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## Cancer Stem Cell Program

Summary of HSCI Research Activities

## HARVARD STEM CELL INSTITUTE

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**The Harvard Stem Cell Institute (HSCI)** is a scientific collaborative established in 2004 to fulfill the promise of stem cell biology as the basis for the cure and treatment of a wide range of chronic diseases and medical conditions. HSCI's unique effort unites experts across the disciplines, schools and departments of Harvard University and all its affiliated research hospitals.

HSCI also sponsors public education programs concerning the scientific, legal and ethical implications of stem cell research, conducts a summer research program for college students, and helps educate high school teachers about stem cell science. HSCI depends upon the vision and generosity of private individuals, foundation and corporate donors to carry on its work, due to current U.S. restrictions on federal funding of embryonic stem cell research.

## Introduction

The following research summaries highlight the key questions and approaches that each HSCI disease program is undertaking to address a particular disease. While each program has the same ultimate goal - to use stem cells to treat injury and disease - the differences among the particular systems, the conditions, and even the cell types dictate distinct paths toward this goal specific to each disease program. These summaries are intended to give you a sense of how each program is approaching its unique scientific challenges.

HSCI focuses on five principal disease areas - cancer, diabetes, nervous system diseases, blood diseases, and cardiovascular disease - with additional working groups in other areas including muscle, kidney, and skin. Each disease program has a leader who is responsible for defining the program objectives, drawing on the resources of the entire community and coordinating the constituent members toward the program goals. The leaders report to HSCI's Executive Committee which is responsible for assessing progress and allocating funding.

The complexities raised by the different diseases call for the vision and expertise of multiple researchers from multiple domains of expertise. Consequently, you will see that these programs highlight the synergy derived from the diverse membership of the HSCI community that extends across the schools of Harvard and the eleven Harvard-affiliated hospitals and research institutions. The union of basic, translational, and clinical research that is enabled by this community is critical to the progress and pace of discovery.

Despite the obvious differences among these disease programs, there are several common objectives guiding the research each is pursuing. One, clearly is the use of stem cells as therapy to cure that particular disease. Another, is finding drugs that could stimulate the growth of existing stem cell populations to accelerate internal repair processes. Yet another, is using stem cells to develop laboratory-based disease models to study the disease or injury *in vitro* and to identify potential therapeutic targets. In the course of pursuing these objectives, each program is able to learn from the others and leverage the resources and know-how from HSCI's core facilities, such as the Therapeutic Screening Center, to advance their program faster.

We are very excited about the progress that HSCI researchers are making toward realizing the promise stem cells hold not only as therapeutics, but also as tools both to find new drugs and to ask questions in the laboratory about the basic biology of disease processes. We hope you find these summaries informative. Please don't hesitate to contact us if you have any questions or if you would like additional information.

Brock Reeve  
*Executive Director*

## Cancer Stem Cell Program

In recent years scientists have learned that cancerous tumors contain small numbers of stem cells that are responsible for their maintenance and growth, suggesting that these cells may become a useful target for new treatments. Yet cancer treatment today relies on relatively blunt and often dangerous instruments – chemotherapy, radiation and surgery – focused on the overall reduction of tumor mass without targeting specific cells. Chemotherapy and radiation treatments are deemed effective when they are less toxic to healthy cells than cancerous cells, and dosage is set at a level where patients may still be exposed to dangerous and potentially lethal side effects. These treatments have been optimized for targeting the entire tumor mass, but often fail to destroy the stem cells that seed the tumor's growth. In order to eliminate a tumor completely it will be necessary to develop new therapies that target and eliminate the stem cells.

Comprehensive knowledge of cancer stem cells will give us a lasting foundation for all future biomedical research on cancer. This knowledge will improve our understanding of the process of tumor initiation, growth, and malignancy and our ability to screen for new, more effective drugs. The implications would stretch from basic research to commercial development of new treatments and prevention. Eventually, with success, new stem cell based approaches will augment or replace existing clinical methods with highly specific and targeted therapies that produce not just remissions but total cures.

