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The Asymmetrex AlphaSTEM Test

Bringing the value of stem cell phenotyping to commercial cell strains supplied for research

Background: Tissue stem cells have essential roles in the renewal, repair, aging, and diseases (*e.g.*, cancer) of vertebrate tissues. These involvements make them a major focus for biomedical research. Ironically, given their importance, tissue stem cells pose many challenges to scientific investigation. Their tissue fractions are extremely low (typically as low as 1 per thousand tissue cells), they are difficult to isolate, and it is difficult to expand their numbers by culturing. A particularly troublesome, long-standing barrier to their investigation has been the lack of a means to count them specifically. This difficulty has persisted for more than half a century because of the well known lack of specific molecular biomarkers that identify tissue stem cells, but not their more numerous progeny, early committed progenitor cells. Asymmetrex recently solved the specific tissue stem cell counting problem with its AlphaSTEM Test technology. Asymmetrex now offers a contract service for determining the specific stem cell fraction of any cell culture population. Commercial suppliers of mammalian cells for research can use this service to determine the number of tissue stem cells in any pre-senescence or pre-crises cell strains. Such stem cell phenotyping will greatly increase the value of these cell strains for all users, particularly those pursuing tissue stem cell research.

The AlphaSTEM Test Service: The AlphaSTEM Test service requires that the client ship to Asymmetrex a single cryopreserved vial containing ideally 1 million cells. Asymmetrex scientists pioneered research to show that, because of their unique asymmetric self-renewal kinetics, during serial culture, the fraction of any type of mammalian tissue stem cells declines (1,2). After obtaining standard viable cell count data from the culturing of evaluated cells on patented serial passage schedules that accelerate the natural decline process, Asymmetrex applies proprietary computational simulation software to determine tissue stem cell number with time (See Fig. 1 below for examples with two ATCC supplied cell strains). In addition to stem cell fraction, the AlphaSTEM Test provides other stem cell-specific cell kinetics factors like generation time, self-renewal rate, and death rate (Table 1). The AlphaSTEM Test is also the first technology that can delineate the cell kinetics parameter of tissue stem cells from those of their committed progeny cells (See Table 1, *Committed Progenitor Cell GT*).

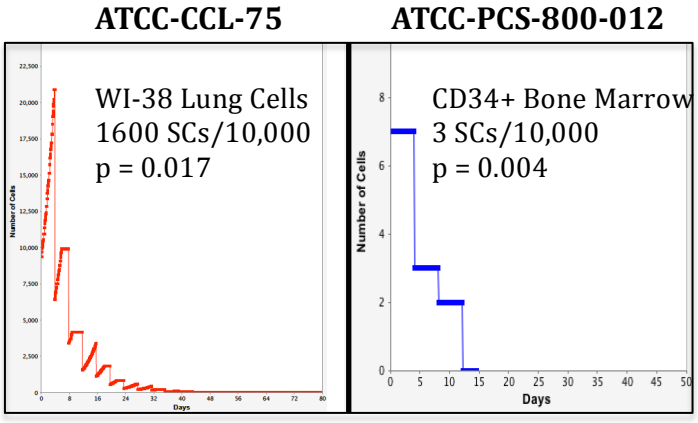

Fig. 1. Examples of AlphaSTEM Test tissue stem cell counting results from serial passage of two ATCC supplied cell strains for research, human WI-38 diploid lung fibroblasts (65,000 total cell input) and human CD34+ bone marrow cells (47,000 total cell input). Note the detection of symmetric self-renewal by lung stem cells (inclined phases), but not hematopoietic stem cells (flat phases), between passages (vertical lines). The decline in tissue stem cell number with successive culture passages is predicted due to continued asymmetric self-renewal by tissue stem cells in culture (1,2).

Table 1. Tissue Stem Cell Kinetics Parameters for ATCC Supplied Cell Strains

Cell Kinetics Factor	WI-38 (p)	CD34+ Bone Marrow (p)
Stem Cell Initial Fraction	0.16 (0.017)	0.00026 (0.004)
Stem Cell Self-renewal Rate	0.24 (0.029)	0.0013 (NS)
Stem Cell Death Rate	0.05 (NS)	0.0014 (NS)
Stem Cell Generation Time (GT)	6.4 h (0.0001)	7.0 h (0.0002)
Committed Progenitor Cell GT	8.0 h (0.011)	6.8 h (< 0.0001)

(NS, not significant)

1. Rambhatla, L., Bohn, S. A., Stadler, P. B., Boyd, J. T., Coss, R. A., and Sherley, J. L. (2001) "Cellular Senescence: Ex vivo p53-Dependent Asymmetric Cell Kinetics," *J. Biomed. Biotech.* 1, 27-36.
2. Paré, J.-F. and Sherley, J. L. (2006) "Biological Principles for Ex Vivo Adult Stem Cell Expansion," in *Current Topics in Developmental Biology*, ed. G. Schatten, Elsevier, Inc. (San Diego), Vol. 73, pp. 141-171.