

AlphaSTEM

A Computer Simulation-Based
Technology for Counting
Adult Tissue Stem Cells

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In Collaboration with AlphaSTAR Corporation
www.alphastar.com

 **asymmetrex**
TECHNOLOGIES FOR STEM CELL MEDICINE

Before AlphaSTEM...

No means to estimate adult stem cell number.

Best developed biomarkers lack sufficient specificity.

For example, CD34 and CD133 biomarkers for hematopoietic stem cells are also expressed by more abundant committed progenitor cells.

Poor specificity biomarkers preclude counting.

Current Needs Met by AlphaSTEM Counting

I. Regenerative medicine

For the first time, determine tissue stem cell dose in transplant treatment preparations

II. Drug development

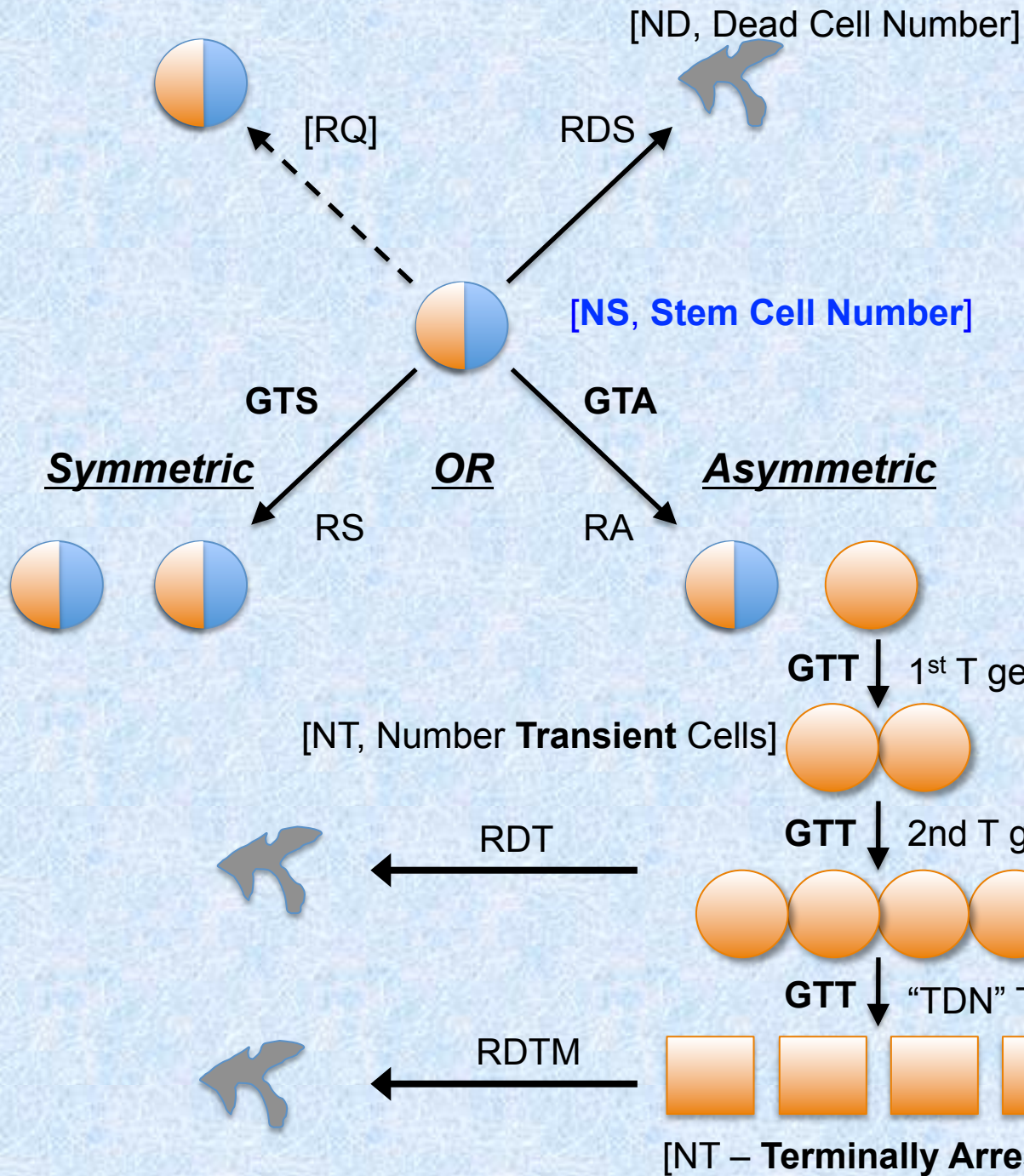
A. Identify “stem cell-safe” drug candidates

B. Identify “stem cell-active” agents

III. Stem cell biology research

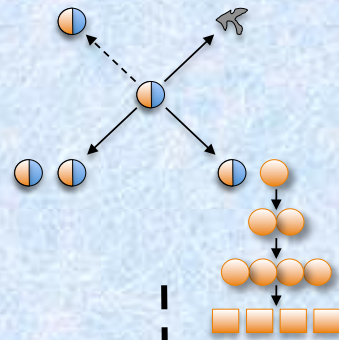
The general principle underlying the
AlphaSTEM Technology
for counting adult tissue stem cells:

