The Fifth Comprehensive Cancer Research Training Program (CCRTP) at Stanford University

presented by
Stanford Cancer Institute
Stanford University School of Medicine

Course Directors:

Amato Giaccia, Ph.D.
Ginna Laport, M.D.
Karl Blume, M.D.

Quadrus Conference Center

2400 Sand Hill Road
Menlo Park, California

September 12-16, 2011

STANFORD
CANCER INSTITUTE

5th Annual

Comprehensive Cancer Research Training Program At Stanford University

Monday, September 12 – Friday, September 16, 2011

at Quadrus Conference Center

Menlo Park, CA

Presented by

Stanford University School of Medicine
Philip A. Pizzo, MD, Dean

Stanford Cancer Institute
Beverly S. Mitchell, MD, Director

Course Directors

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Produced by the Stanford Cancer Institute, June 2011
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Course Overview

In September, 2007, 2008, 2009, and 2010, the first four annual Comprehensive Cancer Research Training Program (CCRTP) courses were held. This educational initiative was exceedingly well received. Selected responses from trainees who attended the first four courses are included in this brochure.

The Stanford Cancer Institute invites again graduate students and post-doctoral students (residents, clinical fellows, research fellows) and junior faculty members from Stanford University, the University of California at San Francisco (UCSF), and San Francisco State University (SFSU) to attend the fifth annual Comprehensive Cancer Research Training Program. In particular, trainees from the respective Schools of Medicine, Schools of Engineering, and the Schools of Humanities and Sciences from the three universities are encouraged to participate. The Stanford Cancer Institute will host this course between Monday, September 12 and Friday, September 16, 2011, at the Quadrus Conference Center at 2400 Sand Hill Road in Menlo Park, California.

This dynamic educational event features a plenary session on a topic of general interest every morning, followed by nine half-day sessions on scientific themes with three presentations each. The 27 didactic lectures on current topics of basic, translational, and clinical cancer research are presented by nationally and internationally renowned faculty from Stanford University and UCSF. Each didactic lecture is followed immediately by a discussion. In addition, registered trainees are invited to present their own research posters on September 14, 2011. A total of 20 posters will be selected. Submissions will be reviewed and selected by the three course directors.

Three months in advance of the course, registered trainees will receive a course syllabus in the mail. The syllabus features an illustrated summary and a reading list for each lecture. The syllabus will be available as a hard copy (figures in black and white) and in an electronic version (figures in color). Trainees are encouraged to use this syllabus in preparation for active participation during lecture discussions.

Evaluation

At the end of each day, the participants are asked to evaluate the presentations that they heard. After the course, the directors will send the participants a limited number of questions allowing formative and summative evaluations of the course. These evaluations will be mailed electronically to the course participants at two time points, i.e., one month following the course and again nine months after completion of the event. Participants are strongly encouraged to provide feedback to the course directors.

Tuition

No tuition fees will be charged. In order for trainees to fully participate in the course, their Laboratory Directors, Division Chiefs, and Department Chairs are asked to provide the trainees with free educational time without clinical responsibilities for the week of September 12 through September 16, 2011. In turn, the organizers expect that the CCRTP trainees will attend the event in its entirety.

Registration

Attendance is limited to 130 participants. Early registration is encouraged. Attendees may register by mail or fax until June 30, 2011. A registration fee of $95.00 is required and may be paid by check or department transfer. Complete registration instructions are located on the registration form in this brochure.

Registered participants will receive a copy of the syllabus three months prior to the course and have access to all lectures, daily breakfast, and lunch, and enjoy a reception at the end of the first day of the course. Each trainee will be awarded a certificate of participation at the end of the course, Friday, September 16, 2011.

Housing

Trainees travelling a long distance may choose to stay at the housing facility of the Stanford Linear Accelerator that is located across the street from the Quadrus Conference Center. The cost per night is approximately $115.00. This expense must be covered by the trainee or their department.

