AN INTRODUCTION TO AlphaSTEM TECHNOLOGY

Accelerating stem cell biomedicine with a first-ever technology for quantitative analysis of adult tissue stem cells

Overview

Asymmetrex, LLC and AlphaSTAR Corporation (**ASC**) have partnered to develop a first-of-its-kind computer simulation-primary cell culture technology with the capability of quantitative analysis of adult tissue stem cells. Because of their essential roles in normal organ and tissue function, initiation of diseases like cancer, and responses to medicines, effects on adult tissue stem cells are crucial determinants of success in tissue biology research, regenerative medicine, and drug development. This wide-ranging importance of adult tissue stem cells in biomedical research and medicine makes the longstanding unmet need for methods for their quantitative analysis all the more significant. The new "AlphaSTEM" technology provides, for the first time, the ability to quantify the number, viability, and unique tissue renewal function of adult tissue stem cells in research samples, treatment preparations, and drug evaluation assays. AlphaSTEM technology is ready for immediate deployment to estimate these important properties of adult stem cells in any mammalian tissue cell preparation, and in particular those from human subjects and patients.

The need – *Quantifying adult tissue stem cell number and function*

Adult tissue stem cells exist in small numbers in organs and tissue, and they are difficult to induce to multiply to higher numbers. These two inherent biological properties result in impure preparations when attempts are made to isolate and purify the cells. As a result, discovering biomarkers capable of specific identification of adult tissue stem cells has been an unyielding challenge. The best currently available tissue "stem cell biomarkers" are also expressed by the much more abundant lineage-committed progenitors that expand from the division of tissue stem cells. So, such biomarkers (*e.g.*, CD34 and CD133) are unable to estimate even the relative stem cell number of different research samples or cell preparations for stem cell transplantation treatments (*e.g.*, bone marrow transplantation). Being unable to estimate tissue stem cell number, these biomarkers are also unable to define the viability and the functional properties of tissue stem cells that might be present.

The solution – *Breakthrough bioengineering embraces verified aerospace innovation*

Asymmetrex, LLC was founded on fundamental knowledge of and bioengineering expertise for adult tissue stem cell kinetics, which encompass the processes by which tissue stem cells renew differentiating tissue cells and maintain their own number in mammalian tissues. Three years ago, Asymmetrex's founder began a partnership with ASC to apply multivariate probabilistic computer simulation techniques to the problem of inferring tissue stem cell properties from primary tissue cell culture data. For over 20 years, ASC has innovated physics-based computer simulation software that determines the failure, failure modes, strength, life, and safety of complex advanced materials for the Aerospace Industry. ASC has won numerous awards, including for its investigative analysis of the Columbia Space Shuttle accident, which earned it NASA's Group Achievement Award in 2004. This vast repository of computer simulation intellectual property was integrated with the adult stem cell kinetics bioengineering expertise of Asymmetrex to solve the adult tissue stem cell detection problem.

The product of this unique partnership is an innovative bioengineering approach that combines fundamental ideas in adult tissue stem cell multiplication modeling with computational simulation methods. The resulting AlphaSTEM technology estimates adult tissue stem cell number, viability, and tissue renewal function from an input of specially prescribed primary cell culture multiplication data. The software is based on a bioengineered mathematical model that describes the total cell multiplication output rates of mammalian tissue cell cultures in terms of the different subtypes of cells present (*e.g.,* stem, lineage-committed, differentiated, dead) and their individual cell multiplication rates. AlphaSTEM technology can also be used to sensitively detect effects of exogenous additives (*e.g.,* drugs) on tissue stem cell properties.

