

*Impact of Dosage Standardization
On Stem Cell Clinical Trials
Operations and Support Needs*

OCT-NE 2022

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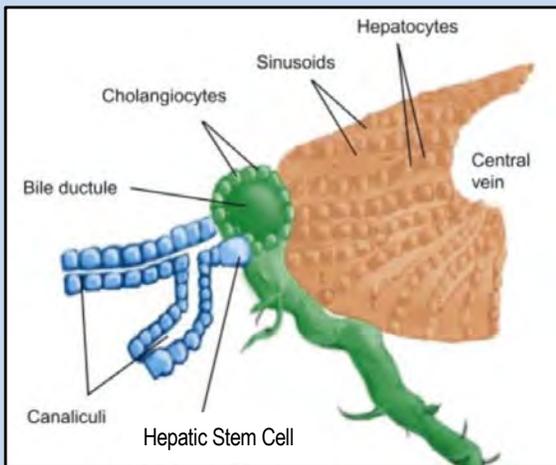
Topics Addressed

- A brief introduction to stem cell clinical trials and stem-gene clinical trials
- A review of current practices for treatment dosages in stem cell clinical trial development and operations
- Predictions for the regulatory horizon of dosage standardization for stem cell clinical trial development
- Emerging technologies for rapid dosing of tissue stem cell treatments that will impact stem cell clinical trial design and operations

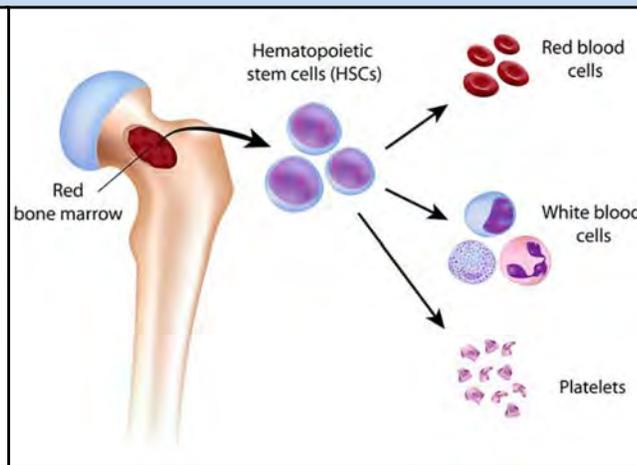
Therapeutic Tissue Stem Cells

- REGENERATIVE MEDICINE -

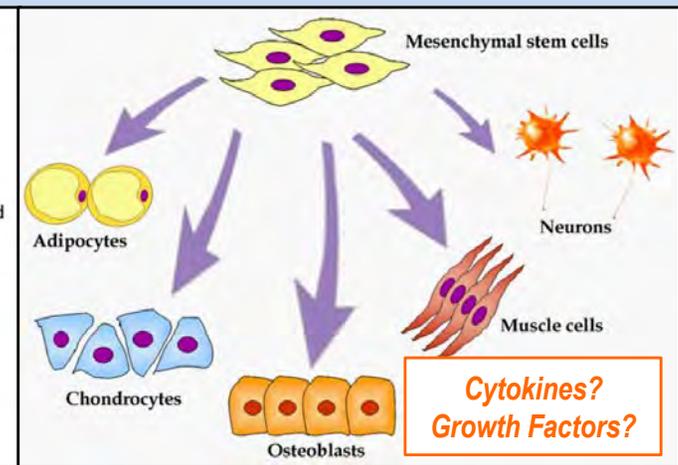
LIVER



HEMATOPOIETIC



MESENCHYMAL



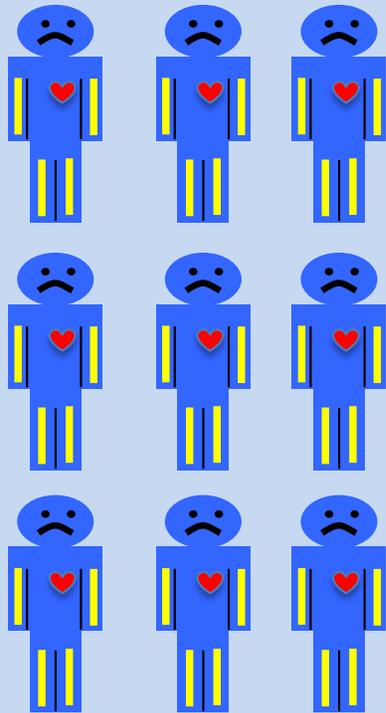
RESIDENT
TISSUE-SPECIFIC
REPLACEMENT

RESIDENT & MOBILE
TISSUE-SPECIFIC
REPLACEMENT
(STIMULATORY?)

RESIDENT & MOBILE
TISSUE-VERSATILE
STIMULATORY

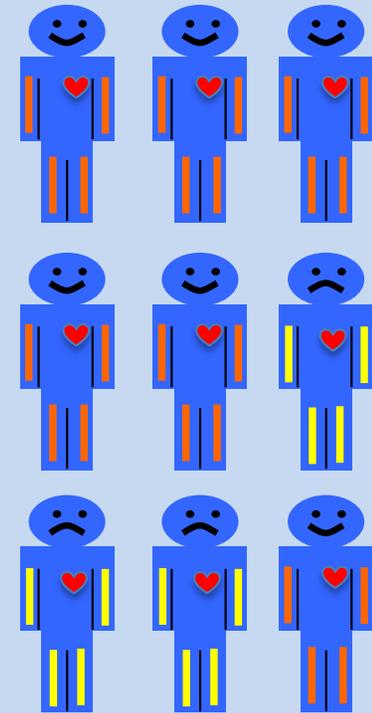
**NOT TO BE CONFUSED WITH EMBRYONIC STEM CELLS (ESCS) OR
INDUCED PLURIPOTENT STEM CELLS (IPSCS)**