September 12, 2011, Monday

7:45am **Breakfast**

8:15am **Introduction into the Course** *Amato Giaccia, Ph.D.*

8:30am **Plenary Lecture 1: Introduction: Cancer and Cancer Research-2011** *Beverly Mitchell, M.D.*

SIGNAL TRANSDUCTION

9:25am **Wnt Signaling in Cancer** *Roeland Nusse, Ph.D.*

10:15am **Coffee Break**

10:35am **Hedgehog Signaling in Regeneration and Neoplasia** *Philip Beachy, Ph.D.*

11:25am **Translational-Clinical Research Based on Signaling Studies** *Jean Tang, M.D., Ph.D.*

12:15pm **Lunch**

TECHNOLOGY AND PERSONALIZED MEDICINE

1:15pm **High-throughput Gene Sequencing and Cancer** *Hanlee Ji, M.D.*

2:05pm **Gene Regulation in Cancer** *Howard Chang, M.D.*

2:55pm **Genome and Immunome Sequencing in Hematologic Malignancies** *Scott Boyd, M.D., Ph.D.*

3:45pm **Reception**

5:15pm **Adjourn**

September 13, 2011, Tuesday

7:45am	Breakfast
8:30am	Plenary Lecture 2: Transplantation of Allogeneic Blood-Forming Cells: History, Achievements and Challenges *Karl Blume, M.D.*

STEM CELLS AND CELLULAR THERAPIES

9:25am	Human Pluripotent Stem Cells *Renee Reijo Pera, Ph.D.*
10:15am	Coffee Break
10:35am	Normal and Cancer Stem Cells *Judith Shizuru, Ph.D., M.D.*
11:25am	Cellular Immunotherapies *Robert Negrin, M.D.*
12:15pm	Lunch

SOCIAL ASPECTS OF CANCER

1:15pm	Social Adaptation after Cancer *Karen Cook, Ph.D./Ginna Laport, M.D.*
2:05pm	Quality of Life Studies in Cancer Patients: Focus on Sleep and Fatigue *Oxana Palesh, Ph.D.*
2:55pm	Palliative Care Research *Vyjeyanthi Periyakoil, M.D.*
4:15pm	Adjourn

September 14, 2011, Wednesday

7:45am	Breakfast
8:30am	Plenary Lectures 3 and 4: Emerging Plasma Accelerator Technologies for Future Particle Therapies *Mark Cappelli, Ph.D.*
9:25am	Small and Large Molecule Therapeutics and Drug Delivery *Paul Wender, Ph.D.*
10:15am	Coffee Break

BIOCOMPUTATION IN CANCER RESEARCH

10:35am	Systems Biology of Cancer *Sylvia Plevritis, Ph.D.*
11:25am	Biocomputation in Genomics *Catherine Ball, Ph.D.*
12:15pm	Lunch

HOW TO...

1:15pm	How to Write a Grant Application *Mildred Cho, Ph.D.*
2:05pm	How to Design a Clinical Trial *Ginna Laport, M.D.*
2:55pm	How to Write a Scientific Paper *Robert Lowsky, M.D.*
4:00pm	Presentation of Research Posters by Course Participants
5:30pm	Adjourn

September 15, 2011, Thursday

7:45am	Breakfast
8:30am	Plenary Lecture 5: Cancer Drug Development by the Pharmaceutical Industry *Sandra Horning, M.D., Genentech*

TRANSLATIONAL/CLINICAL CANCER RESEARCH I

9:25am	Targeting Mutant Ras *Frank McCormick, Ph.D., UCSF*
10:15am	Coffee Break
10:35am	Therapeutic Targeting of Human Acute Myeloid Leukemia Stem Cells *Ravi Majeti, M.D., Ph.D.*
11:25am	Molecular Risk Assessment for Lymphoma *Ash Alizadeh, M.D., Ph.D.*
12:15pm	Lunch