*Infer their number and qualities
based on their output.*

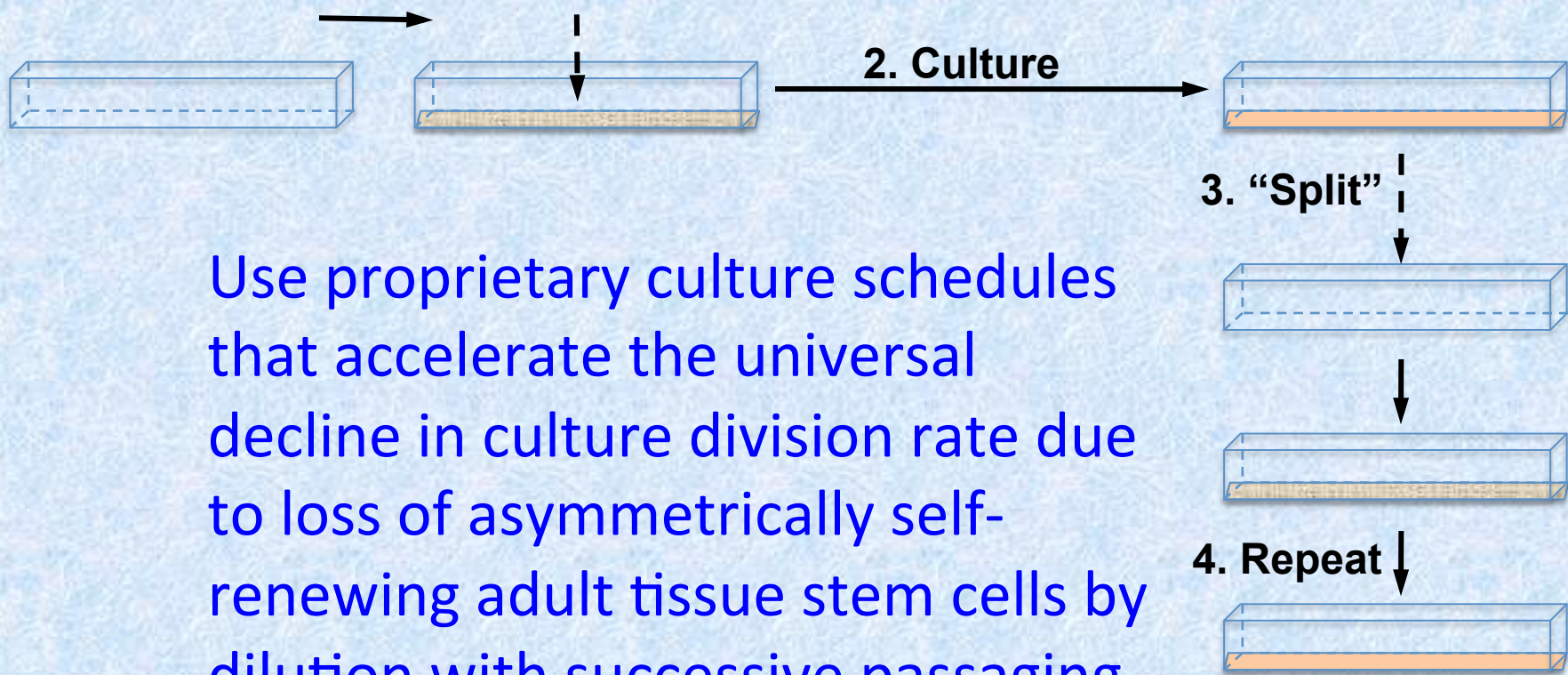


First, developed a computational model for tissue cell growth in culture based on the **unique asymmetric self-renewal** of tissue stem cells.

Next, culture primary cells from *any* human tissue.



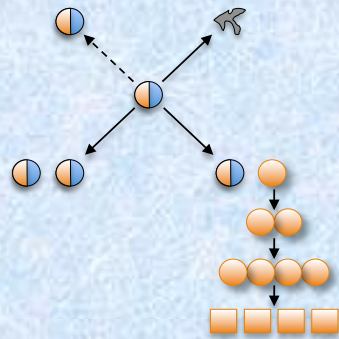
1. Initiate



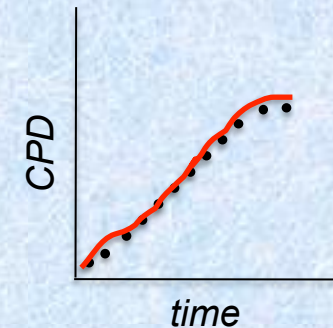
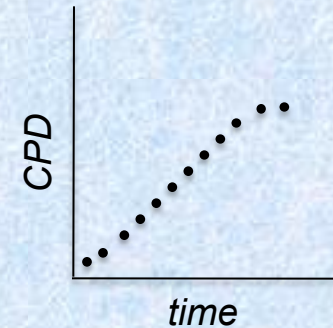
Use proprietary culture schedules that accelerate the universal decline in culture division rate due to loss of asymmetrically self-renewing adult tissue stem cells by dilution with successive passaging.

Simulate, fit, deconstruct, count:

Use total cell count data to generate classical cumulative population doubling (CPD) curves.



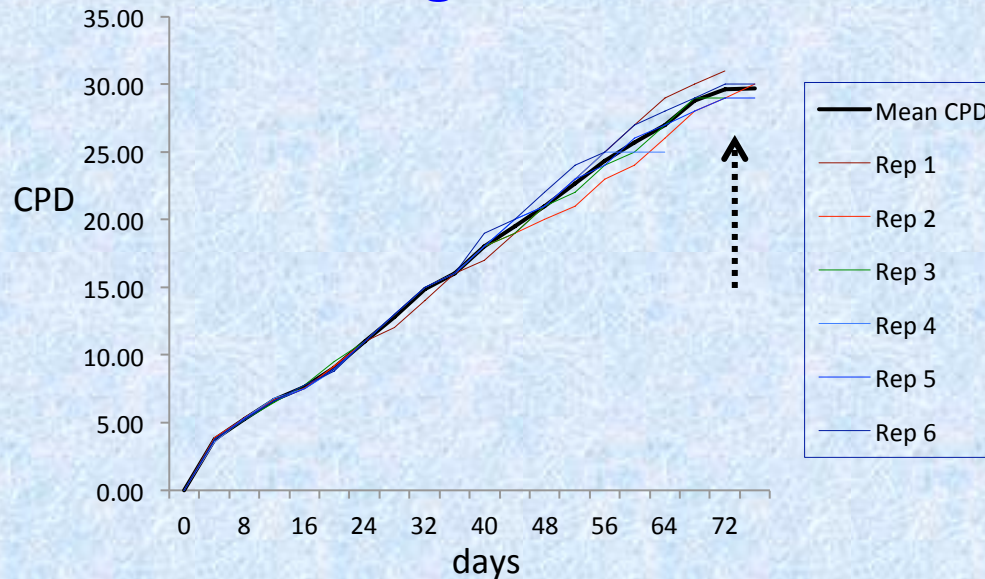
Compute model to find best simulation of the data.



*Deconstruct the best simulation to determine **stem cell number**, viability, and functional status.*

First Milestone: Proof of Adult Tissue Stem Cell Dilution

Human Lung Fibroblast Cultures

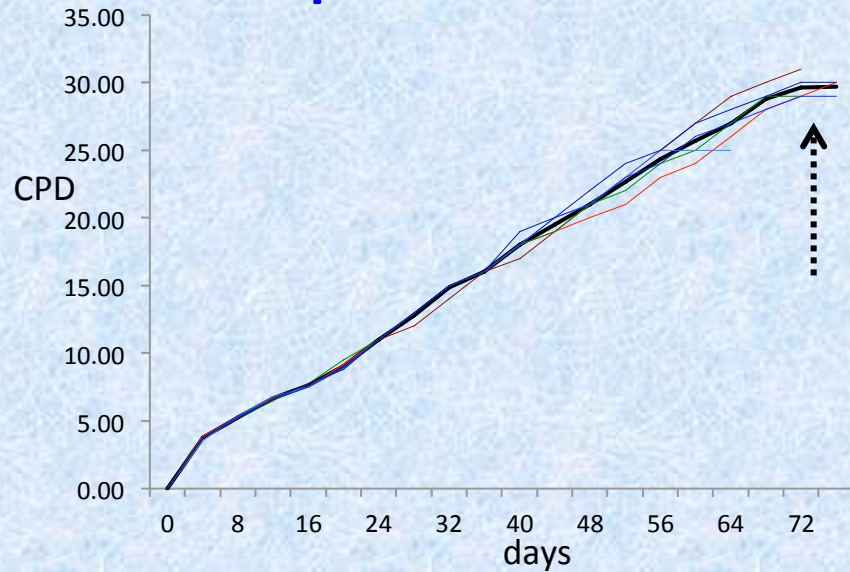


Experimental data (n=6)

