Asymmetrex's AlphaSTEM Test™

For Unique Cell Kinetics Analyses of Therapeutic Tissue Cells in Complex Cell Preparations

Description: Stem cell biotechnology tools company Asymmetrex (http://asymmetrex.com/) partnered with AlphaSTAR Corp., a world leader in computational simulation engineering (http://alphastarcorp.com/), to develop a first-in-kind technology for specific counting of adult tissue stem cells. The new technology is based on tissue stem cells' universal unique property of asymmetric self-renewal. This basis solves the long-standing biomarker specificity problem that continues to confound the counting of tissue stem cells by all other methods previously used, which resulted in known erroneous measures of therapeutic stem cell number and dose.

In addition to specific quantification of tissue stem cells in complex preparations (*e.g.*, primary cultures, manufacturing expansion cultures, treatment samples), the new technology provides, for the first time, access to several important cell kinetics properties of tissue stem cells (*e.g.*, cell cycle time, cell division pattern, death rate) distinct of the analogous cell kinetics properties of other cell types (*i.e.*, committed progenitors and differentiated cells), which generally outnumber stem cells dramatically in all commonly used tissue cell preparations.

The new technology has two key elements: 1) conventional cell culture technology to generate simple total viable cell count data from serially passaged cultures; and 2) the recently developed simulation software package, called the AlphaSTEM Test™ (http://asymmetrex.com/our-services/alphastem-test/). The AlphaSTEM Test™ can be used to *specifically* quantify and determine the specific cell kinetics properties of stem cells from diverse human tissues. Thus far, it has been validated for human liver stem cells, lung stem cells, hematopoietic stem cells (HSCs) from both bone marrow and umbilical cord blood, amniotic fluid stem cells, and bone marrow-derived mesenchymal stem cells (MSCs).

For details of the AlphaSTEM Test™ basis and operation, please, view the free on-demand online videos at the following links.

https://www.regmednet.com/channels/196-webinars/posts/5112-a-first-technology-for-counting-adult-tissue-stem-cells-for-applications-in-regenerative-medicine-and-drug-development

http://asymmetrex.com/asymmetrex-stem-cell-videos/ : Cell and Gene Exchange 2017

Unique Capabilities: The AlphaSTEM Test™ can be used to determine the following properties of complex cell preparations that contain adult tissue stem cells. No other technology has this capability.

Table 1. Cell Kinetics Properties Available From the AlphaSTEM Test™

Tissue Stem Cells	Committed Progenitors	Differentiated
Number *vs*. time	Number *vs*. time	Number *vs*. time
Death rate *vs*. time	Death rate *vs*. time	Death rate *vs*. time
Cell cycle time for symmetric s-r*	Cell cycle time	
Cell cycle time for asymmetric s-r		
Symmetric self-renewal rate		
Asymmetric self-renewal rate		
SC unit generation number**		
Density-dependence of symmetric s-r rate		

*s-r, self-renewal; **number of cell generations between stem cell and terminally differentiated cells produced in culture

Applications for Cell Therapy and Regenerative Medicine: The following is a list of cell therapy and regenerative medicine applications that are enabled by the availability of cell type-specific determinations from the AlphaSTEM Test™.

1. Monitoring changes in specific stem cell number and function during manufacturing and expansion processes

2. Monitoring specific stem cell number and function to optimize expansion processes

3. Use of computer simulation to rapidly explore combinatorial processing factors for optimizing stem cell (or differentiated cell) production

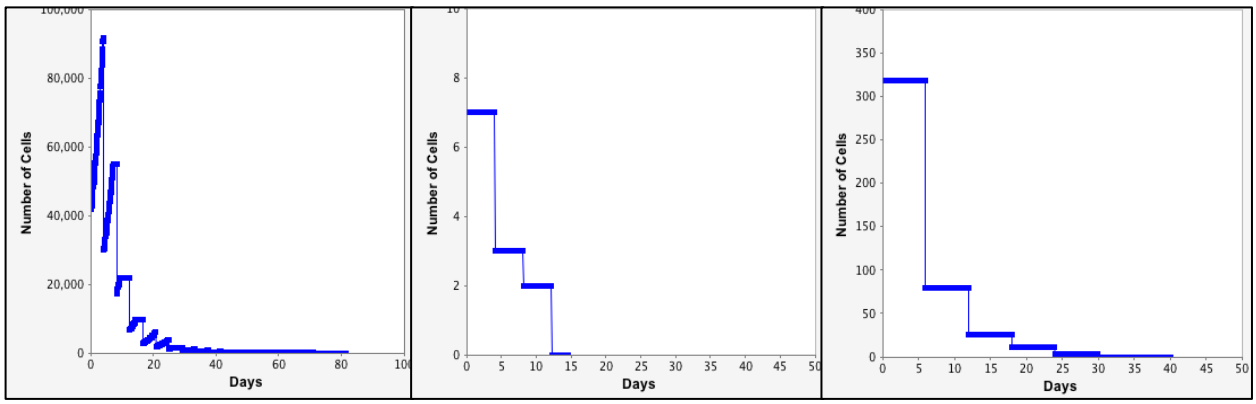
4. Evaluation of effects of agents on stem cell (or differentiating cell) number and function