Tissue Stem Cell Clinical Trials



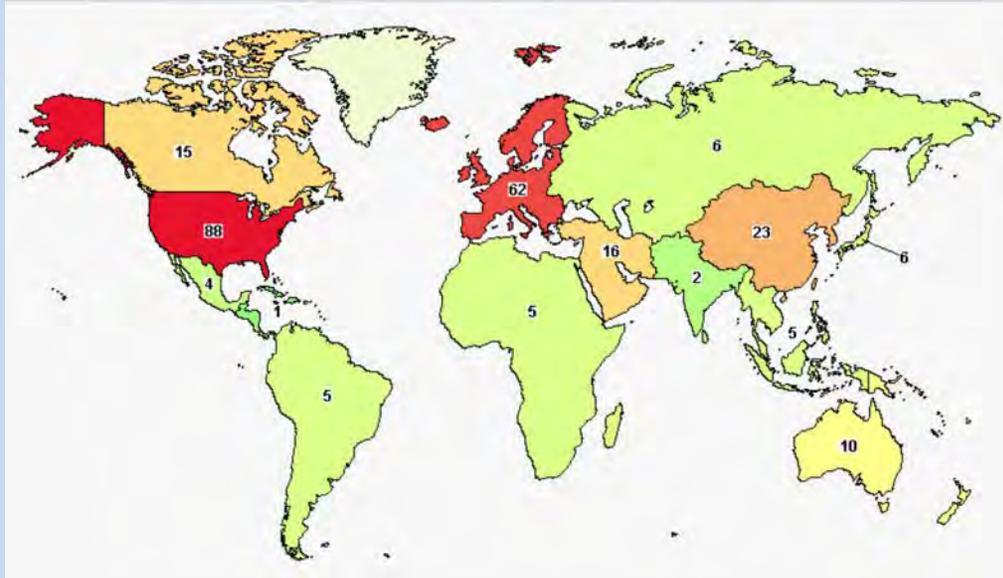
Diseased

1. Natural Tissue Stem Cells (e.g., HSCs, MSCs)
2. Genetically Engineered Tissue Stem Cells "Stem-Gene" (e.g., HSCs)



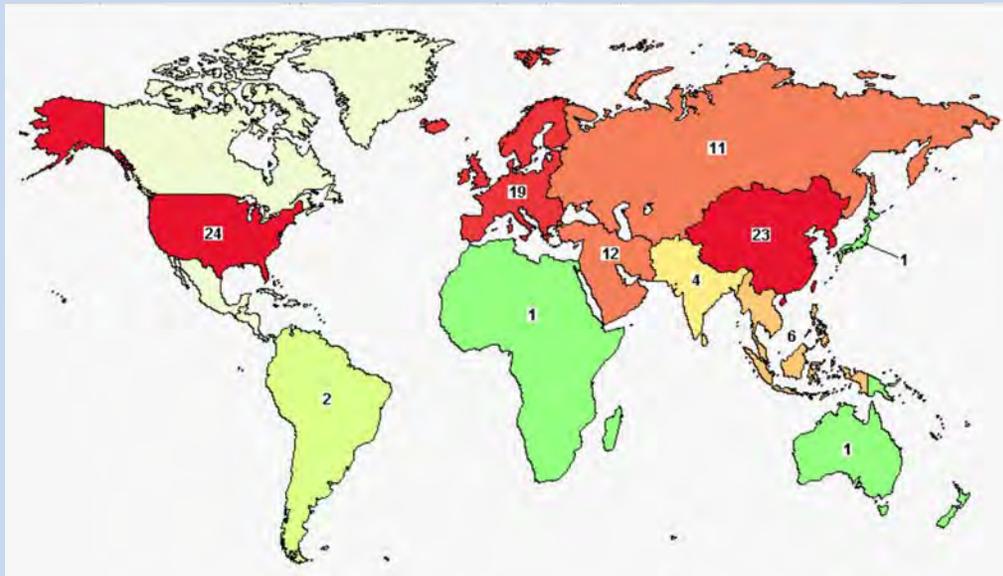
Long-term Cure

Stem Cell Clinical Trials Activity



2017-2022

“HSC transplantation”
182 trials worldwide

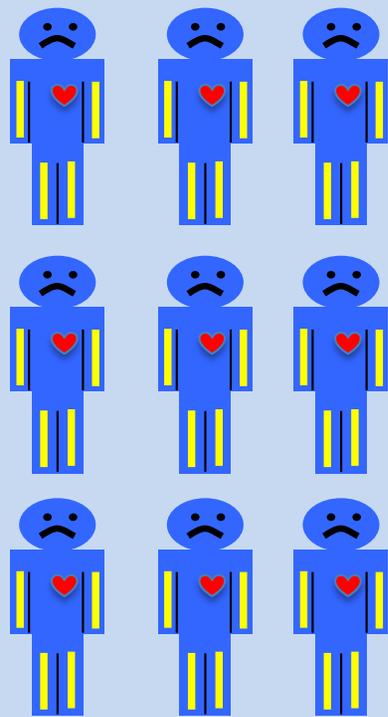


“Mesenchymal stem cells”
102 trials worldwide

<https://clinicaltrials.gov>

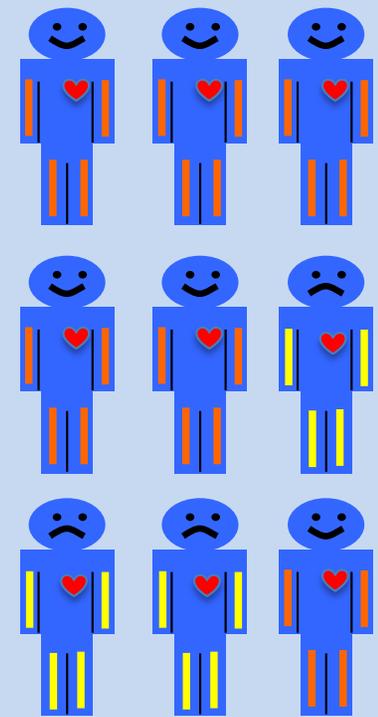
A hidden problem in stem cell medicine

Autologous Cells (including stem-gene)



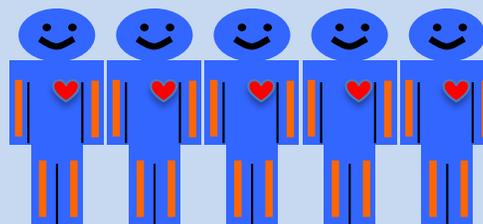
Diseased

DOSAGE?

