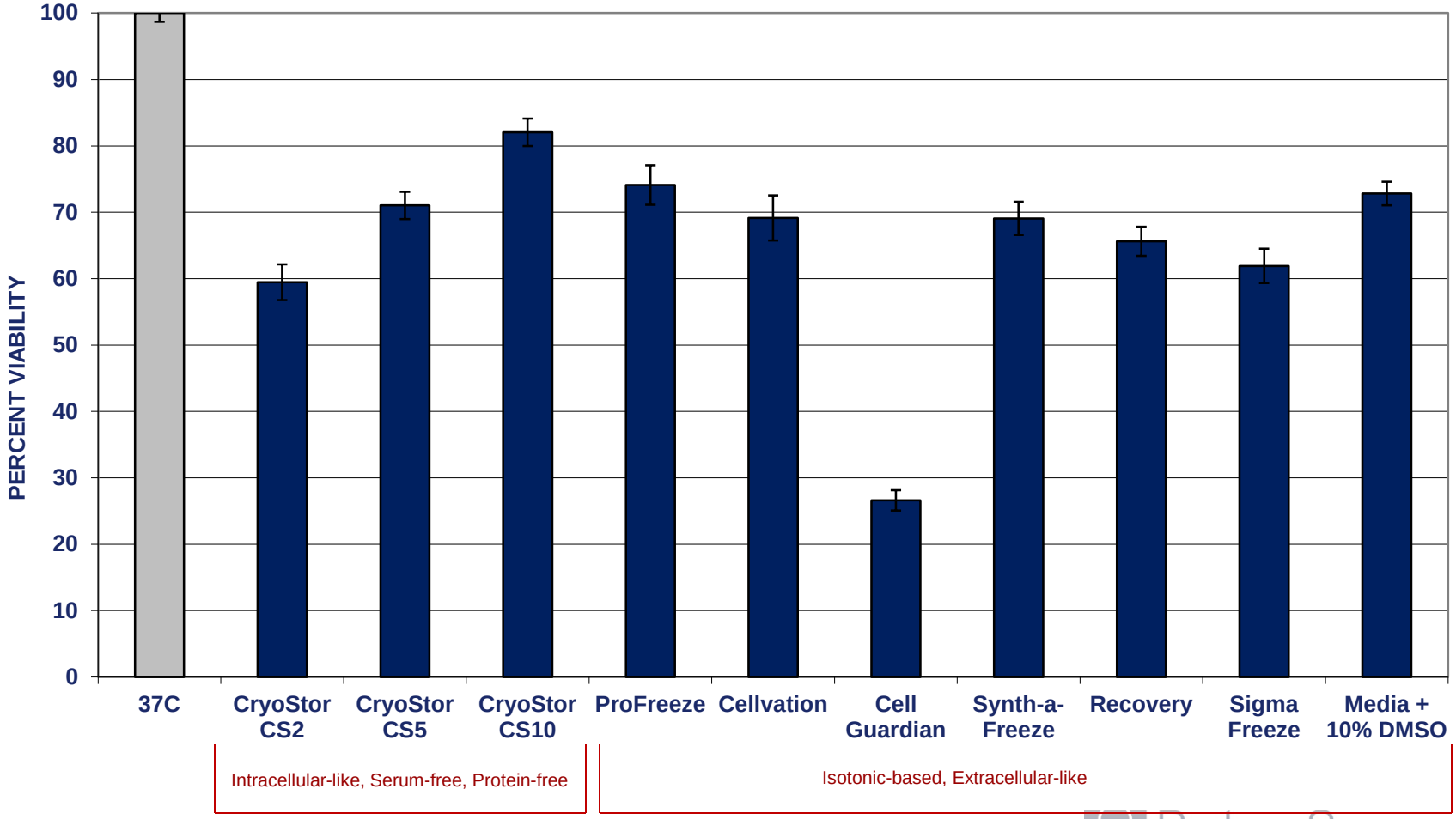
Cryopreservation – Potential Variability

VIABLE RECOVERY OF HUMAN MESENCHYMAL STEM CELLS FOLLOWING CRYOPRESERVATION IN VARIOUS FREEZE MEDIA - 1 DAY POST-THAW ASSESSED WITH ALAMARBLUE