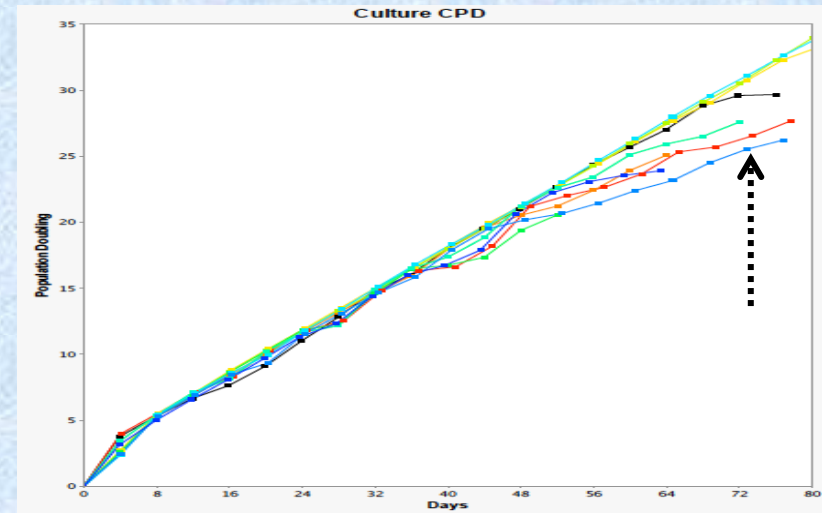
Arrow: Typical for these data, all replicates stop dividing, but at different times and at different maximum CPD.

Demonstration of Stem Cell Dilution with Passage

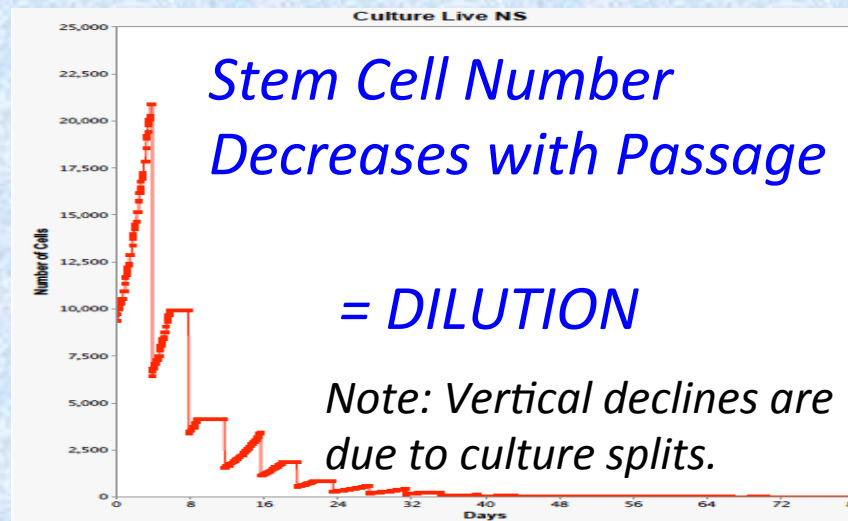
Experimental Data



Simulate and Fit



**Deconstruct
And
Count**



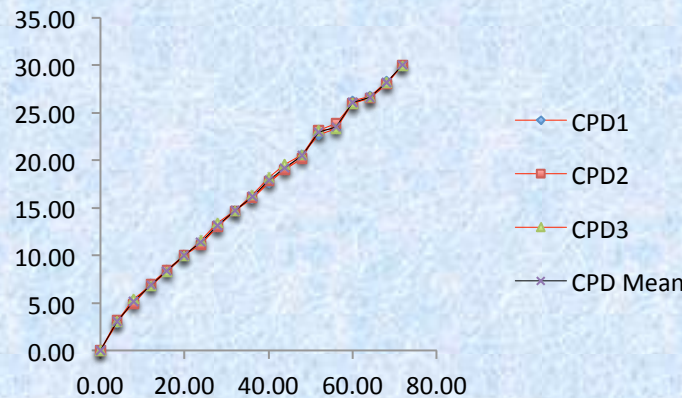
NEW APPLICATION MILESTONES

- I. Counting
- II. Identifying Stem Cell-Safe Drugs
- III. Identifying Stem Cell-Active Agents

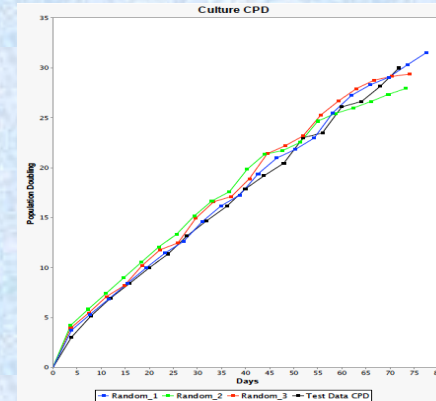
I. Counting

Expanded Liver Stem Cell Strain
(n=3) CPD

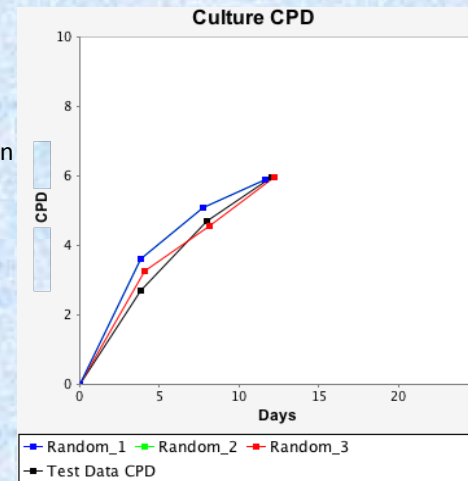
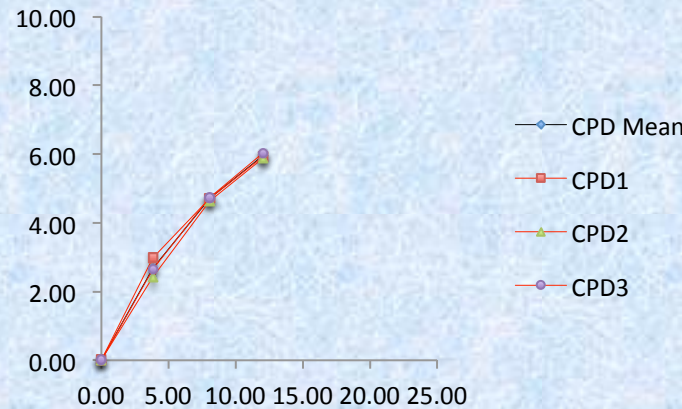
Experimental Data



Simulate and Fit



CD34+ Bone Marrow Cells
(n=3) CPD



Days

Days

Note difference in scales between liver and bone marrow. Each tissue has a specific profile related to different stem cell properties.