5. Determination of the specific stem cell dose and quality of final cell products

Note: *Several of these applications may also be available for immunotherapy cell types. Asymmetrex founders have reported experimental evidence that memory immune cells also display asymmetric self-renewal (Noh et al., 2011, PLoS ONE 6(7): e22077. doi:10.1371/journal.pone.0022077), in which case the AlphaSTEM Test™ could prove effective for improving production of these cell types of recently increased therapeutic interest as well.*

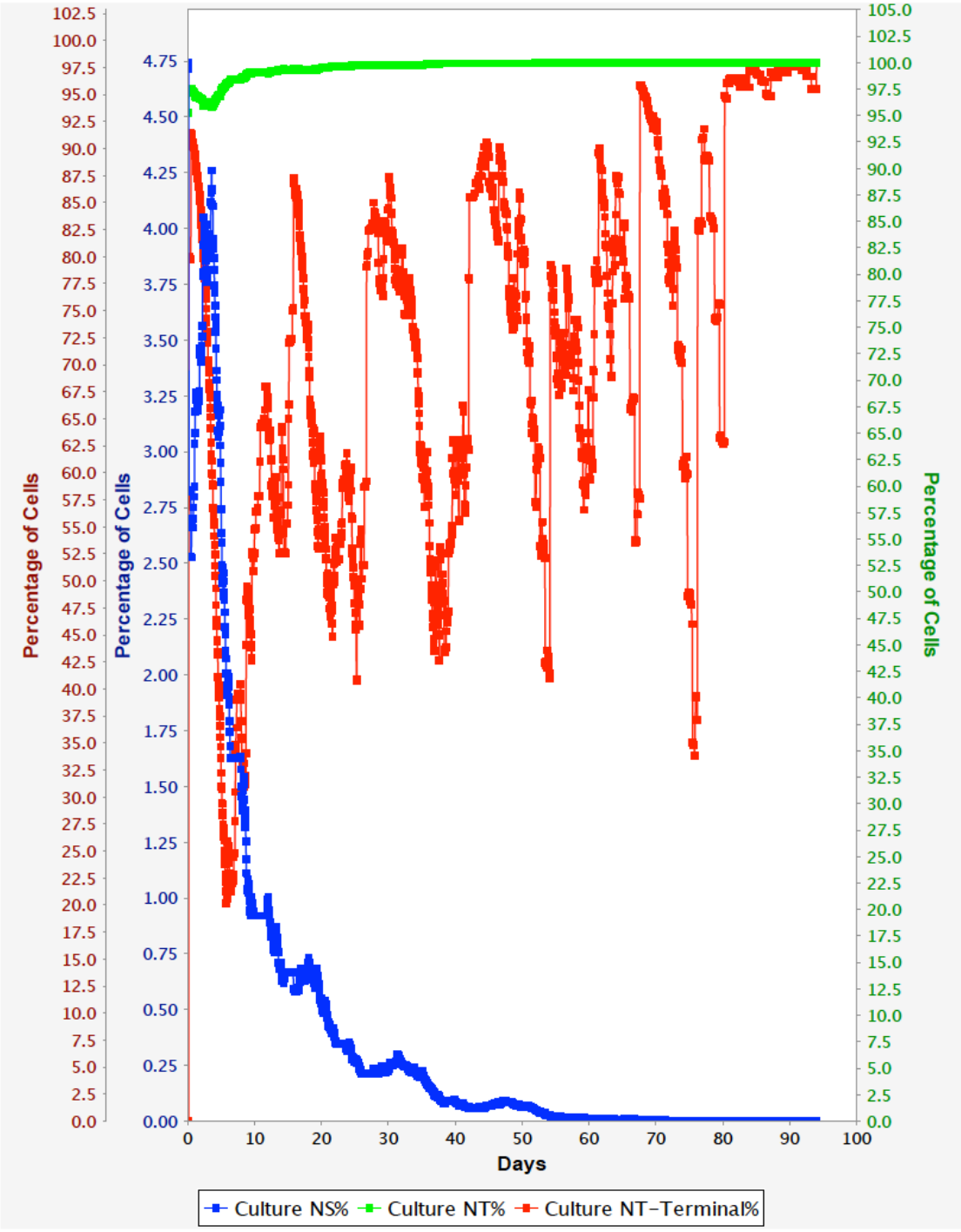
AlphaSTEM Test™ Output Examples: Below are three examples of different types of AlphaSTEM Test™ output data that illustrate some of the unique advantages of the technology.