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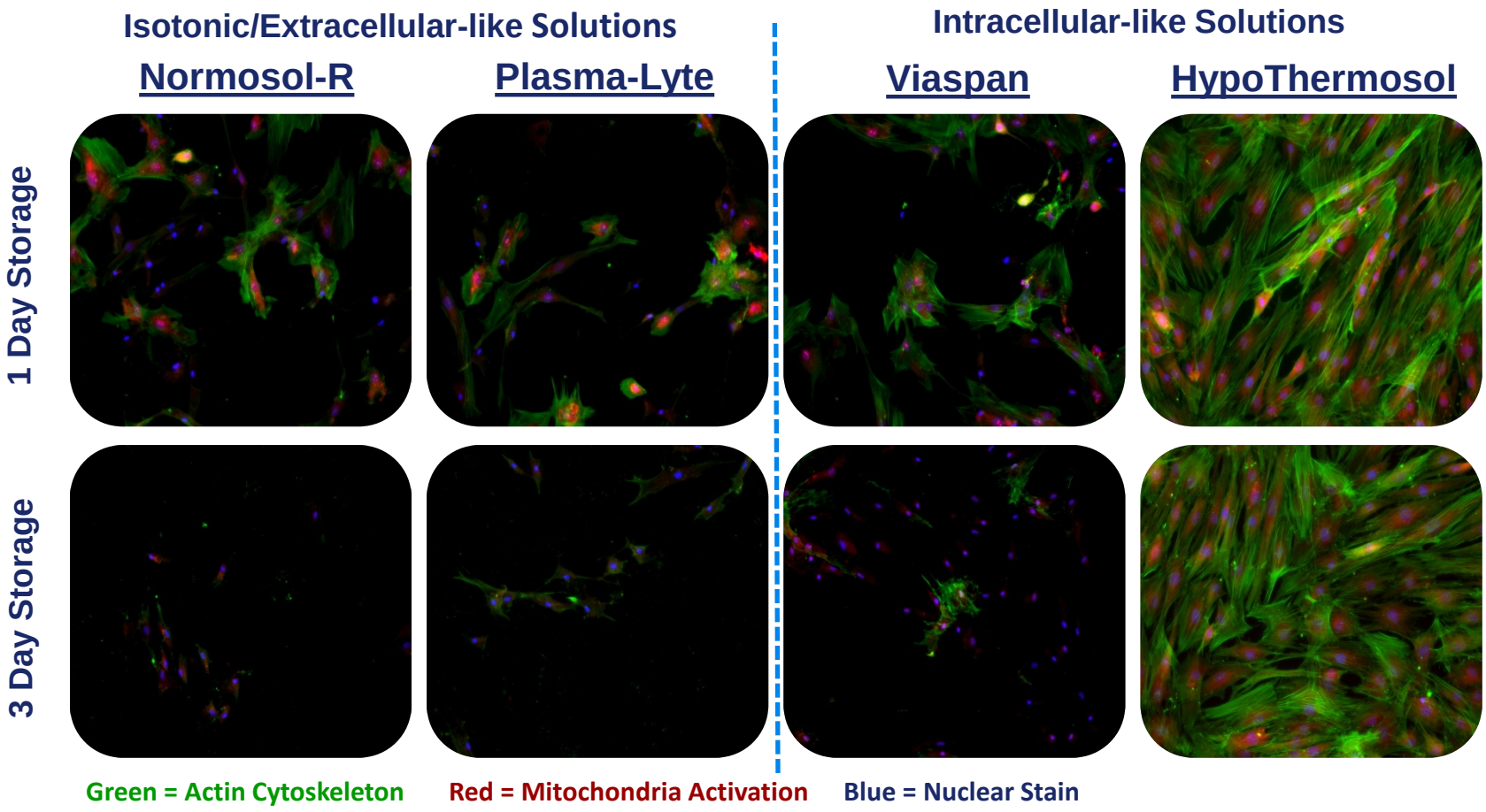
Non-Frozen Shipping/ Storage



What is the Condition of Your Cells When They Arrive for Processing or Reach the Patient?