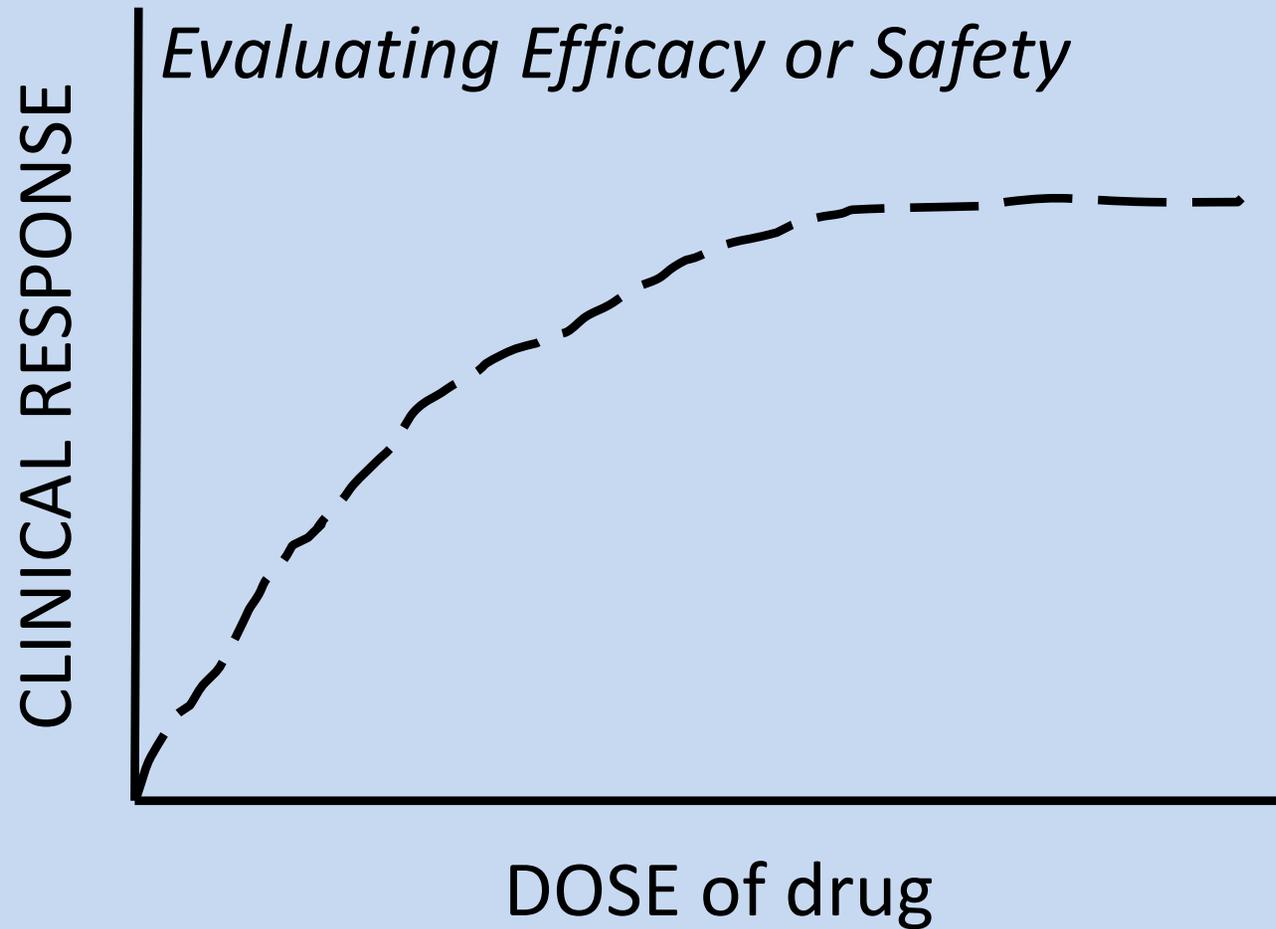


Long-term Cure

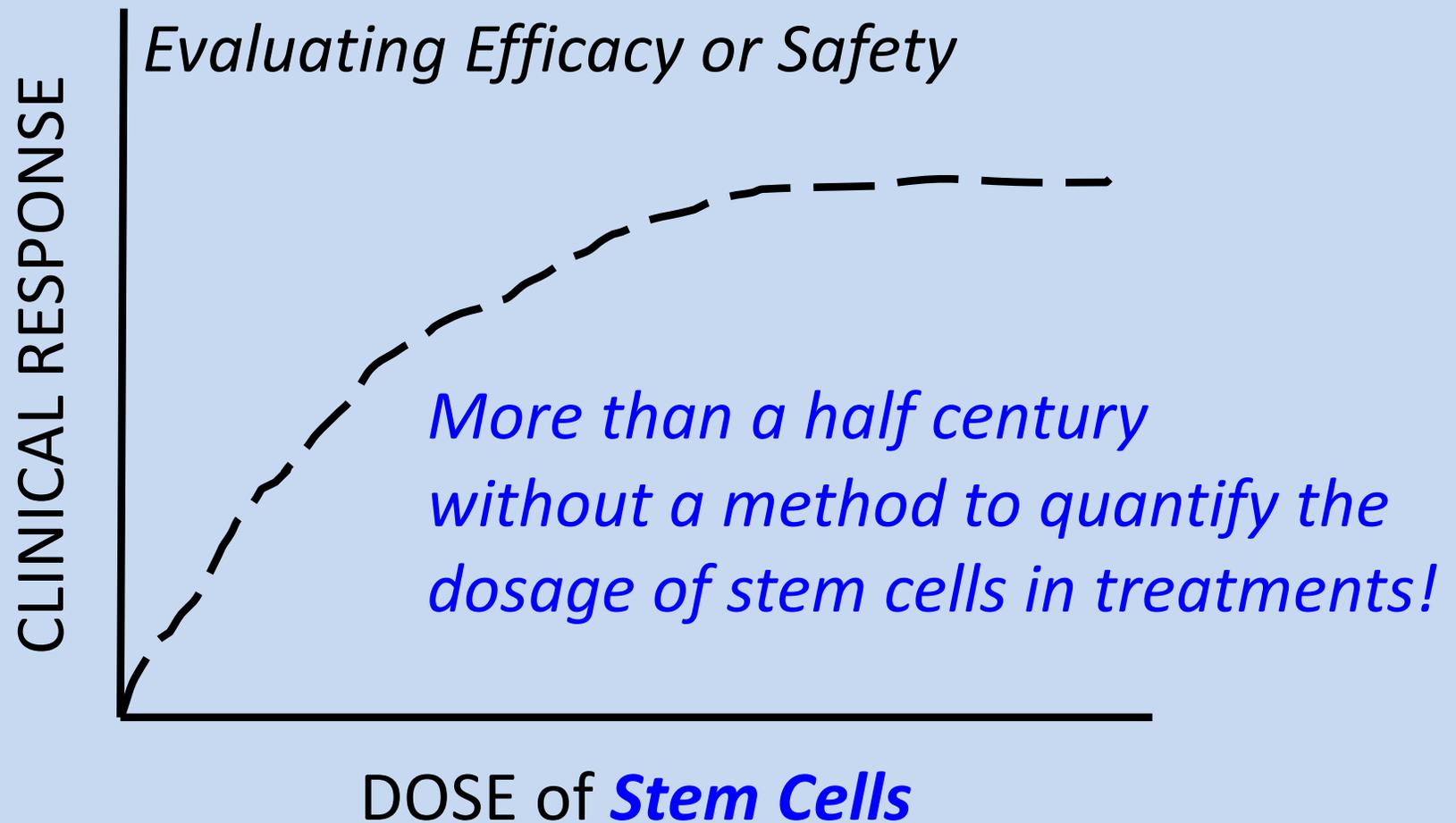
Allogeneic
Cells