Adult Tissue Stem Cell Fraction Determinations From Simulation Deconstructions Are In Good Agreement With Best Available Estimates

Lung Fibroblasts:

Estimated¹ 0.13

AlphaSTEM 0.15 ± 0.03

Expanded Liver Stem Cells: Estimated² 0.22 ± 0.13

AlphaSTEM 0.17 ± 0.03

Cultured CD34+ BM Cells: Estimated³ 0.0002 to 0.001

AlphaSTEM 0.0003 ± 0.0001

¹Rambhatla *et al.* 2001 *J. Biomed. Biotech.* 1, 28-37. Asymmetry time-lapse study

²Asymmetrex unpublished data. Asymmetric colony and chromosome segregation assays

³Ziegler *et al.* 1999 *Science* 285, 1553-8. Mouse repopulation assay

II. Identifying Stem Cell-Safe Drugs

Adult tissue stem cell toxicity is an important cost for drug development. In the U.S., \$50 billion is spent on getting 20-30 approved drugs to market each year.

An estimated 50% of drugs that make it to Phase II clinical trials will fail due to adult stem cell toxicity.

These failures constitutes \$5 billion spent in animal studies and clinical trials that could be saved if a cell-based method to detect adult stem cell toxicity earlier were available. AlphaSTEM technology has this capability. It could save individual Pharma companies hundreds of millions of dollars each year.

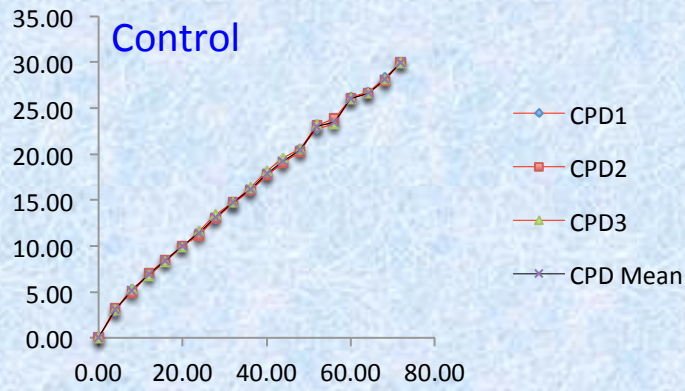
Evaluations for AlphaSTEM Detection of Stem Cell-Toxic Drugs

<u>Agent</u>	<u>Cell Toxicity</u>	<u>Clinical Effects</u>
[Saline]	N/A	Control condition
Idarubicin	SC (CPC?)	Targets hematopoietic progenitors; hepatic failure
Cyclophosphamide	SC and CPC	Cytoxan® (CTX); extremely myelosuppressive
BCNU	SC and CPC	Targets hematopoietic progenitors; late liver toxicity

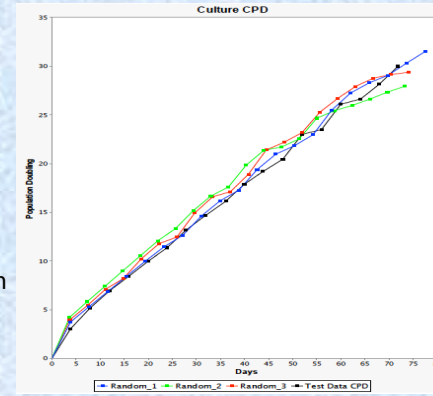
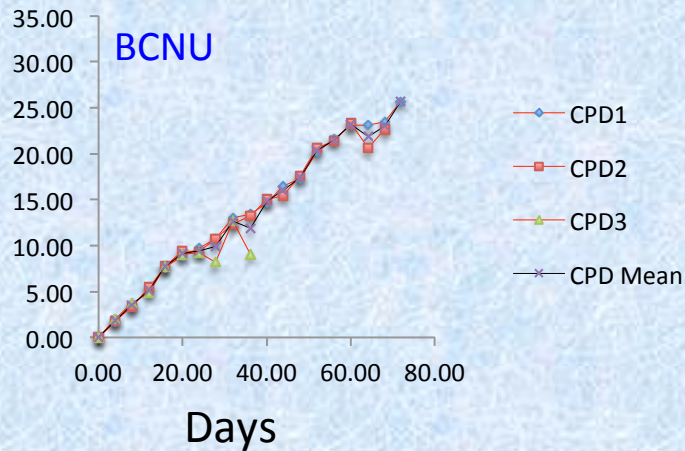
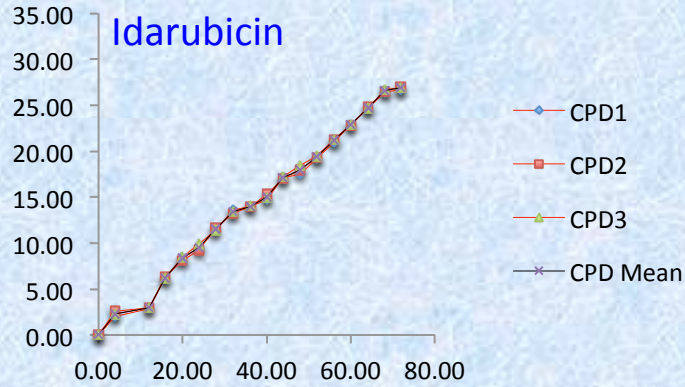
^aSC, tissue stem cells; ^bCPC, committed progenitor cells

Expanded Human Liver Stem Cell Culture Data and Simulations

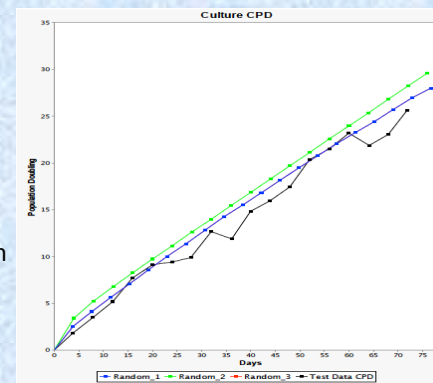
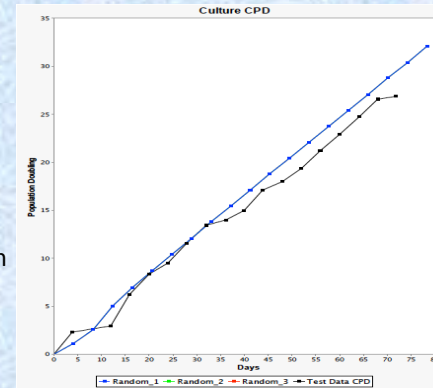
DATA



