The AlphaSTEM Test™ Service

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

Contact:
James L. Sherley, Director
P.O. Box 301179
Boston, MA 02130
E-mail: contact@asymmetrex.com
Phone: 617-990-6819
The Problem

- T75 Flasks
  - 4x
  - 16x
  - 64x

- Tissue Stem Cell (TSC) Expansion Passage
  - P1: Long times, many flasks, high costs,
  - P2: but few stem cells
  - UNQUANTIFIED!

- Long names, many flasks, high costs, but few stem cells

The Solution

- T25 Flasks
  - 2X more stem cells

- 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
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
Why? Asymmetric Division With Serial Culture Dilutes Low Fraction TSCs

Initial TSC fraction = 1/5
Culture 4 generations
1/3 Split
TSC fraction = 1/30

1/3 Split
TSC fraction = 1/64

TSC number ≤ 1
Arrested Culture Few or No TSCs
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.*
Current Biomarkers Lack the Specificity Required to “See” TSCs

All are expressed by committed progenitor cells, too.

e.g., hMSC

CD90
CD44
CD117
CD105
Etc.

Committed Progenitor Cells

CD90
CD44
CD117
CD105
Etc.
The AlphaSTEM Test™ Software Can “See” TSCs Specifically for Counting
How Do We Count Them?

Stem Cell Kinetics Modeling

Aerospace Engineering Computer Simulation

INTEGRATE
A Computational Model for Total Cell Production Based on TSC Asymmetry

Cumulative Population Doublings,

\[ CPD = f(\text{measured factors, unknown factors}) \]

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

**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

\( SC, \) stem cell
\( CP, \) committed progenitor
\( TD, \) terminally differentiated
\( CC, \) cell cycle
Tissue Stem Cell Number From Computational Simulation

The Computational Simulation
(By random, combinatorial search for the best unknown factors.)
Tissue Stem Cell Number From Computational Simulation

Now “Deconstruct”

Evaluate any input factor Independently.
Tissue Stem Cell Number From Computational Simulation

Data mean vs. Simulation mean

The First Tissue Stem Cell Count (Human Lung Cells)
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 Data from Heathman et al., 2016 Cytotherapy, 18: 523-535.
# Specific Counting Validations

<table>
<thead>
<tr>
<th>Stem Cells</th>
<th>Estimated Fraction</th>
<th>AlphaSTEM Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lung Stem Cells</strong></td>
<td>0.13</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td><strong>Liver Stem Cells</strong></td>
<td>0.22 ± 0.13</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td><strong>Bone Marrow Stem Cells</strong></td>
<td>2exp-4 to 1exp-3</td>
<td>2.6exp-4 ± 5.5exp-5</td>
</tr>
<tr>
<td><strong>CD34⁺ Umbilical Cord Blood</strong></td>
<td>0.025 to 0.0003</td>
<td>0.08 ± 0.06</td>
</tr>
<tr>
<td><strong>CD34⁻ Umbilical Cord Blood</strong></td>
<td></td>
<td>&lt; 1.2e-4</td>
</tr>
</tbody>
</table>

1 Time-lapse; 2 Time-lapse and molecular asymmetry analyses; 3 SRC
Cell Kinetics-Specific Analyses

Transient Cells and Terminal Cells

Terminal Differentiated Cells

Stem Cells
Quantifying Effectors Of Tissue Stem Cell Kinetics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Liver</th>
<th>CD34+ BM</th>
<th>CD34+ BM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Xs (+)</td>
<td>BCNU (-)</td>
</tr>
<tr>
<td><strong>Stem Cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial Fraction</td>
<td>0.28 (0.014)</td>
<td>2.6e-4 (0.004)</td>
<td>3.5e-3 (0.001)</td>
</tr>
<tr>
<td>Symmetric Rate</td>
<td>0.24 (0.048)</td>
<td>1.3e-3 (NS)</td>
<td>3.2e-3 (0.037)</td>
</tr>
<tr>
<td>Sym CC Time</td>
<td>30h (2e-4)</td>
<td>7.8h (&lt;1e-4)</td>
<td>9.4h (NS)</td>
</tr>
<tr>
<td>Asym CC Time</td>
<td>16h (1e-4)</td>
<td>7.0h (2e-4)</td>
<td>6.6h (NS)</td>
</tr>
<tr>
<td><strong>Committed Progenitors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC Time</td>
<td>18h (3e-4)</td>
<td>6.8h (&lt;1e-4)</td>
<td>8.2h (NS)</td>
</tr>
</tbody>
</table>

*Xs, xanthosine*
The Problem

- T75 Flasks
- Expansion Passage
- P1: Long times, many flasks, high costs, but few stem cells UNQUANTIFIED!
- P2: 4x
- P3: 16x
- P4: 64x

The Solution

- hMScs
- T25 Flasks
- With AlphaSTEM Test™:
- Shorter times, fewer flasks, lower costs, and more stem cells QUANTIFIED!
The AlphaSTEM Test™ Service

Fits into existing workflow. Asymmetrex does the work.

Cell Count Data From Each Passage (Client or Asymmetrex)

AlphaSTEM Test™ Computational Simulation

Production Kinetics Quality Dose More efficient expansion