The AlphaSTEM Test™ Service

Providing Specific Counting of Therapeutic Tissue Stem Cells and Optimization of Their Expansion

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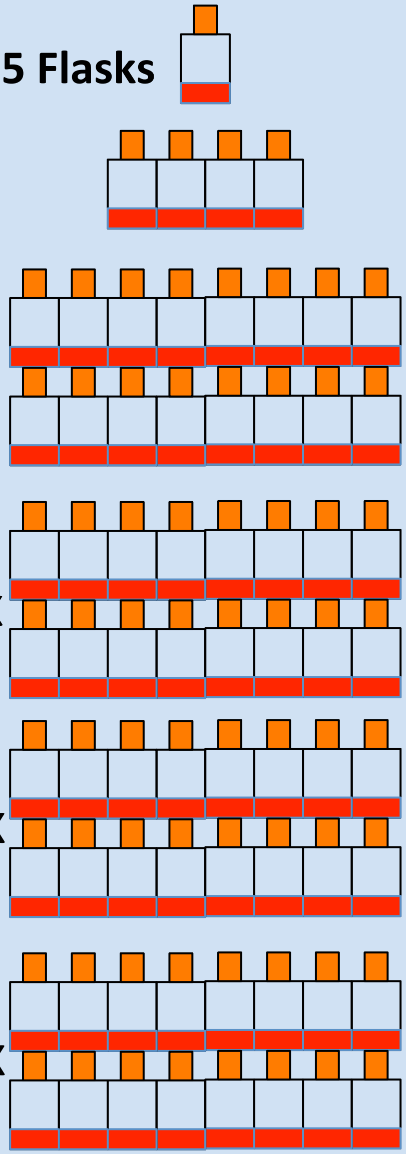
The Problem

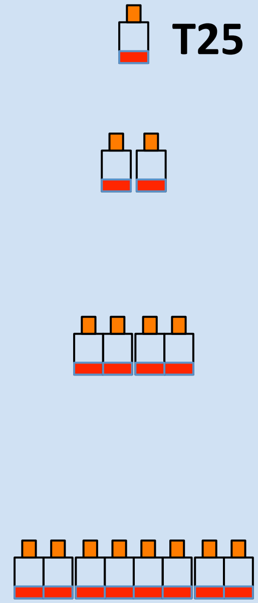
The Solution

Tissue Stem Cell (TSC)
Expansion Passage

T75 Flasks



T25 Flasks



4x

16x

64x

P1 Long times,
many flasks,
high costs,
P2 but few stem cells
UNQUANTIFIED!

P3

P4

P5

With AlphaSTEM Test™ and Design

2X more stem cells

1/32 the culture media

1/24 the culture area

1/2 the time

Lower costs with more TSCs

QUANTIFIED

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Adult TSC Production Challenges

1. <1% of cells in TSC preparations are stem cells.
2. TSC activities decline with serial expansion culture.

Currently, the production process is "blind." Can't specifically count TSCs.

Time and money are lost:

- *Inefficient timing of expansion steps*
- *Cultures stop growing earlier than desired*
- *Poor performance of cultures sold to customers*

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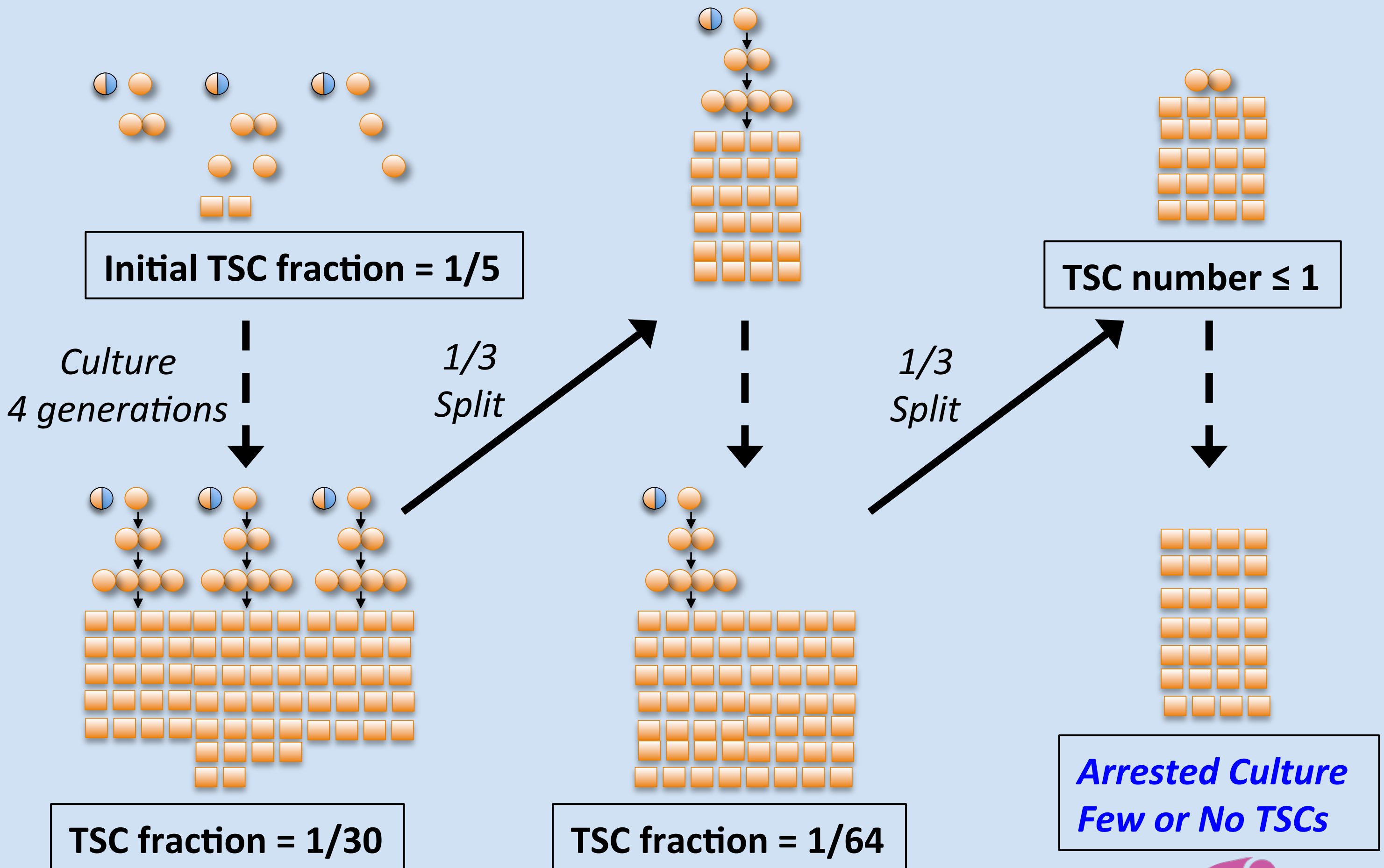
Why? Asymmetric Division With Serial Culture Dilutes Low Fraction TSCs