TRANSLATIONAL/CLINICAL CANCER RESEARCH II

1:15pm	Prostate Cancer: The Challenge of Screening and Prognostication *James Brooks, M.D.*
2:05pm	Innovative Approaches to the Treatment of Non-Small Cell Lung Cancer *Joel Neal, M.D.*
2:55pm	Breast Cancer: Epidemiology and Risk Factors *Allison Kurian, M.D., M.Sc.*
4:00pm	Adjourn

September 16, 2011, Friday

7:45am	Breakfast
8:30am	Plenary Lecture 6: The Case for Investments in Innovation and Discovery During a Time of Resource Constraint: Cancer as a Model *Philip Pizzo, M.D.*

SCREENING FOR CANCER TARGETS

9:25am	Mechanisms for Maintaining Genome Stability at the Replication Fork *Karlene Cimprich, Ph.D.*
10:15am	Coffee Break
10:35am	Cancer Biomarkers and Molecular Therapeutics *Dean Felsher, M.D., Ph.D.*
11:25am	Clinical Cancer Trials Based on Target Selection *James Ford, M.D.*
12:15pm	Closing Remarks *Amato Giaccia, Ph.D.* Certificates to Trainees
12:30pm	Lunch
1:15pm	Adjourn

Cancer and Cancer Research-2011

Beverly S. Mitchell, M.D.
Director, Stanford Cancer Institute
Stanford University

The "War on Cancer" - 40 Years Later

Cancer Centers developed out of the Nixon Administrations "war on cancer" that led to the development of the National Cancer Institute as a semi-autonomous arm of the National Institutes of Health. Since that time, approximately $90 billion dollars have been spent by the NCI on cancer research. There has been considerable concern that the "war on cancer" has not provided the promised results in terms of cures. Yet, overall cancer mortality has been declining since the 1990's. The purpose of this talk is to briefly review what we have achieved in cancer research, raise the question of how we might have achieved more, and open discussion as to what we might hope to accomplish in the future.

Is Cancer an Impossibly Difficult Problem?

No, but it is immeasurably more complicated than is perceived by the public and than has been communicated by researchers and advocates in the field.
A few reasons for the complexity:

- Many different tissue types are involved
- Different cells are dependent on different signaling pathways
- Multiple genetic lesions co-exist in the cancer cell
- Genomic instability amplifies the number of genetic lesions
- The cancer stem cell model remains under investigation
- Mechanisms of metastasis remain to be elucidated

What Recent Successes have been Achieved?

- New targeted therapeutics
  - Melanoma, lung cancer, lymphomas, breast cancer, multiple myeloma
- Emphasis on prevention/early detection
  - Breast cancer, lung cancer, colon cancer
- Understanding dependence of cancer cells on metabolic pathways, oncogenes
- Genomic analysis

What Barriers Continue to Exist?

- Lack of ideal model systems
- Complexity of biologic systems- eg regulation of RNAs, epigenetics, network of signaling pathways
- Difficulty in identifying and pursuing "big" ideas
- Expense, difficulty, and enrollment of patients in clinical trials
- Expense of research in general and funding "priorities"
- Cost of clinical care