Applications

Tissue Stem Cell Toxicology: *Early prediction of drug candidate failure could save $4 billion/yr*

A crucial unmet need in the pharmaceutical industry is a convenient and effective method for assaying drug effects on tissue stem cells. In particular, drugs that are toxic to adult stem cells produce severely

debilitating side effects. For example, the dose-limiting bone marrow failure induced by many cancer chemotherapy drugs is due to toxic effects against blood-forming tissue stem cells that reside in the bone marrow. Such toxicities can be life threatening, erasing any therapeutic benefits, and curtailing the commercial success of a promising new drug candidate. An estimated 20% of drugs that fail preclinical animal testing and initial Phase I and Phase II clinical trials do so because of intolerable toxicity. At least half of these failures have features of adult stem cell toxicity. These toxicities may even emerge after a new drug has successfully reached the marketplace. Some seemingly idiosyncratic deadly drug toxicities that emerge after a new drug has entered into general medical practice – with increasing numbers of patients and more extended doses – may also be related to chronic toxicity against adult tissue stem cells. So, tissue stem cell-toxic agents are a significant cause of expensive drug candidate failures. AlphaSTEM technology could identify these much earlier in the drug development process, leading to greatly reduced drug development costs, accelerated drug development, and increased drug safety. Current estimates indicate that full implementation of AlphaSTEM technology by major U.S. pharmaceutical companies could save the industry as much as $4 billion each year.

Regenerative Medicine: *First-ever quality control for tissue stem cell transplantation treatments*

AlphaSTEM technology can provide presently lacking quality control data for new regenerative medicine clinical trials as well as significantly improve approved stem cell transplantation treatments (*e.g.*, cord blood and bone marrow transplantation). Currently, the most crucial factor for success in stem cell transplantation therapy – sufficient number of high functioning adult tissue stem cells – is an unknown throughout regenerative medicine practice. AlphaSTEM technology can fill this longstanding void. AlphaSTEM technology can also be used to identify agents that increase the therapeutic properties of adult tissue stem cells to improve the effectiveness of tissue stem cell transplantation treatments.

New Market Development: *A powerful new tool for accelerating progress in emerging genetic therapies*

Because of the crucial function of tissue stem cells in long-term maintenance of organ and tissue function, they are key elements for success in gene therapy and newly emerging gene-editing therapeutics. In fact, many current prototype genetic therapy trials target hematopoietic stem cells (HSCs) for gene-editing. Unfortunately, current editing procedures and the transplantation of edited cell preparations proceed with out knowledge of the number of HSCs present. This state of affairs is certainly compromising the success of these early trials. Asymmetrex is now working to develop AlphaSTEM technology service relationships with companies in the gene-therapy and gene-editing therapeutics space.

State of the technology – *Ready for immediate application*

The development of the AlphaSTEM computer simulation software is completed, and essential technological benchmarks have been achieved. The technology very accurately describes primary tissue cell culture data in the published literature, which in one case had previously defied explanation for more than five decades. New cell culture data were also used to validate AlphaSTEM technology's ability to precisely simulate the cell multiplication output data of pre-senescent human lung cell cultures, expanded human liver stem cell cultures, and human bone marrow cell cultures enriched for blood stem cells. AlphaSTEM technology accurately matched the best available estimates of the stem cell numbers in these cultures that span three orders of magnitude.

AlphaSTEM's ability to use specialized cell culture data to identify specific effectors of tissue stem cell function was confirmed by evaluating the effects of three known stem cell-toxic agents (chemotherapy drugs BCNU, Cytoxan, and Idarubicin) and one previously described stem cell-activating agent (the purine nucleoside xanthosine). AlphaSTEM technology very clearly discerned the respective effects of these agents using both human liver stem cells and human hematopoietic stem cells.

The opportunity – *AlphaSTEM technology is poised to accelerate stem cell biomedicine*

Asymmetrex and ASC are open to discussing adoption of AlphaSTEM technology with institutions and companies in all spheres of stem cell biomedicine. These include the pharmaceutical and biopharmaceutical industries, cord blood banks, HSC transplantation centers, regenerative medicine companies, and gene therapy and gene-editing companies. In particular, pharmaceutical and regenerative medicine companies constitute major commercialization targets, because of the immediate positive impact that implementation of AlphaSTEM technology is predicted to have on their business success.