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Adult TSC Production Needs

1. Need: A simple to use method to count TSCs specifically and monitor changes in quality and number during production.
2. Need: A method to determine the quality and dose of the final TSC-containing product.

The ability to see and follow TSCs during production will lead to process optimizations that save time and money while increasing TSC production.

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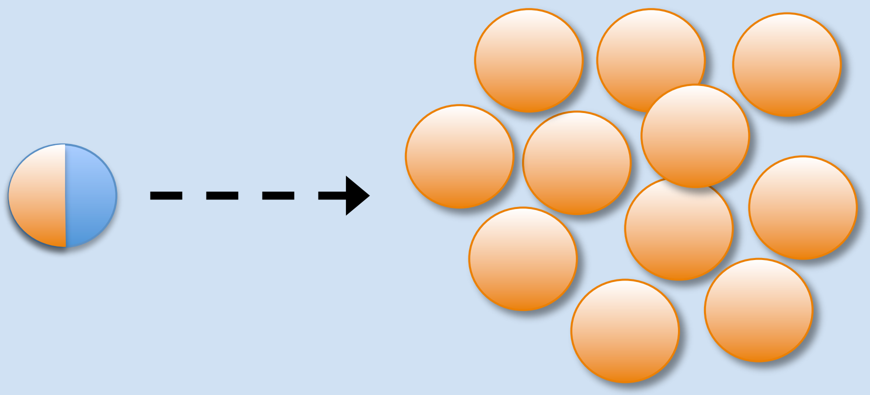
Current Biomarkers Lack the Specificity Required to "See" TSCs

All are expressed by committed progenitor cells, too.

e.g., hMSC



Committed Progenitor Cells

CD90
CD44
CD117
CD105
Etc.

CD90
CD44
CD117
CD105
Etc.