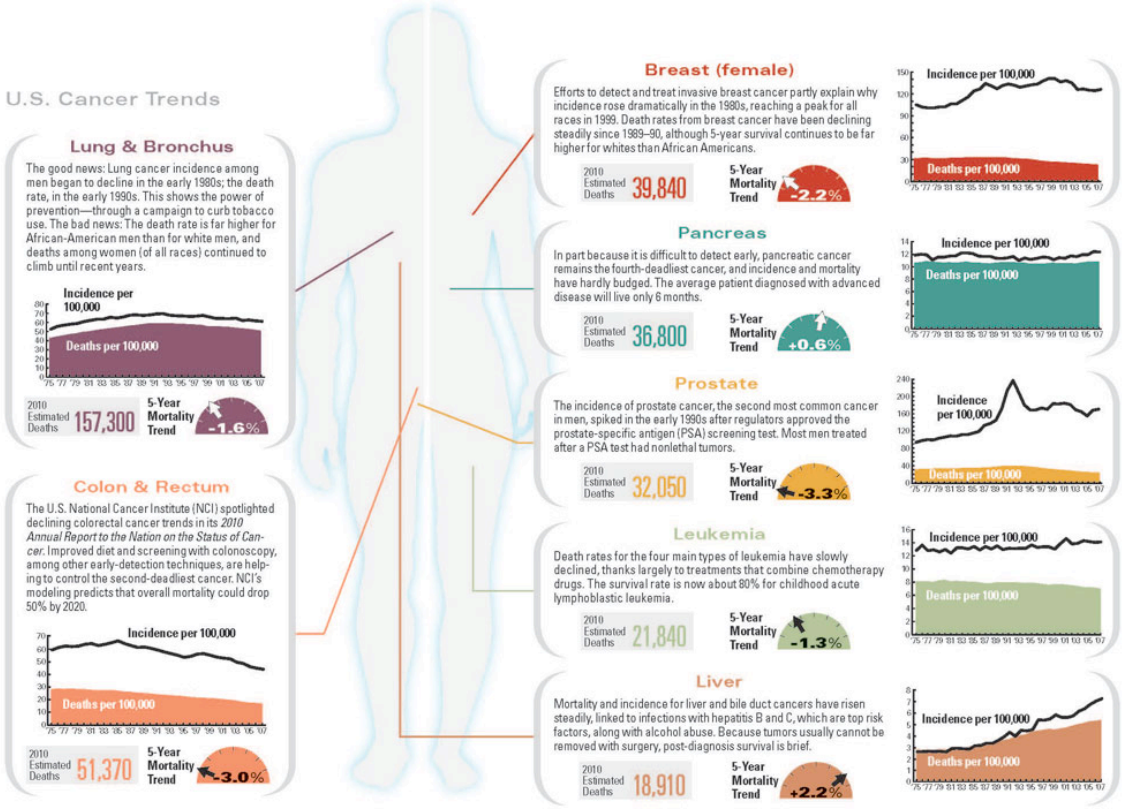
Where Does the Future Lie?

- Increased emphasis on prevention, early diagnosis
- Improved quality of care
- Understanding the cancer genome and epigenome
- Understanding the role of the immune system in cancer development and treatment
- Developing better algorithms to understand signaling pathways
- Use of combinations of therapies
- Emphasis on both basic and "Translational" Research

Definition: Translational Research is the process of applying ideas, insights and discovery generated through basic science research to the diagnosis, treatment or prevention of human disease.

Translational research also refers to the wider spectrum of patient-oriented research that embraces innovations in technology and biomedical devices as well as the study of new therapies in clinical trials. It also includes epidemiological and health-outcomes research and behavioral studies that can be brought to the bedside or ambulatory setting.

Incidence and Mortality Data for seven cancers taken from Science, March 25, 2011:



Subjects for discussion: What is the best way to facilitate cancer research, given a shrinking annual investment by the NCI? How do participants in the CCRTP perceive the opportunities and impediments to pursuing and contributing to cancer research activities at Stanford?

References:

1. The Emperor of all Maladies: A Biography of Cancer Siddhartha Mukherjee

2. Guy Faquet: The War on Cancer: an anatomy of failure and a blueprint for the future. Springer, 2008.

3. Cancer Crusade at 40. Science Magazine, March 25, 2011 Issue

Wnt Signaling in Cancer

Roel Nusse, Ph.D.
Howard Hughes Medical Institute
Professor and Chair, Developmental Biology
Stanford University

Wnt proteins comprise a large family of highly conserved growth factors that control important developmental and homeostatic processes. Wnt genes are widely implicated in developmental events and human diseases. Wnts and their signaling pathways have therefore become the subject of intense investigation over the last two decades. Recently, Wnt signaling has been associated with stem cell fate and these observations have added fuel to both these already very active fields. Through biochemical, genetic, and genomic approaches, our knowledge of Wnt proteins, their production and signaling properties, and their evolutionary history has expanded rapidly.