Dose Matters in Medicine



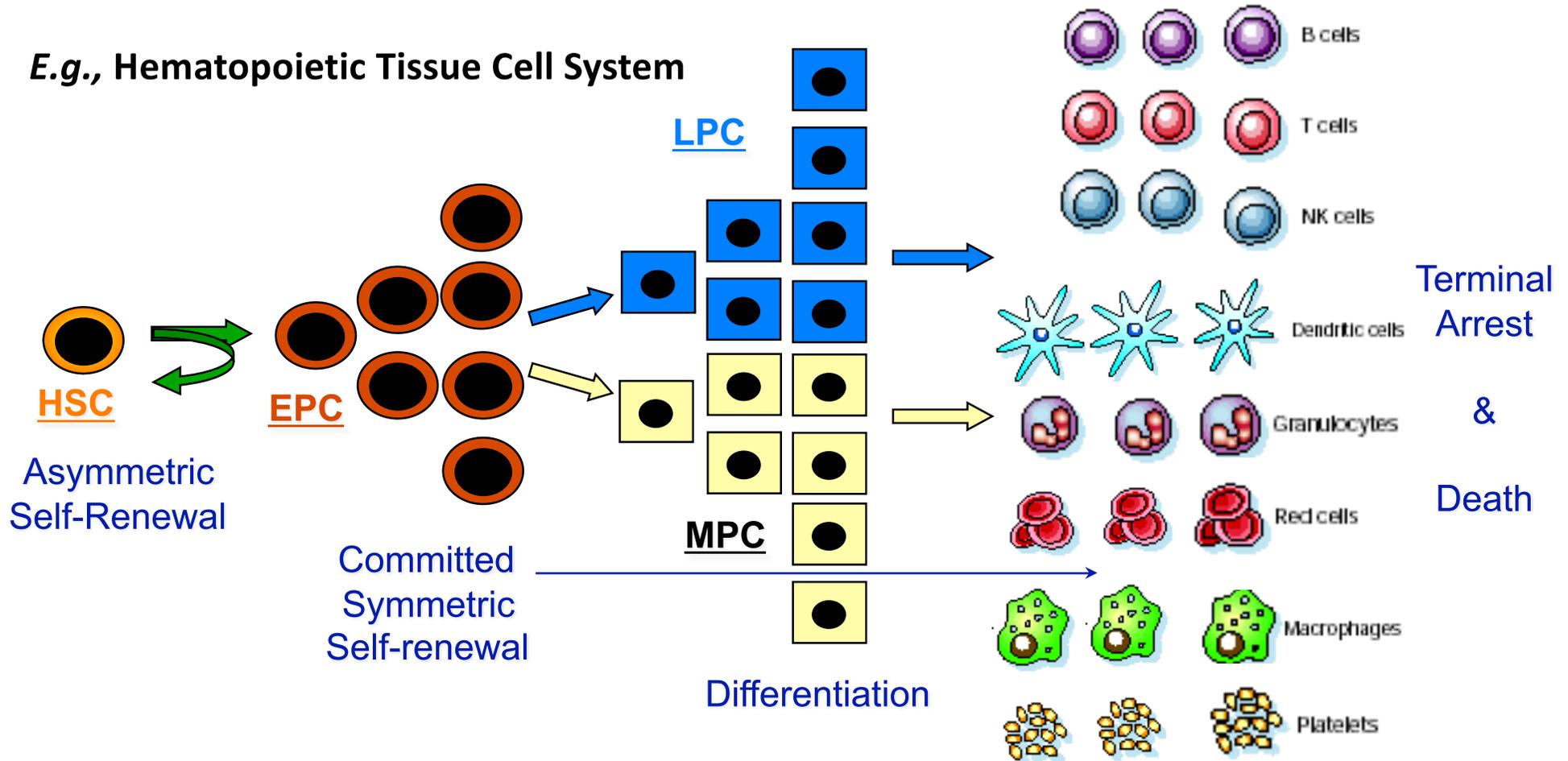
Dose Also Matters in Stem Cell and Stem-Gene Medicine