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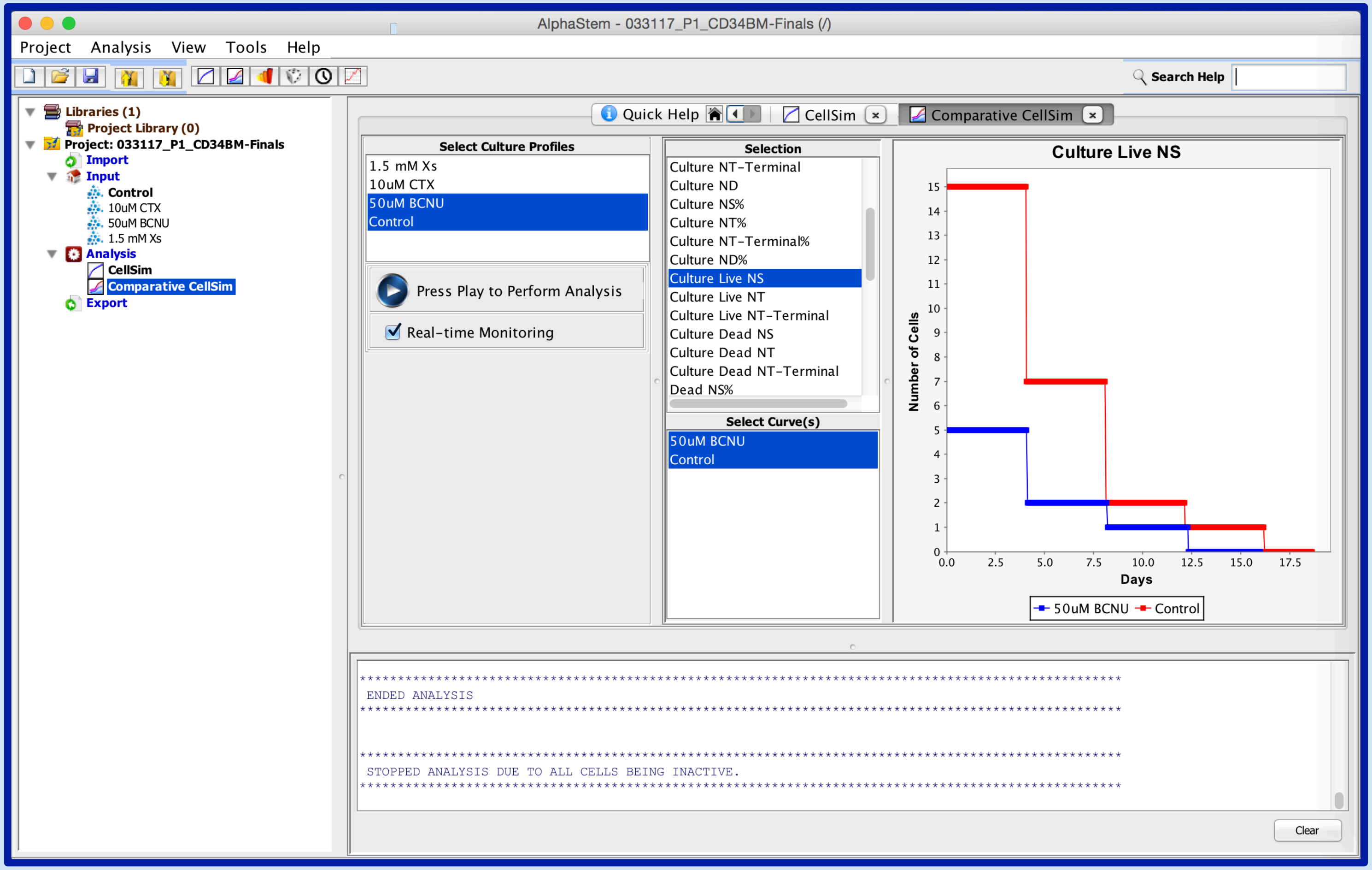
The AlphaSTEM Test™ Software Can "See" TSCs *Specifically* for Counting



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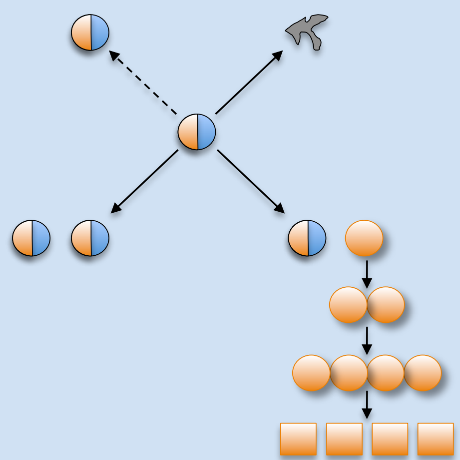
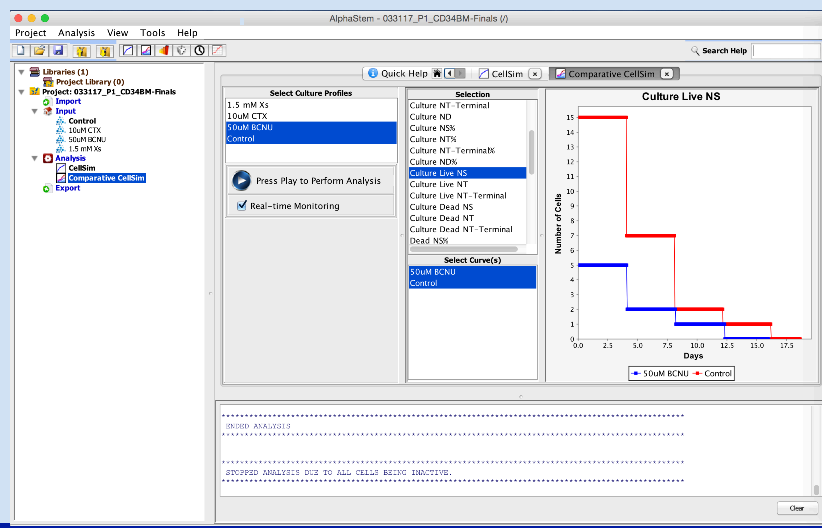
How Do We Count Them?



INTEGRATE

Stem Cell Kinetics Modeling

Aerospace Engineering Computer Simulation



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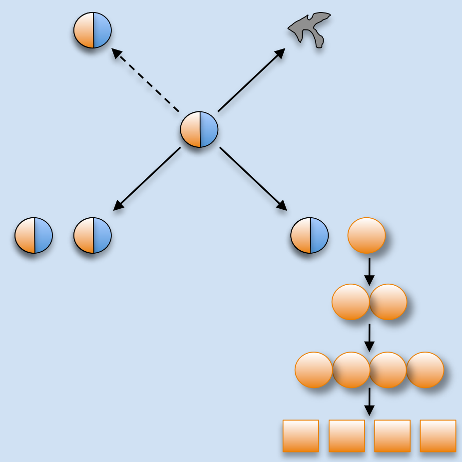
A Computational Model for Total Cell Production Based on TSC Asymmetry