The identification of cancer stem cell or stem-like cells has been accomplished in a very small number of tumors. Undertaken by individual laboratories, this effort can be enormously time-and resource-intensive. Since the methods and technology needed to discover these cells is very similar for all tumor types, a larger, collaborative effort studying multiple tumors in parallel might be a more effective approach. The multi-investigator, multi-laboratory HSCI Cancer Stem Cell Program provides such a framework, allowing the sharing of data resources, combining expertise for improvements in technology, and increasing the scale and scope of the effort.

The HSCI Cancer Stem Cell Program, under the leadership of Gary Gilliland at Brigham and Women's Hospital, is composed of an integrated group of basic scientists, clinical researchers, and clinical practitioners from scientific and medical institutions across the Harvard community. Together, they will define the cancer stem cells responsible for tumor generation and maintenance in many of the major types of human cancer. Their work will expand on the current knowledge of stem cell involvement in leukemia, breast cancer, and tumors of the central nervous system. The HSCI Cancer Stem Cell Program has three primary aims:

1. Identify cancer stem cells in all tumor types
2. Further characterize the biology of cancer stem cells
3. Use these insights to develop novel therapeutic approaches to curing cancer

## Identify cancer stem cells in all tumor types

### **Project 1. Build a system for tissue procurement and banking as a source of cancer stem cells**

The collaborative environment of HSCI is closing the gap between research and patient involvement. Much of our experimental material includes tumor tissue from cancer patients undergoing surgery in cooperation with hospital administrations. Personnel involved in cancer tissue collection are required to check surgery schedules, visit clinics to obtain patient consent prior to surgery, and then collect tissue in the operating room. Fresh tumor samples are then transported to our Cancer Stem Cell Core Laboratory for disaggregation and flow sorting (a technology used to separate and purify cell types). The isolated human tumor cells are then used to identify stem cell surface markers and generate antibodies that will help purify cancer stem cells from subsequent tumor samples.

## Define the unique biological characteristics of cancer stem cells

### **Project 1. Optimize the ability to culture cancer stem cells *in vitro***

After purification, the ability to maintain cancer stem cells in culture is the next important step in advancing the field. We will analyze the ability of cancer stem cells to self renew *in vitro* in the presence and absence of stromal support (the connective tissue and cellular framework that helps to support and nourish growing cells). Optimal stromal support systems will be explored using tissue-engineering approaches, while other supporting elements, such as growth factors, will be identified.

### **Project 2. Study the growth of tumors and the role of cancer stem cells in animal models**

Animal models are being used to explore cancer stem cell biology as it may occur in a living organism. Cancer cells obtained from human tissue banks are injected into non-obese diabetic/severe combined immunodeficient (NOD-SCID) mice in order to observe and analyze the formation of tumors. NOD-SCID mice provide an ideal model for studying cancer because they have genetic mutations that make them immunodeficient – they readily accept transplanted foreign cells, and have a higher-than-normal incidence of tumor formation. The injected mice are monitored daily for tumor development using non-invasive imaging methods. Zebrafish models are also being used to examine the role of genetic mutations in the emergence of cancer stem cells.

**Project 3. Study of known self-renewal pathways in cancer stem cells**

We are examining the roles of signaling pathways in the biology of cancer stem cells using reporter molecules that track the activity of specific genes. The pathways studied include Wnt/ $\beta$ -catenin, Hedgehog, BMP, and Notch – all implicated in the process of self renewal required for stem cells to survive. We are also studying Bmi-1, a cancer-related gene (oncogene) believed to be involved in pluripotency (the ability to form many different cell types), as well as other important oncogenes and tumor suppressors.

**Project 4. Take genomic approaches to cancer stem cell analysis**

Genetic, epigenetic (non-mutational alterations to DNA), and proteomic profiles capture “snapshots” of the molecular events that occur within a cell. We examine these profiles in cancer stem cells using DNA expression arrays, signal transduction profiling assays, as well as genome-wide location analysis, which indicate which parts of the DNA are being controlled by specific DNA binding proteins. A comparison of results between cancerous and normal tissue stem cells will reveal the key molecular signatures that breed cancerous tumors. Using this approach, we will study each of the major tumor types including: blood-derived cancers such as multiple myeloma, and solid tumors from the lung, prostate, kidney, liver, pancreas, stomach, colon, and ovary.