Tissue Stem Cells Are Hard To Count

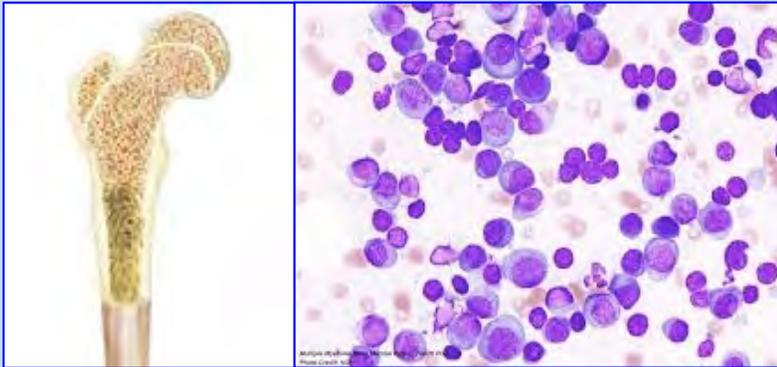
Why? Tissue Cell Subtype Heterogeneity

E.g., Hematopoietic Tissue Cell System

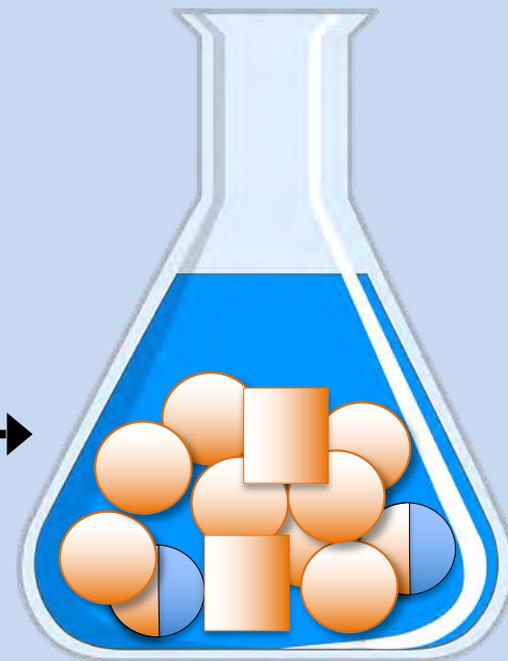


Tissue stem cell preparations are always cell-type heterogeneous

Organs and Tissues



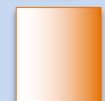
Treatment Preparations



Isolation

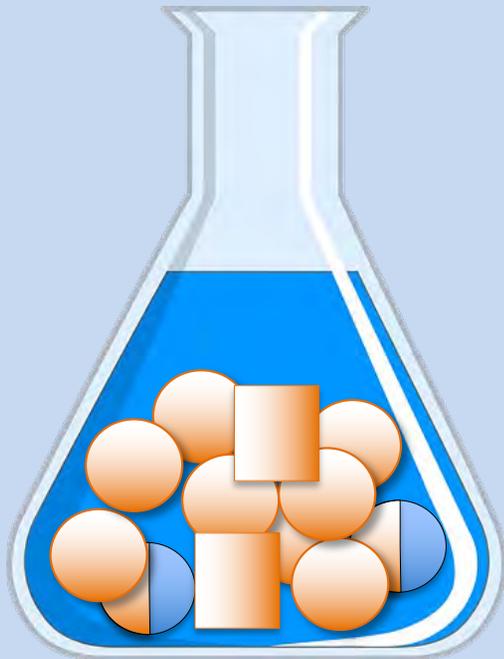
Manufacturing

Supply →

-  = Tissue Stem Cell
-  = Committed progenitor cell
-  = Mature cells

All Tissue Stem Cell Preparations
(e.g., HSCs, MSCs)

The “Stem Cell Dose”



All Tissue Stem Cell Preparations
(e.g., HSCs, MSCs)