Cumulative Population Doublings,

***CPD** = f(measured factors, unknown factors)*

Measured Factors

Input cell number
Maximum cell number
Split interval
Split fraction
Total cell counts
Average viability
Variances
(Est. variances)



***SC**, stem cell*
***CP**, committed progenitor*
***TD**, terminally differentiated*
***CC**, cell cycle*

Unknown Factors

SC Number
CP Cell Number
TD Cell Number
SC Viability
CP Viability
TD Viability
SC Symmetric Rate
SC CC Times
CP CC Time
CP Division Number

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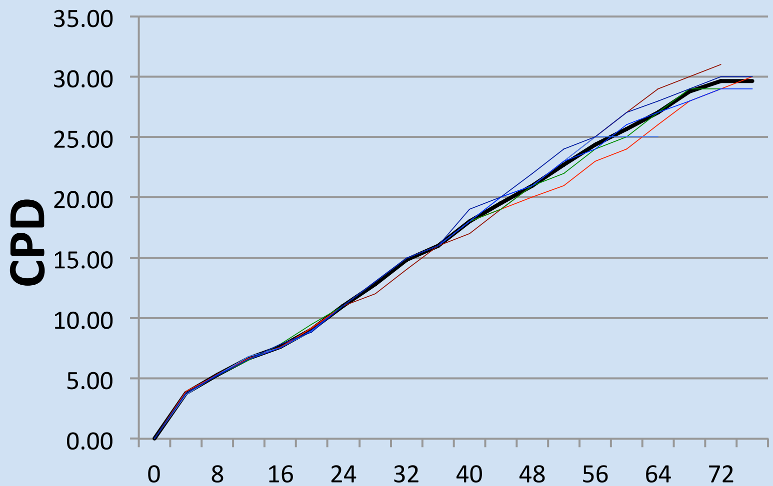
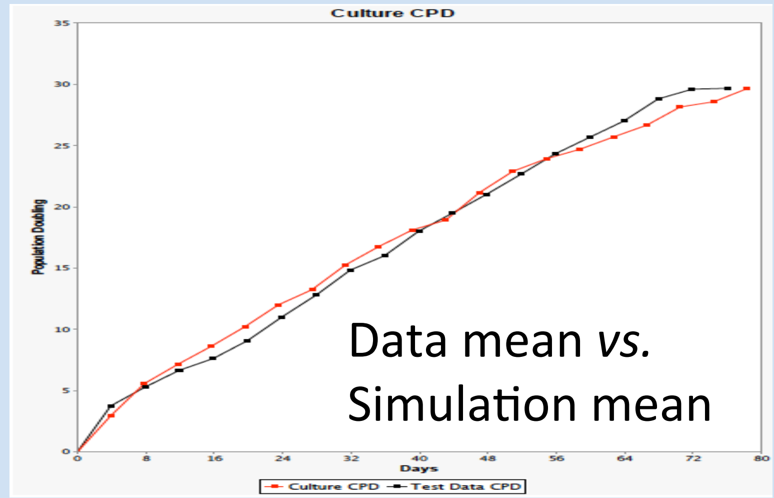
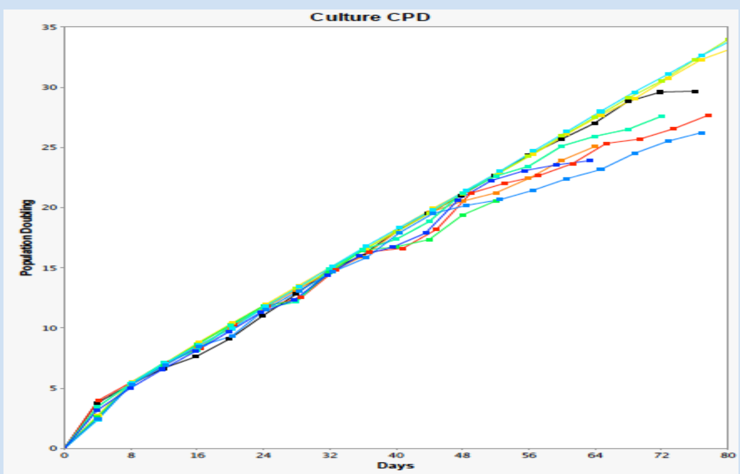
Tissue Stem Cell Number From Computational Simulation







The Computational Simulation

(*By random, combinatorial search for the best unknown factors.*)

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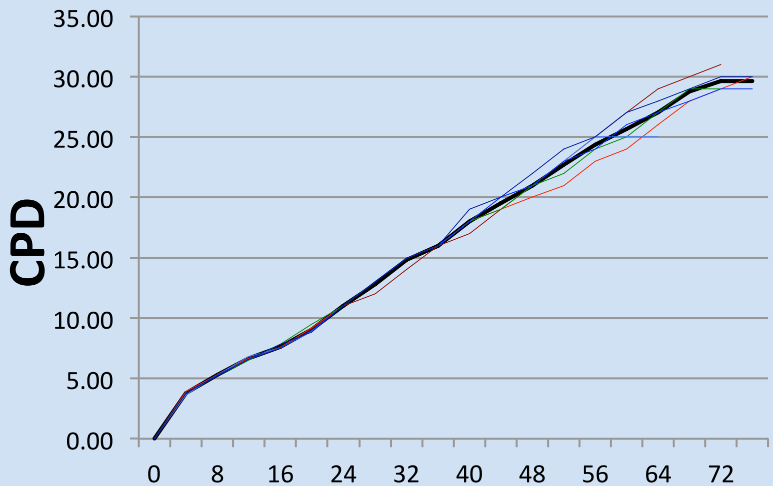