Example 1 – Use of the AlphaSTEM Test™ to determine the specific stem cell numbers in serial cultures from different human tissues. The total number of viable cells input at the start of each culture (0 hour) was 325,000 (expanded liver stem cells), 65,000 (HSCs in $CD34^+$-selected bone marrow cells), and 375,000 (MSCs in primary bone marrow). The characteristic decline in tissue stem cell number with successive culture passages is predicted due to continued asymmetric self-renewal by tissue stem cells in culture. Note the detection of symmetric self-renewal by liver stem cells (inclined phases) between passages (vertical lines), but not for HSCs or MSCs (flat phases). This important biological distinction is quantified by the symmetric self-renewal rate (see table in Example 3 below), which is only available with use of the AlphaSTEM Test™.



Liver Stem Cells **Bone Marrow HSCs** **Bone Marrow MSCs**

Example 2 – Example of cell type-specific cell kinetics output from the AlphaSTEM Test™. During serial passage, the technology can be used to monitor the individual cell kinetics of stem cells (**NS**, middle y-axis), total transiently amplifying progenitor cells and differentiated cells (**NT**, right y-axis), and terminally-arrested differentiated cells (**NT-Terminal**, left y-axis). The data shown are from an analysis of human lung tissue cells. The cell count data are presented as the % of total culture cells.



Example 3 – Comparison of cell kinetics properties for different types of tissue stem cells and different culture conditions determined with the AlphaSTEM Test™. Note that human liver stem cells have some cases of cell kinetics properties that are quite different than for bone marrow-derived HSCs, including a much higher fraction and a significant symmetric self-renewal rate. The AlphaSTEM Test™ showed the primary effect of BCNU, a cancer chemotherapeutic agent known to cause chronic bone marrow failure, to reduce the number of HSCs significantly during the first culture period.

Cell Kinetics Property	**Liver**	**BM-HSCs**	**BM-HSCs + BCNU***
Stem cells			
Initial Fraction	**0.28 (0.014)**	**0.00026 (0.004)**	**0.00013 (0.0009)**
Death Rate	0.042 (0.041)	0.0014 (NS)	0.0 (NS)
Symmetric Cell Cycle Time	30h (0.0002)	7.8h (< 0.0001)	8.2h (NS)
Asymmetric Cell Cycle Time	16h (0.0001)	7.0h (0.0002)	7.6h (NS)
Symmetric self-renewal rate	**0.24 (0.048)**	**0.0013 (NS)**	0.0 (NS)
SC unit generation number	15 (0.0001)	13 (< 0.0001)	14 (NS)
Committed progenitor cells			
Death Rate	0.009 (NS)	0.02 (< 0.0001)	0.01 (< 0.0001)
Cell Cycle Time	18h (0.0003)	6.8h (< 0.0001)	6.4h (NS)

*BCNU = 50 microM BCNU, a hematopoietic stem cell toxin; values are mean (p); NS, not statistically significant at the p = 0.05 level. BCNU p values refer to comparisons with the control condition.

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TECHNOLOGIES FOR STEM CELL MEDICINE

AlphaSTEM Test™ Validation Highlights

I. Specific and Accurate Adult Tissue Stem Cell Counting Validations

A. Comparison to time-lapse analyses for asymmetrically self-renewing cells

B. Comparison to immunofluorescence microscopy for asymmetrically self-renewing patterned cells

C. Comparison to gold standard for estimating human blood stem cells after transplant into mice

D. Research manuscript under review (*pre-print available on request*)

II. Drug Evaluation Validations

A. Chronic organ failure drugs successfully detected as tissue stem cell-toxic (3 of 3 tested)
- Cytoxan (cyclophosphamide)
- Idarubicin
- BCNU (carmustine)

B. Known and proposed tissue stem cell-activating agents confirmed (2 of 2 tested)
- Xanthosine
- Human platelet lysate

C. US patent 9733236 (2017) issued for the use of the AlphaSTEM Test™ to detect tissue stem cell-active agents.

III. Cell Therapy Application Validations

Human tissue stem cells confirmed to date
- Liver stem cells
- Lung stem cells
- Corneal stem cells
- Bone marrow blood stem cells
- Umbilical cord blood stem cells
- *Adipose-derived mesenchymal stem cells*
- Bone marrow-derived mesenchymal stem cells
- Umbilical cord mesenchymal stem cells
- Amniotic fluid stem cells

For more information contact:

James L. Sherley, M.D., Ph.D.
Director
Asymmetrex
jsherley@asymmetrex.com
617-990-6819