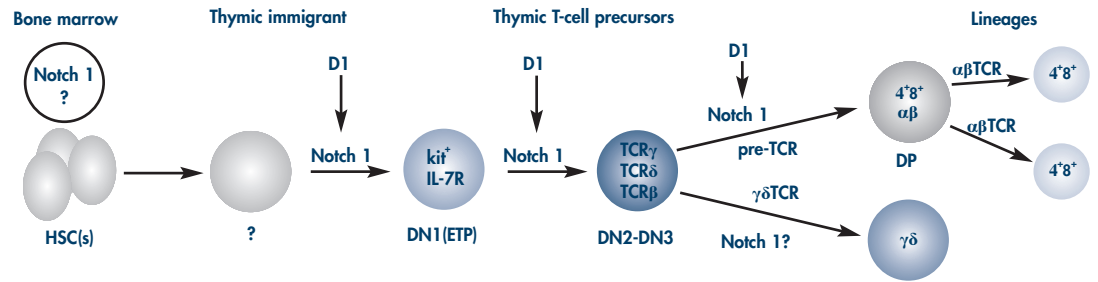
**Discover novel therapeutics targeting cancer stem cells****Project 1. Identify the shared molecular characteristics of cancer stem cells that may represent potential drug targets**

We are using the results of genomic studies to determine the differences between cancerous and normal tissue stem cells at the molecular level, in order to better understand the processes leading to malignancy and identify therapeutic targets.

Several potentially target-rich molecular pathways are already known to be involved in the formation of cancer stem cells, such as the Notch, Wnt, and Hedgehog pathways. We will be looking at treatment strategies that force stem cells to exit self-renewal programs driven by these and other pathways.

Mutations in the Notch pathway have been associated with a number of leukemias, particularly, T-cell acute lymphoblastic leukemia (T-ALL). The Notch pathway is essential for T-cell development and probably hematopoietic (blood forming) stem cell self-renewal (see Figure 1). Work by Jon Aster, Steve Blacklow, and Tom Look at Brigham and Women’s Hospital, Dana Farber Cancer Institute, and Harvard Medical School found that mutations in the NOTCH1 gene that can keep the pathway in a constantly active state are found in nearly 60% of adult and pediatric T-ALL. This makes the Notch pathway an extremely attractive therapeutic target. NOTCH1 mutations appear to be sensitive to gamma-secretase inhibitors, and their use is being explored in Phase 1 clinical trials led by Daniel DeAngelo and Lewis Silverman with Merck.

**FIGURE 1.**  
**The Notch pathway is essential for T-cell development and probably hematopoietic stem cell self-renewal. Shown here is the likely role of the Notch pathway in these processes. Reprinted with permission from Grabher et al., Nature Reviews Cancer (2006) 6, 347-359.**



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In addition to their roles in embryonic pattern formation, the Hedgehog, along with the Wnt pathway, have been found to have a role in renewal of tissue stem cells in a number of organs including blood, muscle, and the nervous system. Genetic and cell culture experiments indicate that the pathways promote self-renewal by stimulating proliferation. In addition to the role of these pathways in normal stem cell self-renewal, it is significant that many cancers correlate with increased activity of these pathways. Inhibitors of these pathways, such as cyclopamine, which acts on Hedgehog signaling, are being screened for potential anti-cancer properties.

**Project 2. Identify the pathways and genes that regulate cancer stem cell self-renewal**

We are working to identify pathways, genes and other factors that regulate stem cell self-renewal using short-hairpin RNA (shRNA) as a tool to silence specific genes within the cell. shRNAs are short RNA sequences that are complementary to the target gene and trigger the degradation of its messenger RNA before it can help make protein. By analyzing the loss of function that occurs when a gene is silenced by shRNA, we can begin to determine the role of each gene in the self-renewal pathway. We are also studying the role of pathway components by adding them into the cell through retroviral transduction, which integrates the new genes into the chromosome.

**Project 3. Screens for small molecules that specifically target cancer stem cells**

We are screening chemical libraries to find compounds that effectively target the molecular pathways of the cancer stem cell.

The overall goal of the approaches outlined here is to enable a fundamental paradigm shift in the treatment of cancer. This transition will move clinicians from a virtually blind assault on replicating cells, to a specific, minimally toxic approach that actually targets the source. Such an approach will allow us to speak of cures, rather than remissions, and countless lives could be saved.

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