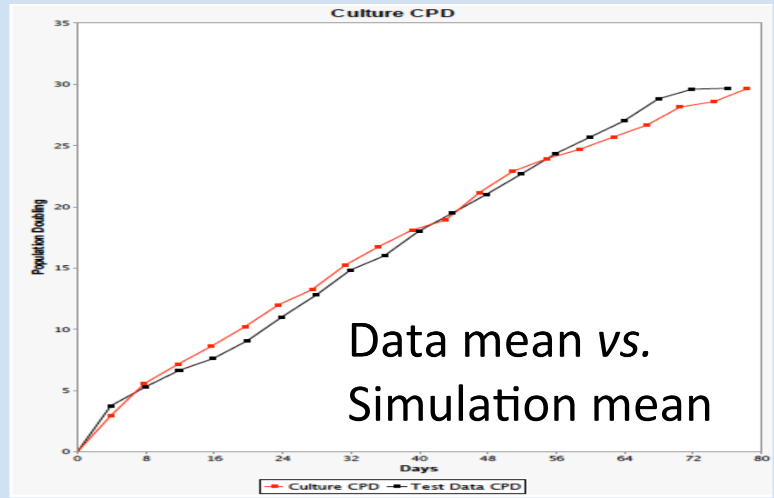

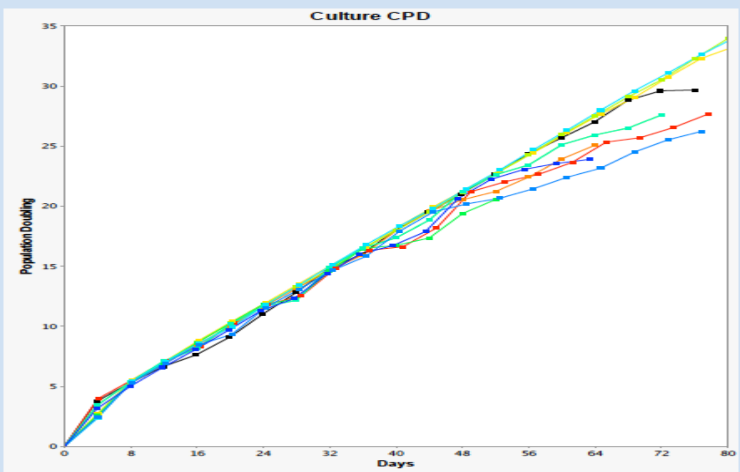

Now "Deconstruct"

Evaluate any input factor Independently.

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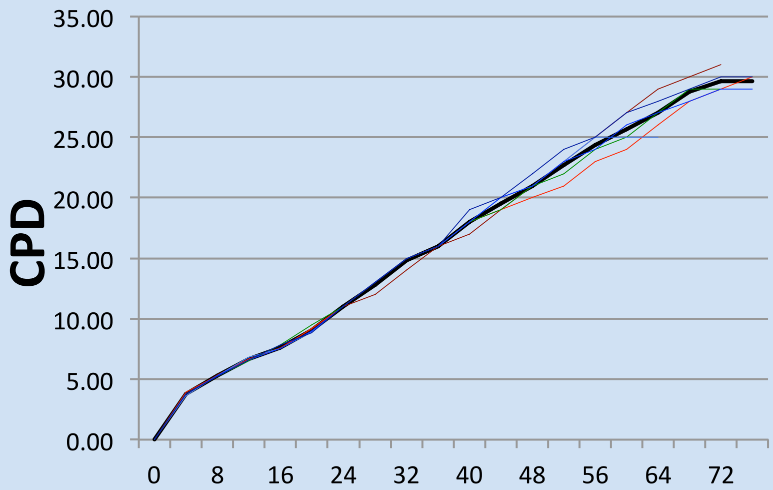