The stem cell dose = 2
NOT 12



= Tissue Stem Cell

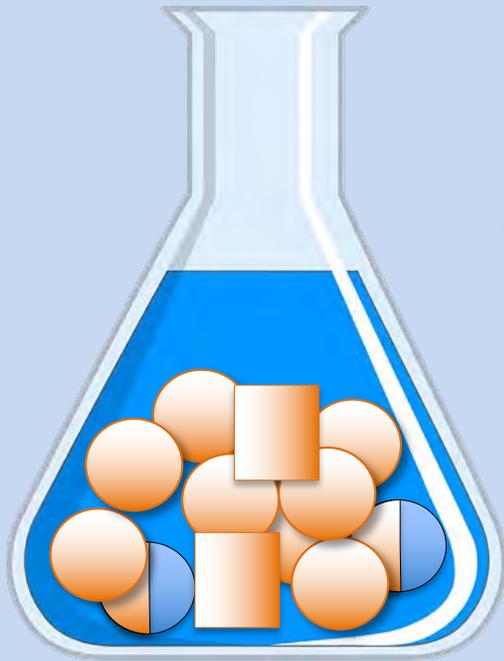


= Committed progenitor cell



= Mature cells

Something to Understand About Tissue Stem Cell Preparations



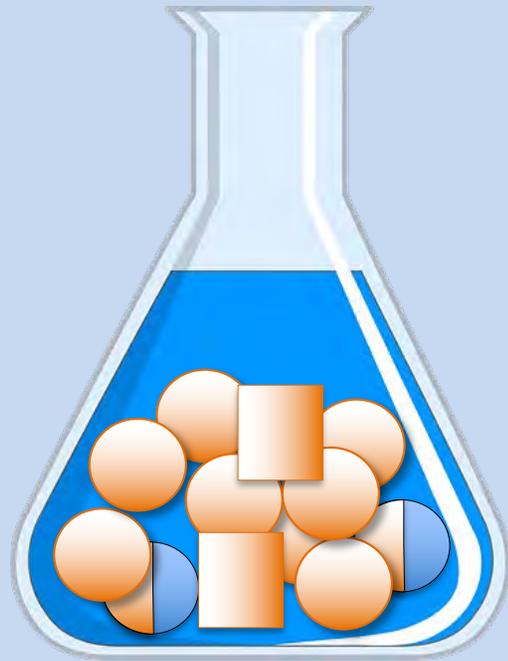
All Tissue Stem Cell
Preparations
(e.g., HSCs, MSCs)

For Stem Cell and Gene Therapies:

-  = Long-lived. Durable cures
-  = Short-lived. No durable cures.

-  = Tissue Stem Cell
-  = Committed progenitor cell
-  = Mature cells

No Previous Method for Quantifying Stem Cell Dosage Routinely



All Tissue Stem Cell Preparations
(e.g., HSCs, MSCs)

Common Misconceptions

1. Flow cytometry (*No = 10*)
No tissue stem cell specific-biomarkers
2. Colony Forming Unit (**CFU**) Assay (*No = 10*)
3. Enzyme-specific assays (*No = 10*)
4. SCID mouse repopulating cell (**SRC**) assay
Yes = 2, but impractical!



= Tissue Stem Cell



= Committed progenitor cell



= Mature cells

SRC Assay? No, too impractical

Yes, stem cell dosage = 2

But: 1) only available for HSCs



All Tissue Stem Cell Preparations
(e.g., HSCs, MSCs)



2) One count requires 30-40 mice!

3) Takes 16 weeks to complete!

Too expensive, protracted, unreliable

IMPRACTICAL for routine use

Why Stem Cell Dosage Is Needed

Stem Cell Suppliers

- For monitoring and optimizing tissue stem cell biomanufacturing
- For engineering tissue stem cells for stem-gene therapies

Stem Cell and Stem-Gene Clinical Trial Sponsors

- Knowing stem cell dosage for development and treatment
- Increasing statistical power to detect tissue stem cell efficacy

Pharma and Biopharma Sponsors

- Drug evaluations for stem cell-active and stem cell-toxic drugs

<https://asymmetrex.com/a-stem-cell-count-would-have-made-it-better/>

Critical clinical needs for stem dosage

Est. >40,000 umbilical cord HSC transplants in 25 years.

However, a continuing 12-18% engraftment failure rate attributed to insufficient HSC dosage.



Many studies and trials focused on increasing HSC dosage.

Chronic Organ Failure Is A Major, Painful Cost for Pharma

- *About half of drug failures in phase II and phase III due to safety (20-30%) are caused by **chronic organ failure**.*
- *The U.S. Pharma industry loses **\$4-5 billion** each year on these late phase drug development failures.*

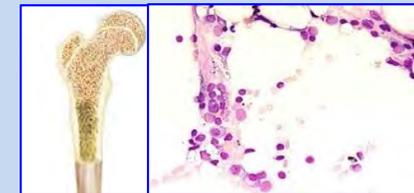
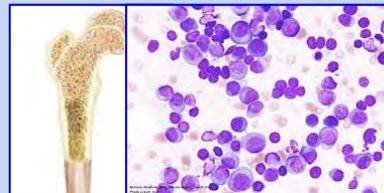
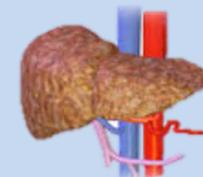
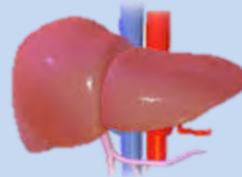
Half of Chronic Organ Failures Are Caused By Tissue Stem Cell Toxicity

Examples

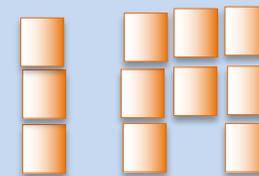
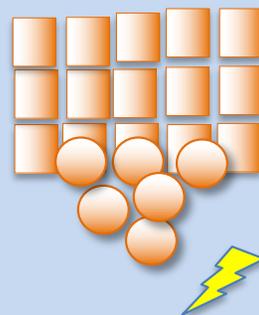
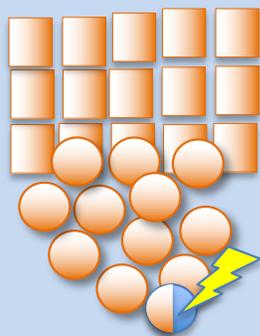
Liver

Normal

Failure!



Bone Marrow



 = Stem Cell Toxin

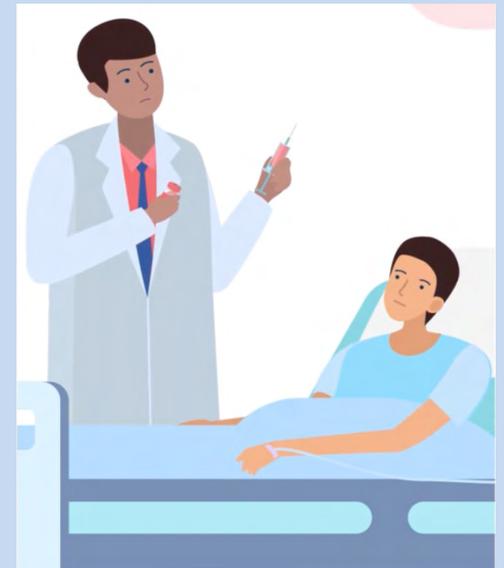
Current Technologies for Early Detection of Chronic Organ Failures Are Animals, Trial Subjects, And Patients

Presently, not detected until...