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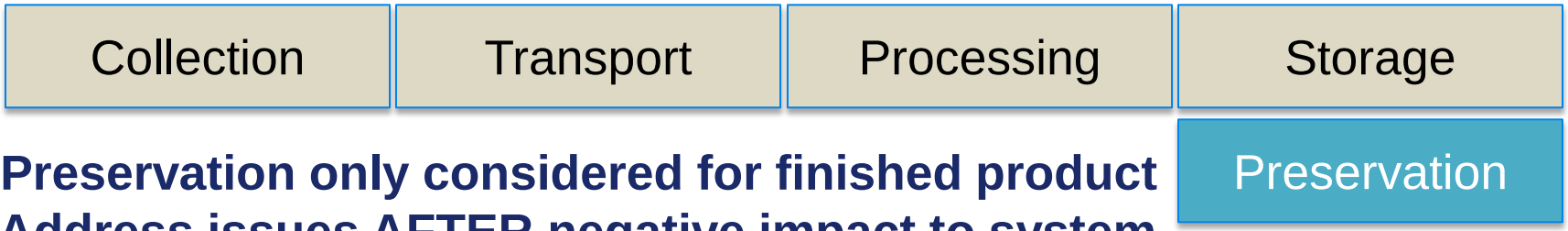
Best Practices Perspectives

Taking Preservation Upstream

Conventional Practice



- **Preservation only considered for finished product**
- **Address issues AFTER negative impact to system**

Recommended Best Practice

- **Preservation considered from source material to finished product**
- **Do Not wait until late clinical or commercial stage to optimize!**

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Quality/ Regulatory Considerations

Biopreservation media solutions are considered Ancillary/Excipient materials

- Ancillary - used in the process and removed prior to clinical application
- Excipient – remains within final cell product at clinical application
- Not a Drug
- Not a Medical Device

Is it cGMP Manufactured?

Are there FDA Master Files available for cross reference?

Does it have Fully Defined Components and Finished Product?

- Protein-Free
- Serum-Free
- USP/Multicompendial/Highest-quality components

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Potential Home Brew Impact

- **Traditional in-house formulated biopreservation media products may not be optimized for performance, quality, scalability, and efficiency**
- **Lower component costs, 'free' labor, and limited release testing are often traded off for reduced performance and quality**
- **Overall system performance may be initially and cumulatively limited due to reduced efficacy of home-brew formulations**

- Non-GMP process
- Historical formulation
- Batch variability
- Limited release testing
- R&D grade components

Potential Home-Brew Impact

Poorer quality
Inefficient workflow
Limited preservation
Clinical effects
Low scalability
Not optimized for best practices

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How Assist Qualification of Proprietary Reagent -

1. Gap analysis (data-driven) – current system or outline desired system. Is there a need/desire for improved biopreservation within the system?
2. Does your biopreservation system currently use serum or proteins, manually make up a cocktail, etc.?
3. Decision to qualify the new reagent with scientific feasibility studies (multiple assays and multiple time points)

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How Assist Qualification of Proprietary Reagent -

4. Quality/Regulatory considerations –

   grade of components

   whether GMP manufactured

   what is the Regulatory footprint

   are there FDA Master Files available

   if outside US/FDA, how share proprietary information – CDA, consult

   is an audit required

   should there be a Quality Agreement

   can the biopreservation solution also be used as the excipient vehicle for the cell/tissue therapy product

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How Assist Qualification of Proprietary Reagent -

5. Business considerations –

   what is the cost of the reagent

   what is the value proposition to the cell/tissue product

   what is the security of availability – License/Escrow Agreement

   should there be a Supply Agreement

There should be consideration whether the reagent is pre-formulated ready-to-use (pre-tested, GMP) or a cocktail requiring user manipulation (lot-to-lot risks). The manipulation part falls under Quality scrutiny for impact to the process, but the pre-formulation might save time/labor and need to be considered for monetary savings.

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Stability/ Biopreservation Process
Excipient/ Ancillary Reagent Considerations

Ease of Use	• Pre-formulated with DMSO • Sterile filtered/ USP sterility tested • Ready-to-use packaging	• No end user manipulation required • Reduced contamination risk • Easily integrates into current protocols
Performance	• Unique ionic balance • Multiple pH Buffers • Antioxidants • Mitigates Apoptosis & Necrosis • Osmotic support components • Defined energy substrates	• Improved viability • Improved quality • Reduced post-preservation cell death • Faster/better attachment • Better yields of functioning cells
Quality & Regulatory	• Serum-Free and Protein-Free • USP components • cGMP production • Bioassay and Biosafety tested • FDA Master Files	• Reduced contamination risk • Fully defined media • Lot-to-Lot consistency • Quality assurance • Reliable performance • Support for clinical applications

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