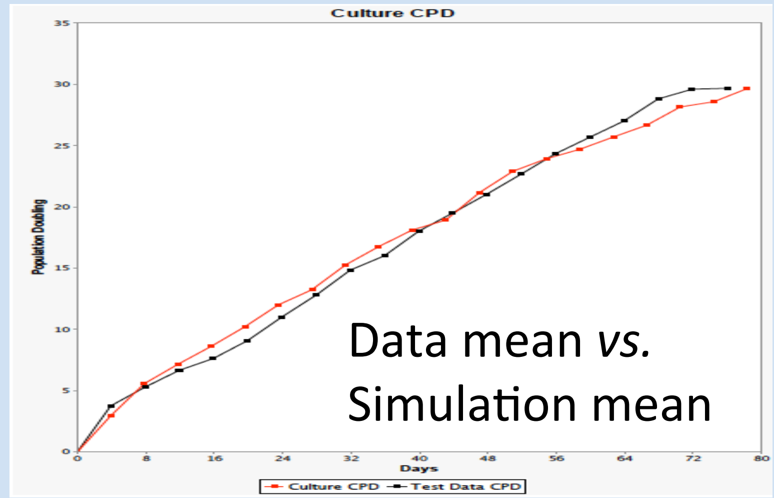

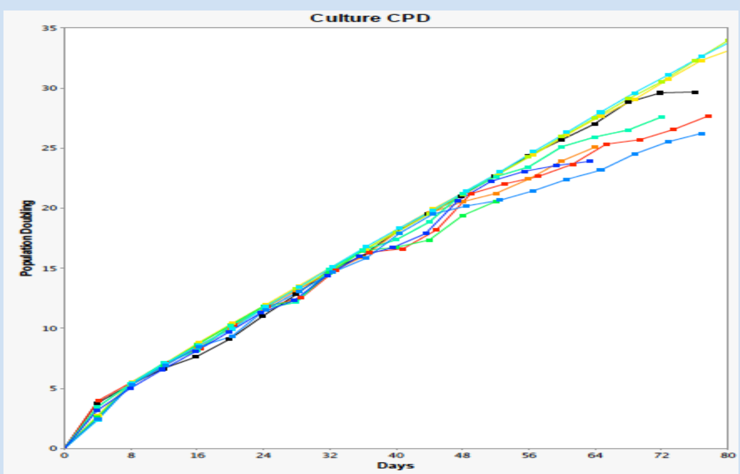

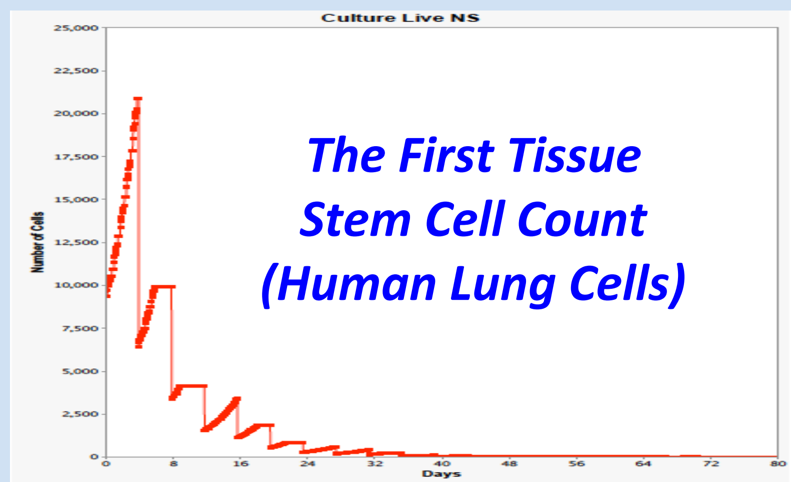

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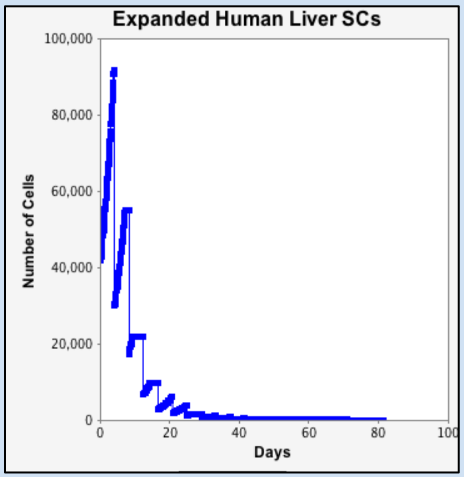
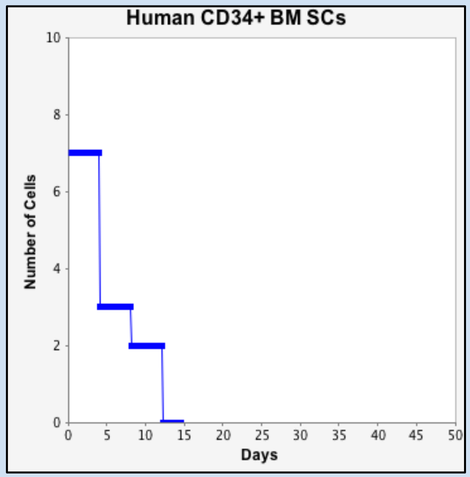
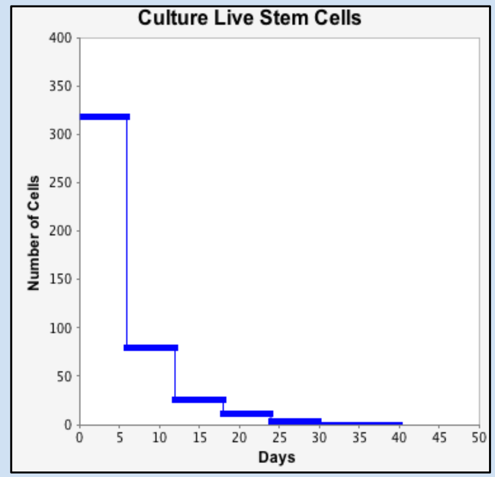
Dilution by Asymmetric Self-Renewal Kinetics is a Universal Feature of Adult Tissue Stem Cells In Culture



Expanded Liver Stem Cells



Bone Marrow Hematopoietic Stem Cells



Bone Marrow Mesenchymal Stem Cells[1]

[1] Data from Heathman *et al.*, 2016 *Cytotherapy*, **18**: 523-535.