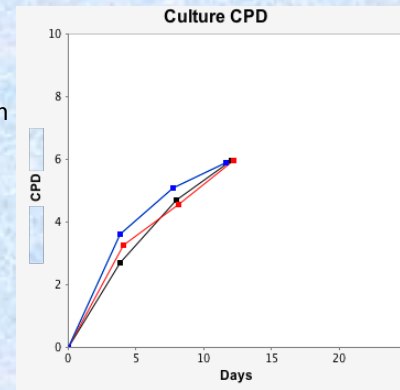
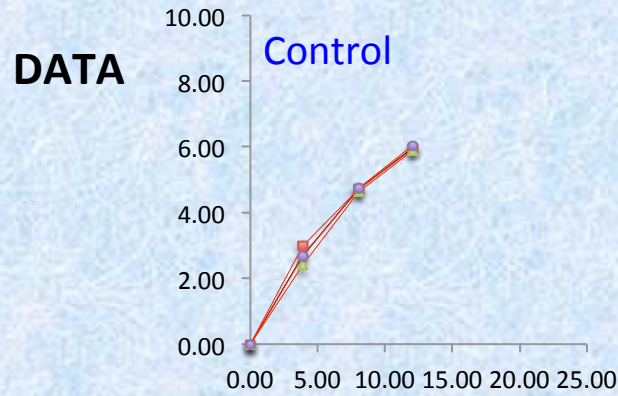
CPD



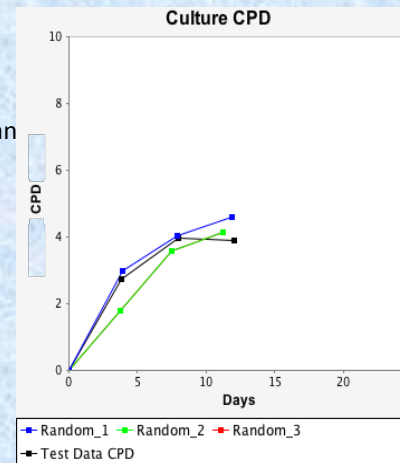
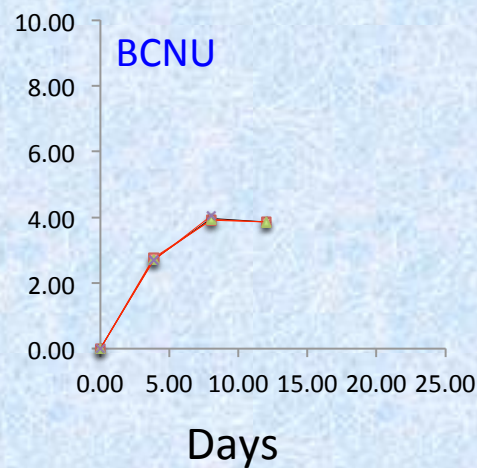
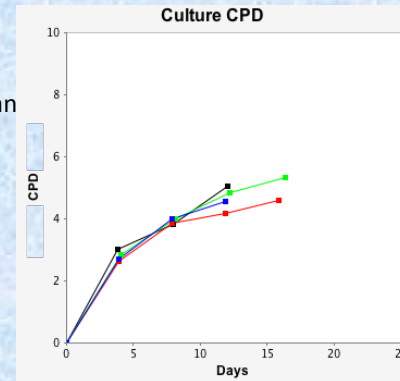
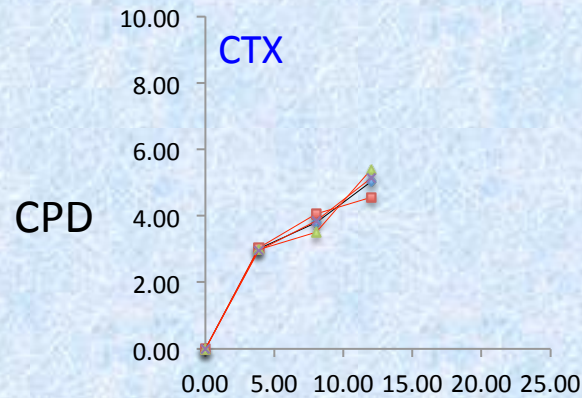
**Simulation
vs.
CPD Mean Data**



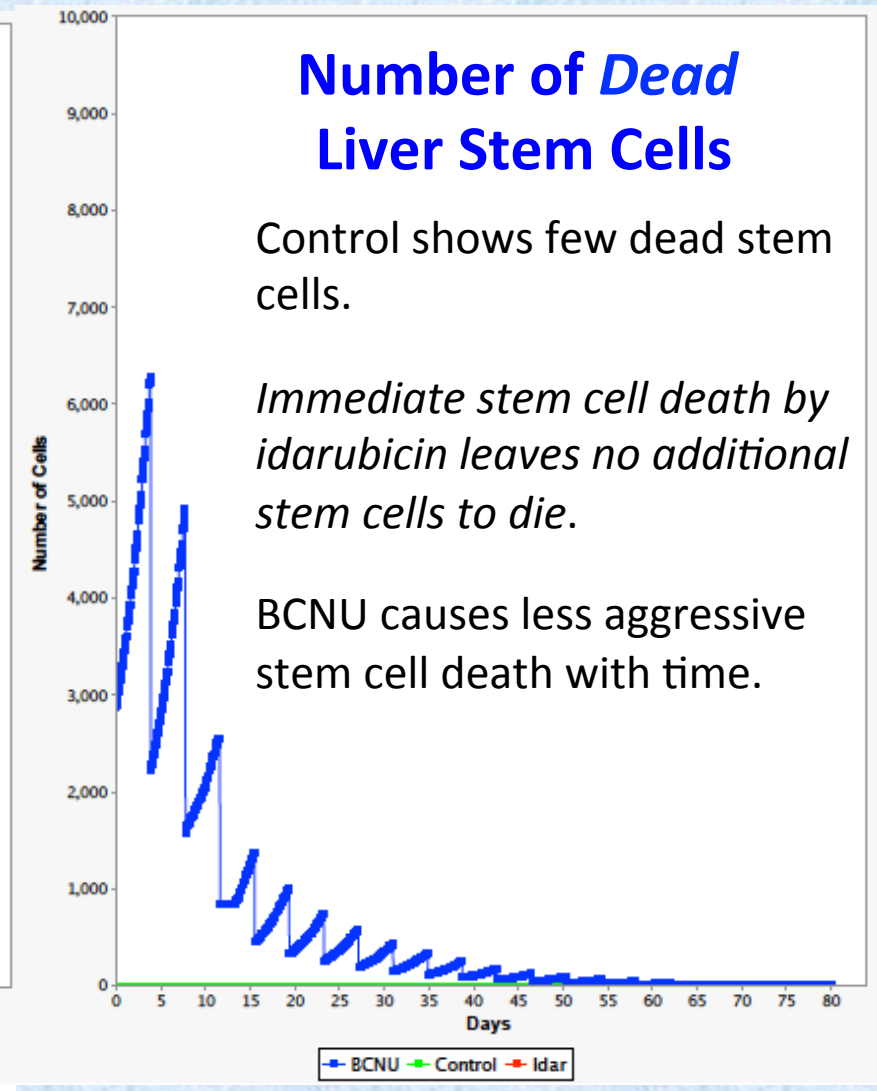
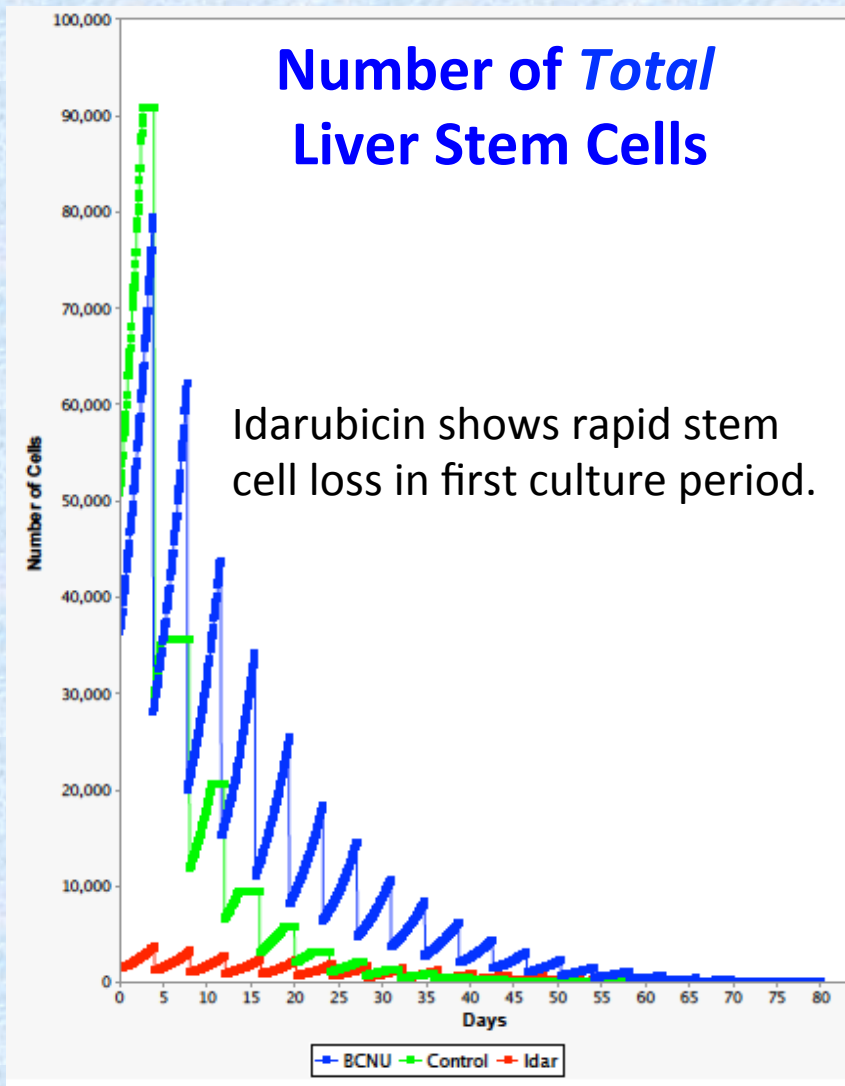
CD34⁺ BM Cell Culture Data and Simulations