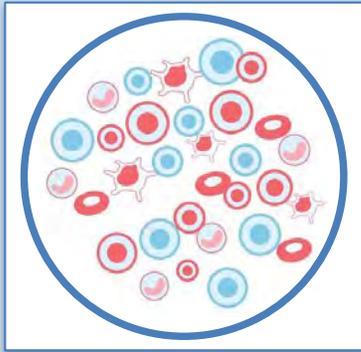
animal studies  (maybe),

phase II and III clinical trials,

or even later, post-marketing.

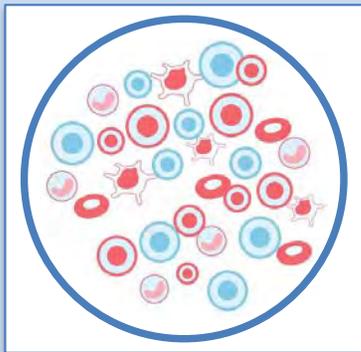


For Pharma Sponsors and Tox CROs: If there were a means to quantify stem cells...



Control
----->

**NORMAL STEM
CELL FRACTION**



SC-toxic Drug
----->

**REDUCED STEM
CELL FRACTION**

**Primary human
tissue cell cultures**

*Would be much faster and
much less expensive!*

The Regulatory Landscape for Stem Cell Clinical Trials Is Changing



2020 FDA SCB Needed Standards

Standards Needs - Clinical Trials

STANDARDS DEVELOPMENT FOR
REGENERATIVE MEDICINE THERAPIES

Clinical Trials

C21 CLINICAL TRIAL INTERPRETATION WITH UNKNOWN CELL-SPECIFIC DOSES

 Cell Therapy  Gene Therapy  Tissue Engineering

FUNCTIONAL AREAS

Cell dose is a measure of the viable cells present in a given treatment, which can vary within a trial and across trials for different therapies.

CHALLENGE: The mechanisms for cell activity are complex and poorly understood, and cell counts may vary over time, which makes it difficult to count cells and establish standard, effective doses and routes of administration (ROA) in clinical trials. This leads to inconsistent trial results that are hard to interpret and replicate and may not be sufficiently reliable to progress to the next phase of clinical trials.

-  Diagnostic and Quality Control Standards
-  Analytical & Testing Methods Standards
-  Product Quality and Characterization Standards
-  Production and Compliance Control Standards
-  Good Practice Standards
-  Clinical Trial Standards

POTENTIAL FOR STANDARDIZATION

STANDARD OBJECTIVE

Broaden **understanding of cell activity and variation over time** to establish guidelines to identify reliable mechanisms for administering safe, efficacious doses.

POSSIBLE AREAS TO STANDARDIZE

- Cell counting methods/technologies
- Optimal timing for dose assessment
- Qualifying ROAs
- Dose preparation methods

RELATED EFFORTS

- Efforts around cell counting (including an [SCB standard advancement project](#)) can ensure accurate counts are measured when comparing doses across trials.
- USP published a [CD34+ Cell Enumeration System Suitability Reference Standard](#), as well as [USP chapter <177>, Flow Cytometric Enumeration of CD34+ Cells](#).



NEXT STEPS

- Conduct comparative ROA and dosage studies.
- Assess common causes of inconsistent doses.

Needed: Stem Cell Dosage Technology For Standardizations

Stem Cell Supply Products

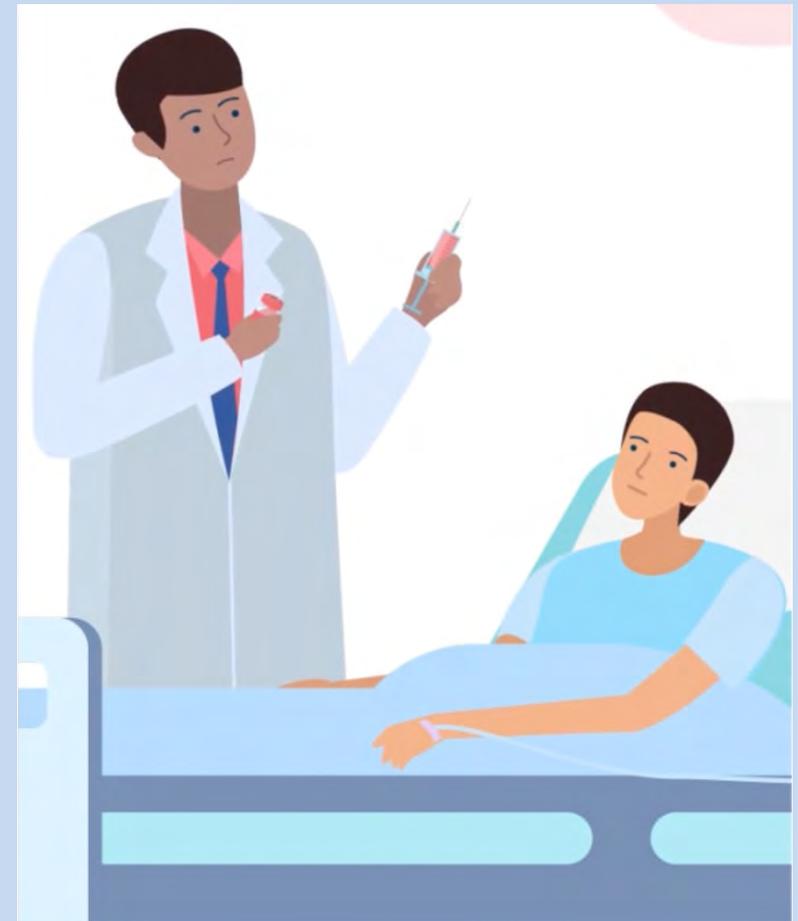
- Donor bone marrow HSCs
- Donor apheresis HSCs
- Donor bone marrow MSCs

- Donor tissue stem cells
 - Cornea stem cells
 - Liver stem cells

- Umbilical cord blood HSCs
- Umbilical cord tissue MSCs

- Placental tissue stem cells
- Amniotic membrane stem cells

DOSAGE?