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Specific Counting Validations

Lung Stem Cells	Estimated Fraction[1]	0.13
	AlphaSTEM Test	0.15 ± 0.03
Liver Stem Cells	Estimated Fraction[2]	0.22 ± 0.13
	AlphaSTEM Test	0.17 ± 0.03
Bone Marrow Stem Cells	Estimated Fraction[3]	2exp-4 to 1exp-3
	AlphaSTEM Test	2.6exp-4 ± 5.5exp-5
$CD34^+$ Umbilical Cord Blood	Estimated Fraction[3]	0.025 to 0.0003
	AlphaSTEM Test[4]	*0.08 ± 0.06*
$CD34^-$ Umbilical Cord Blood	AlphaSTEM Test[5]	**< 1.2e-4**

[1] Time-lapse; [2] Time-lapse and molecular asymmetry analyses; [3] SRC

[4] Data from Durand *et al*., 1994 *Blood*, **84**: 3667-3674.

[5] Data from Engelhardt *et al*., 1997 *Blood*, **90:** 182-193.

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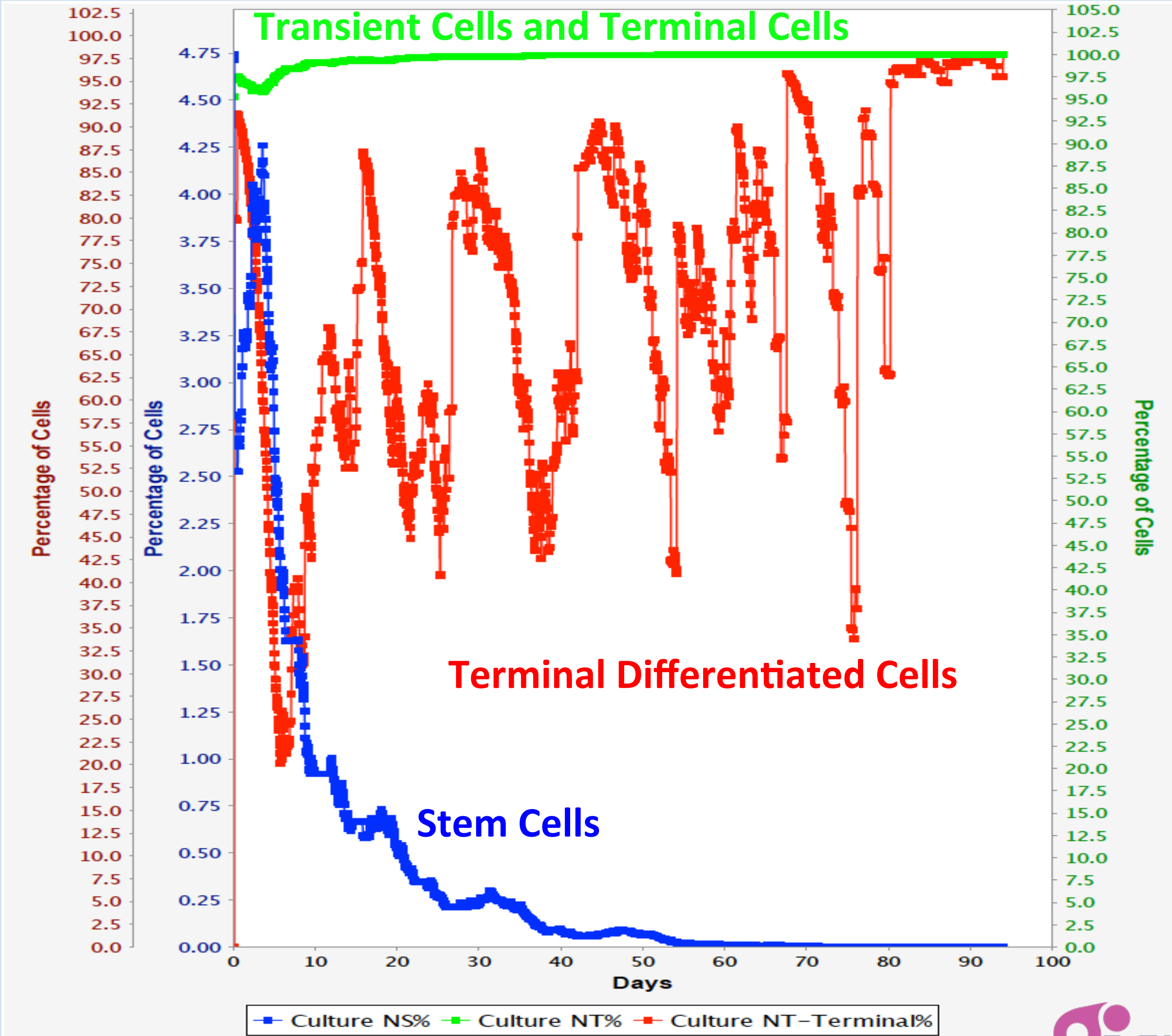
Cell Kinetics-Specific Analyses