**Simulation
VS.
CPD Mean Data**



Deconstruction Detects Tissue Stem Cell Toxicity



Summary of CD34+ Bone Marrow Cell Stem Cell Toxicity Detection

Relative Stem Cell Death Rate

Control	1
BCNU	40
CTX	90*

**Also simultaneously distinguishes CTX's induction of HSC proliferation associated with growth factor mobilization and CTX's toxicity against committed progenitor cells.*

BCNU, Idarubicin, CTX

If AlphaSTEM technology were available previously, these drugs would have been identified as stem cell-toxic drug candidates before animals studies or clinical trials – saving time, money, and patients from toxicity.

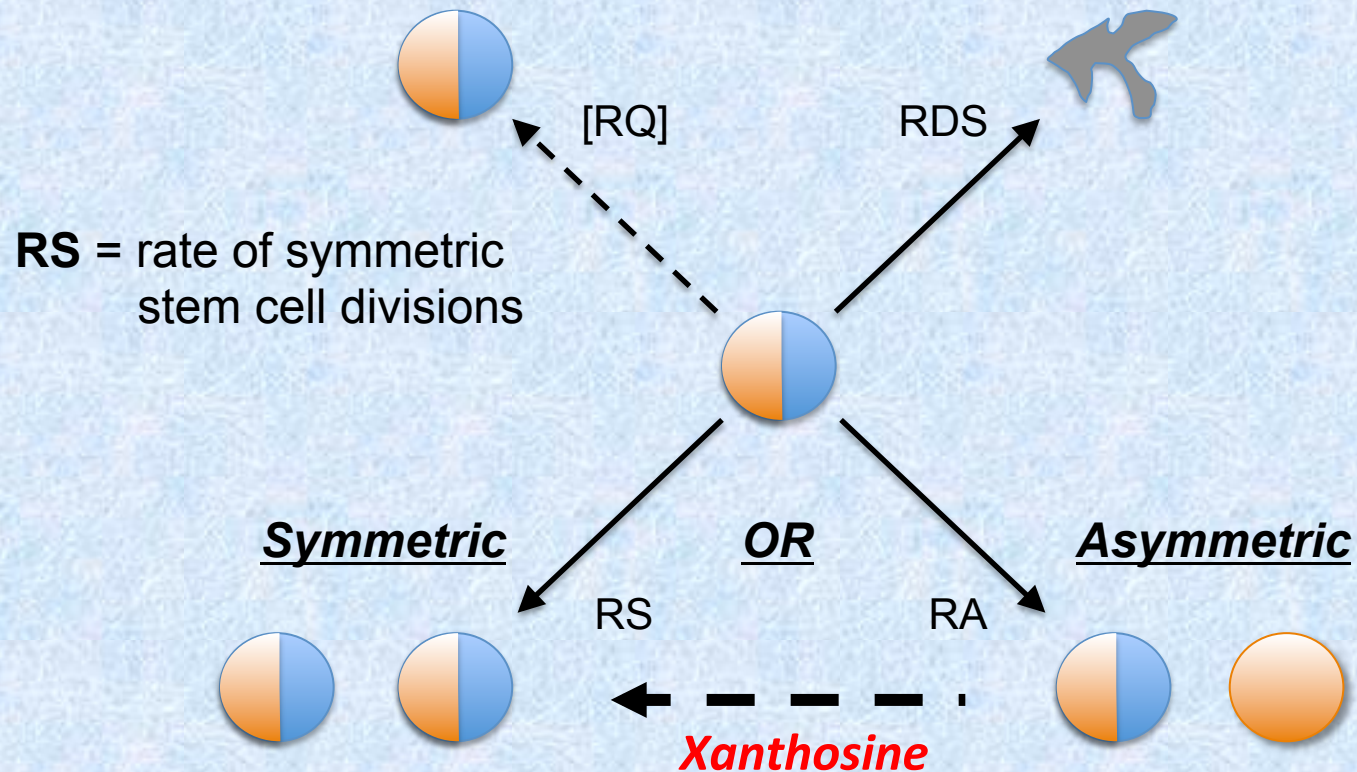
Like saline in the present study, AlphaSTEM technology can identify *stem cell-safe* drugs, which are not toxic to tissue stem cells. *Stem cell-safe* drugs have a higher likelihood for successful development, because they are cleared for a major cause of drug failure.

III. Identifying Stem Cell-Active Agents

Agents that increase adult tissue stem cell proliferation and/or viability have potential for use in stem cell research and regenerative medicine. Such agents might also be important to identify because of carcinogenic properties.