Between samples, suppliers, patient cohorts, treatment sites, clinical trials

2020 Report of a First Method to Quantify Stem Cell Dosage Routinely

Open Access

Research Article

A Computational Simulation Technology for Specific Counting of Perinatal and Postnatal Human Tissue Stem Cells for Transplantation Medicine

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Special Issue: Isolation and Characterization of Adult Therapeutic Cells

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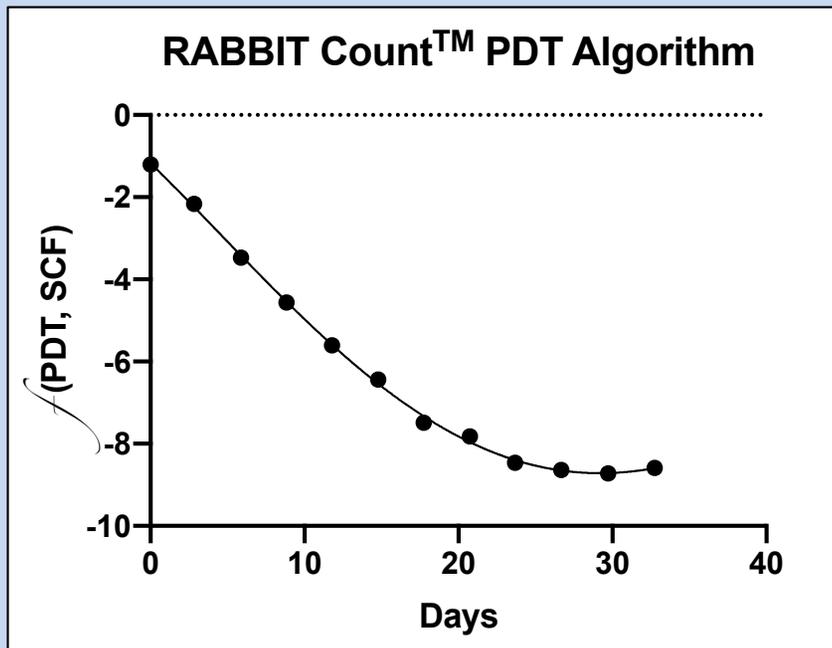
“Kinetic Stem Cell (KSC) Counting”

<https://asymmetrex.com/wp-content/uploads/2020/09/obm.transplant.2003117.pdf>

Rapid Stem Cell-Counting Algorithms

Operation:

1. Culture sample for 72 hours.
2. Enter total cell count data into online rapid-counting algorithm calculator.
3. Obtain stem cell dosage instantly.



Note: Data for CD34⁺ umbilical cord blood HSCs

SCF, stem cell-specific fraction; **PDT**, population doubling time

Human stem cell dosages quantified to date

- Bone marrow hematopoietic stem cells¹
- Mobilized peripheral blood hematopoietic stem cells^{1,2}
- Umbilical cord blood hematopoietic stem cells^{*1,2}
- Umbilical cord tissue mesenchymal stem cells
- Bone marrow-derived mesenchymal stem cells
- Adipose-derived mesenchymal stem cells
- Oral-derived mesenchymal stem cells
(bone, gingival, dental pulp)
- Liver hepatic stem cells
- Lung interstitial stem cells
- Cornea stem cells
- Amniotic membrane stem cells

1. CD34⁺-selected

2. Unfractionated

Purple: Approved therapies and clinical trials

Red: Clinical trials

A growing portfolio of online calculators



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ONLINE RAPID TISSUE STEM CELL COUNTING CALCULATOR PORTALS

GENERIC RAPID-COUNTING CALCULATOR PORTALS

Asymmetrex's RABBIT Count algorithms for rapid counting of tissue stem cells are provided as online calculator portals. Calculator portals are now available for the following generic tissue stem cell types. *Contact Us* to set up your own online *Rapid-Counting Calculator Portal*.

- Unfractionated human umbilical cord blood HSCs
- Human umbilical cord blood CD34+ HSCs
- Human bone marrow CD34+ HSCs
- Human mobilized peripheral blood CD34+ HSCs
- Human adipose-derived MSCs

Additional calculator portals for a wide variety of tissue stem cells are in development.

CUSTOMIZED RAPID-COUNTING CALCULATOR PORTALS

Asymmetrex® can also develop proprietary online calculator portals for tissue stem cell banking and production processes. *Please inquire*.

Rapid-Counting Calculator Portals are designed for convenient routine determination of the stem cell-specific fraction of cultured tissue cell preparations. A sample calculator portal console is shown below. From simple inputs of 72-hour cell culture total cell count data, the calculator returns the stem cell-specific fraction (SCF) instantaneously.



Counting Instruments Beginning to Appear



CELL CULTURE | COUNTING | IMAGING | FLOW CYTOMETRY | ANALYSIS | **NOW: RAPID TISSUE STEM CELL COUNTING**

CASY Cell Counter & Analyzer-PLUS

Multi-Parameter - Accurate – Reproducible

Cell Counting & Quality Control – *Hand in Hand*

Tissue Stem Cell-Specific Quantification – Accurate and Fast



10.11.22

Accelerating Stem Cell Science

20

The Landscape of Stem Cell Medicine Is Changing: *Stem Cell Dosage is Here*