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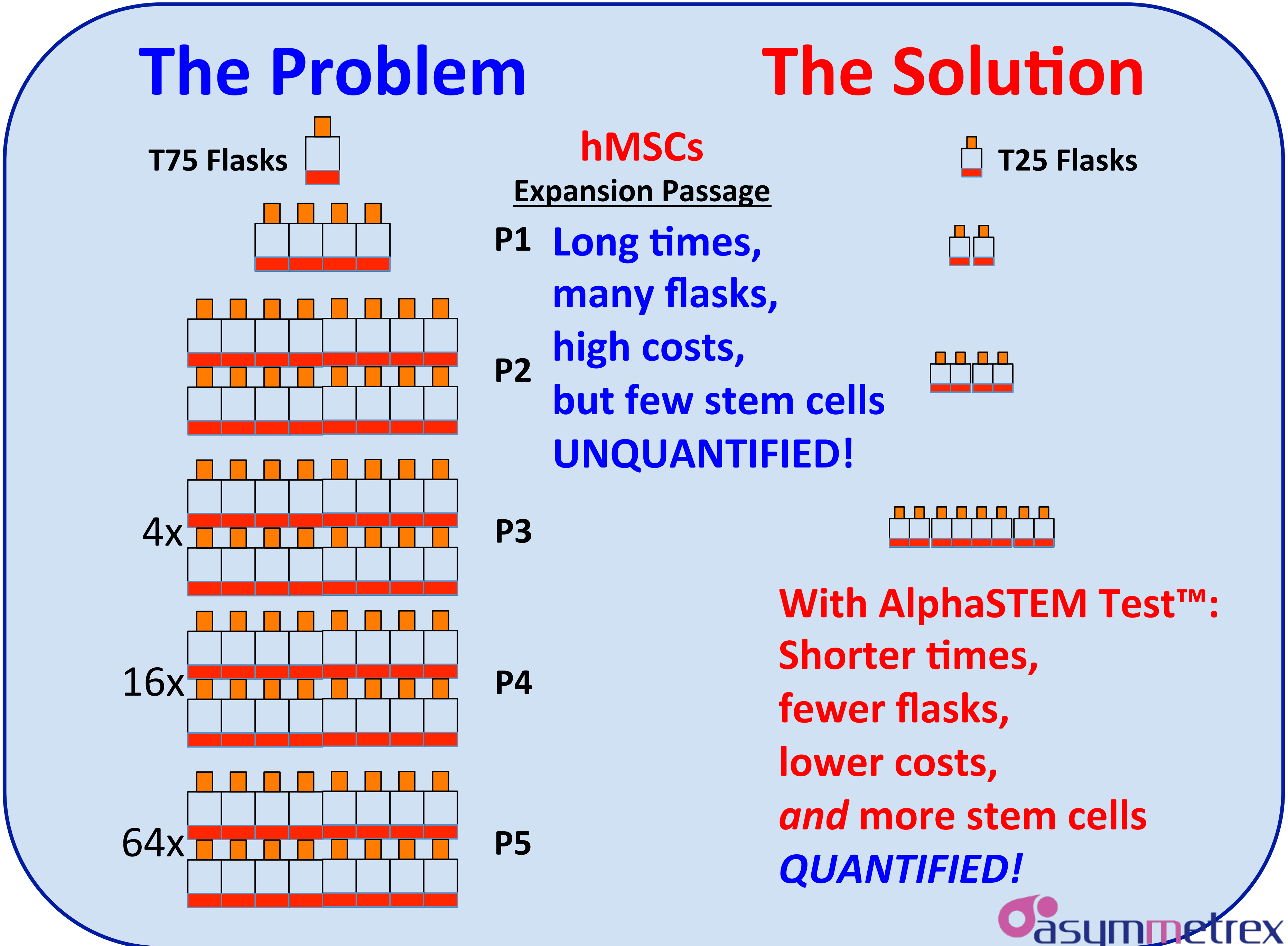
Quantifying Effectors Of Tissue Stem Cell Kinetics

			CD34+ BM	
Parameter	**Liver**	**CD34+ BM**	**Xs (+)**	**BCNU (-)**
Stem Cells				
Initial Fraction	0.28 (0.014)	2.6e-4 (0.004)	3.5e-3 (0.001)	1.3e-4 (0.001)
Symmetric Rate	0.24 (0.048)	1.3e-3 (NS)	3.2e-3 (0.037)	0.0 (NS)
Sym CC Time	30h (2e-4)	7.8h (<1e-4)	9.4h (NS)	8.2h (NS)
Asym CC Time	16h (1e-4)	7.0h (2e-4)	6.6h (NS)	7.6h (NS)
Committed Progenitors				
CC Time	18h (3e-4)	6.8h (<1e-4)	8.2h (NS)	6.4h (NS)

***Xs**, xanthosine*


The Problem
The Solution
T75 Flasks
hMSCs
Expansion Passage
T25 Flasks
P1
Long times,
many flasks,
high costs,
but few stem cells
UNQUANTIFIED!
P2
4x
P3
16x
P4
64x
P5
With AlphaSTEM Test™:
Shorter times,
fewer flasks,
lower costs,
and more stem cells
QUANTIFIED!
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The AlphaSTEM Test™ Service

Cell Count Data From Each Passage

(Client or Asymmetrex)

AlphaSTEM Test™ Computational Simulation

Production Kinetics

Quality

Dose

More efficient expansion

Fits into existing workflow.
Asymmetrex does the work.

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