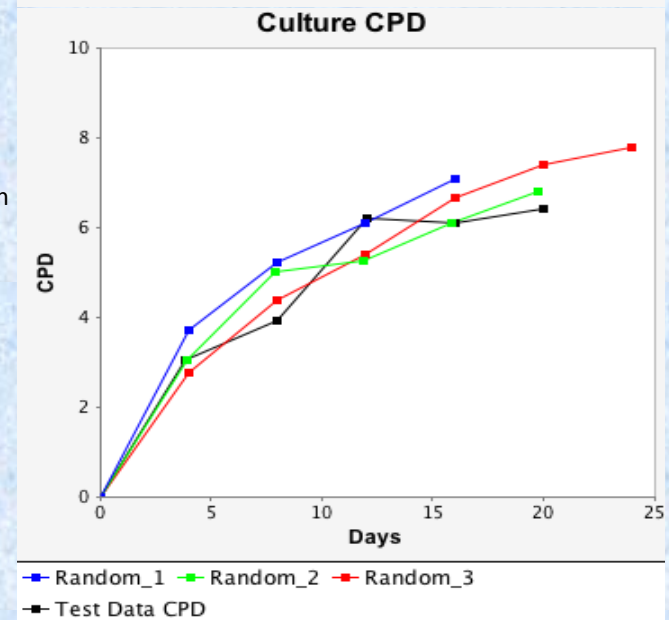
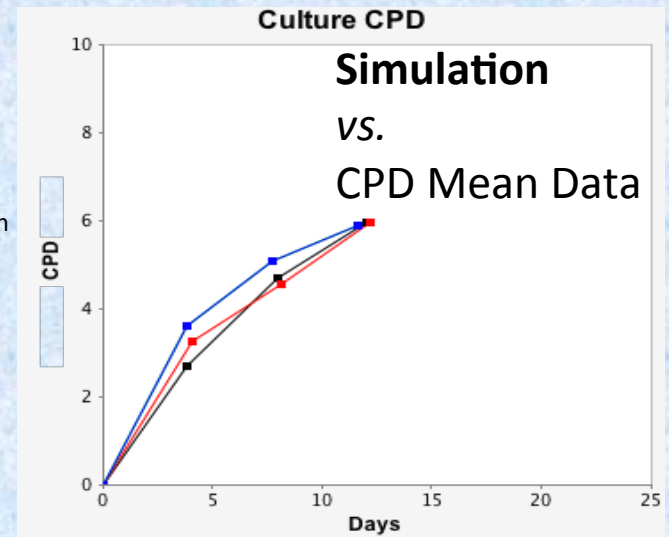
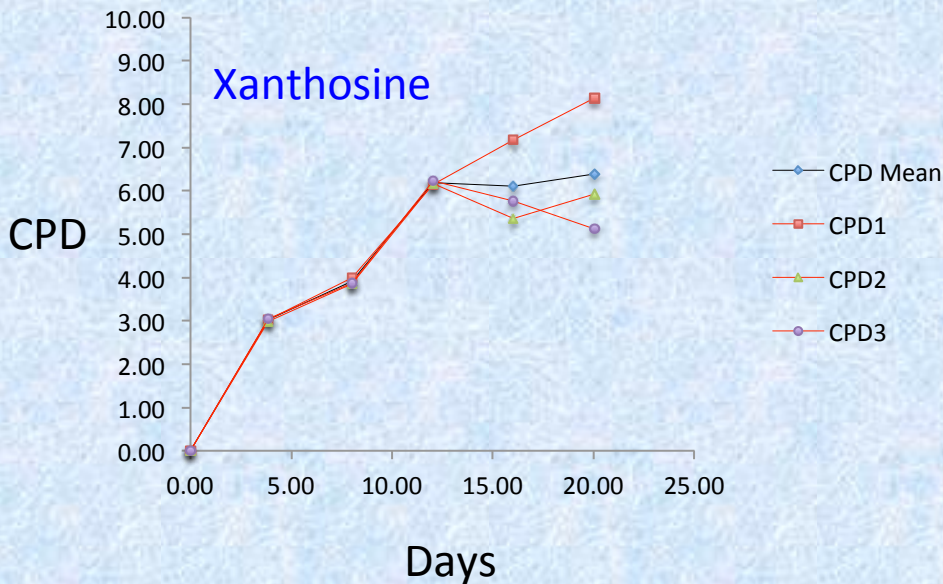
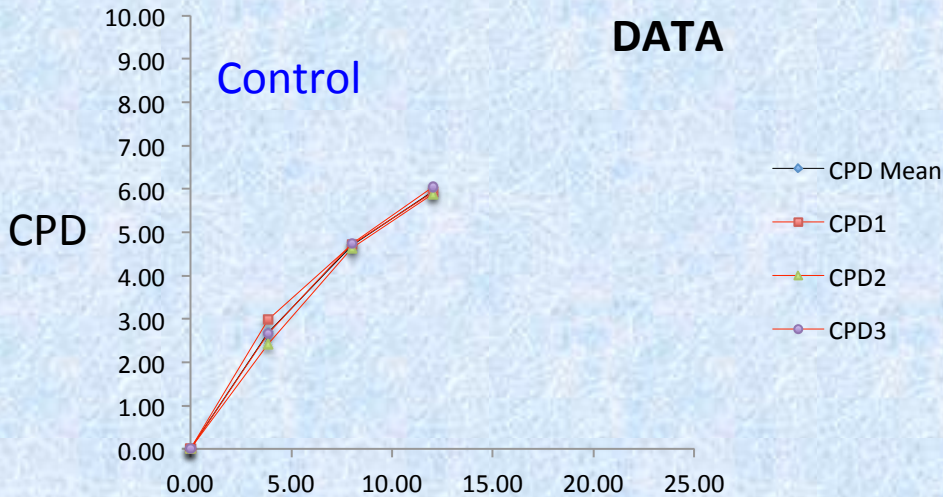
AlphaSTEM technology can also be used to identify such “stem cell-active” agents.

Use of Xanthosine to Validate AlphaSTEM Detection of Adult Tissue Stem Cell-Active Agents

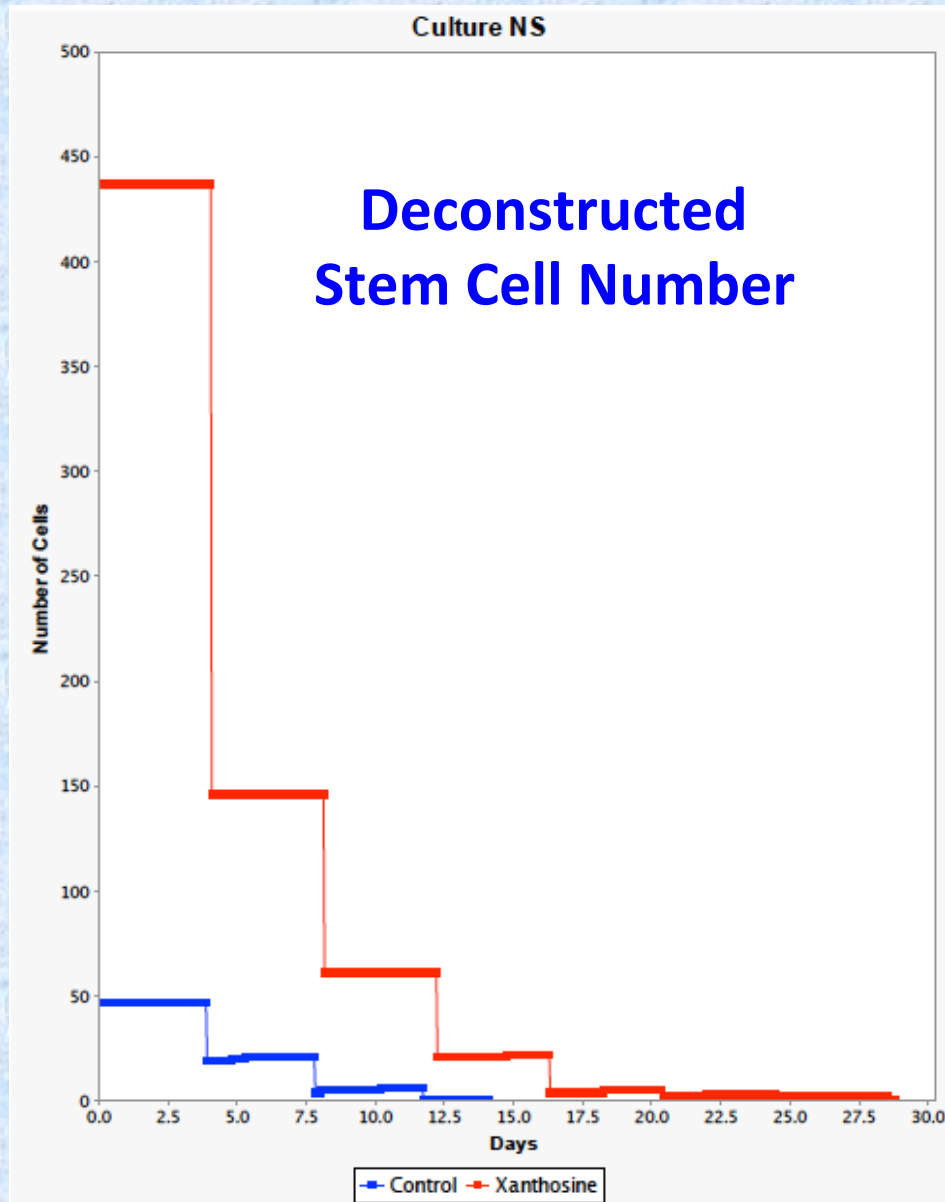


Xanthosine is a guanine ribonucleoside known to shift a variety of adult tissue stem cell types from asymmetric self-renewal to symmetric self-renewal. Such shifts in self-renewal pattern increase the number of stem cells.

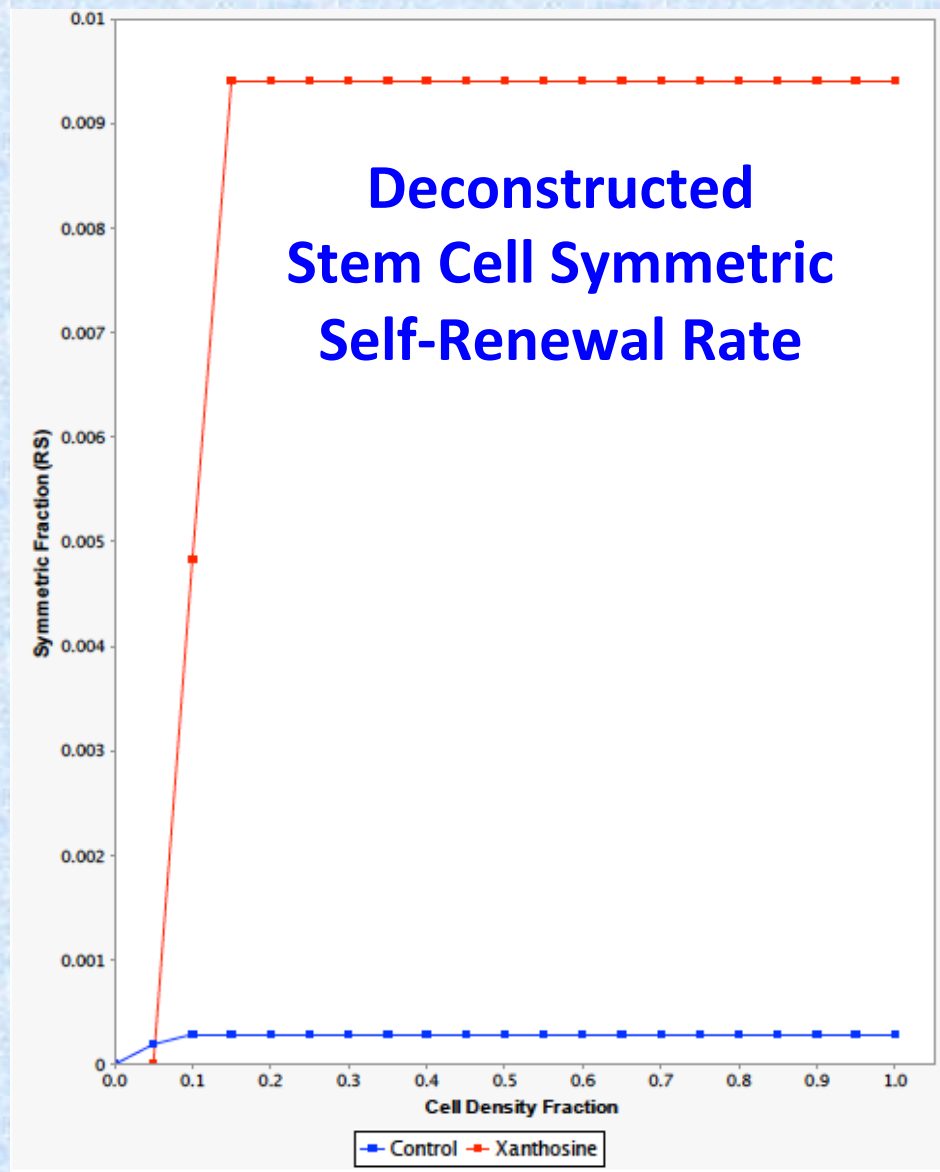
CD34⁺ BM Cell Culture Data and Simulations with Xanthosine



Xanthosine Increases Tissue Stem Cell Number



Xanthosine Increases Symmetric Self-Renewal Rate (RS)



WHO CAN BENEFIT FROM AlphaSTEM?

- A. Pharma and bio-pharma companies
- B. Pharma contract research organizations
- C. Regenerative medicine companies
- D. Umbilical cord blood banks
- E. Clinical centers and laboratories
- F. Academic centers and laboratories
- G. Patients

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