Membership in the Wnt family is defined by amino acid sequence rather than functional properties. It is therefore not too surprising that Wnts have been associated with a number of different activities and downstream signaling pathways. The defining event in canonical Wnt signaling is the cytoplasmic accumulation of β-catenin and its subsequent nuclear translocation and activity. Under non stimulated conditions, a β-catenin destruction complex formed by proteins that include Axin, Adenomatous Polyposis Coli (APC) and glycogen synthase kinase-3b (GSK-3) keeps cytoplasmic levels of β-catenin low through phosphorylation by GSK-3 (Figure 1). Phosphorylated β-catenin becomes ubiquitylated and is targeted for degradation by the proteasome. Following Wnt binding to a receptor complex composed of members of the Frizzled (Fz) family of seven transmembrane, serpentine receptors and low density lipoprotein (LDL) receptor-related protein (LRP), the Axin/APC/GSK-3 complex is inhibited, leading to a block in β-catenin phosphorylation by GSK-3. Hypophosphorylated β-catenin accumulates in the cytoplasm and is translocated to the nucleus where it regulates target gene expression through partnerships with the TCF/LEF family of transcription factors (Figure 1).

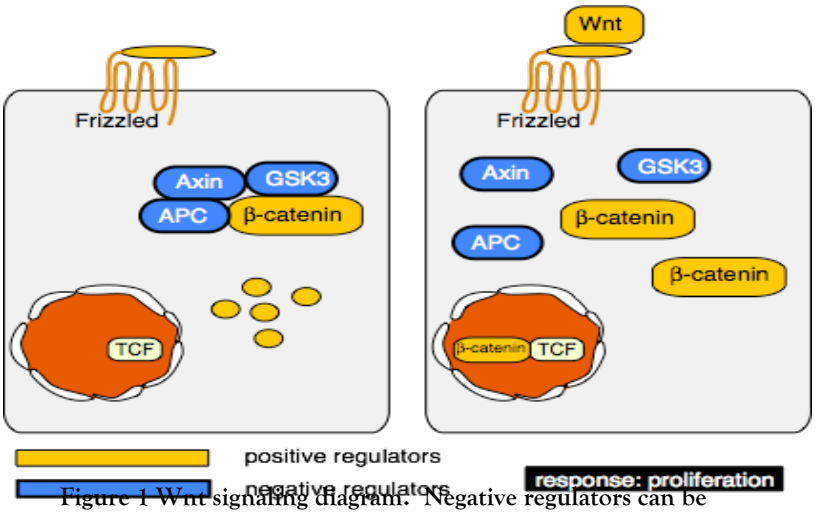


Figure 1 Wnt signaling diagram. Negative regulators can be found to be muted in cancer, leading to activation of the Wnt pathway.

Several components of the Wnt signaling pathway have been implicated in human tumors or experimental cancer models. Wnt-1 was found as an oncogene activated by the Mouse Mammary Tumor Virus in murine breast cancer. APC was first isolated as a tumor suppressor gene in human colon cancer. After establishing that APC and β-catenin bind to each other , activating mutations in the human β-catenin gene were found in human colon cancer and melanomas. These mutations alter specific β-catenin residues important for GSK3 phosphorylation and stability. Interestingly, mutations in the human AXIN1 gene were reported in human hepatocellular carcinomas. These discoveries underscore the important predictive value of these pathways: by establishing how genes control signaling (e.g.

whether they act as repressors or activators) one can make educated guesses how these gene might contribute to human cancer. A repressor can be a tumor suppressor gene and an activator a dominant oncogene. The same principle applies to other pathways, such as the Hedgehog- Patched pathway and the TGF beta pathway.

In the presentation, I will highlight the various functions of Wnt signals in normal tissue homeostasis. There will also be a discussion on the role of Wnt signaling in human cancer, in cancer stem cells and in cancer genetics.

References

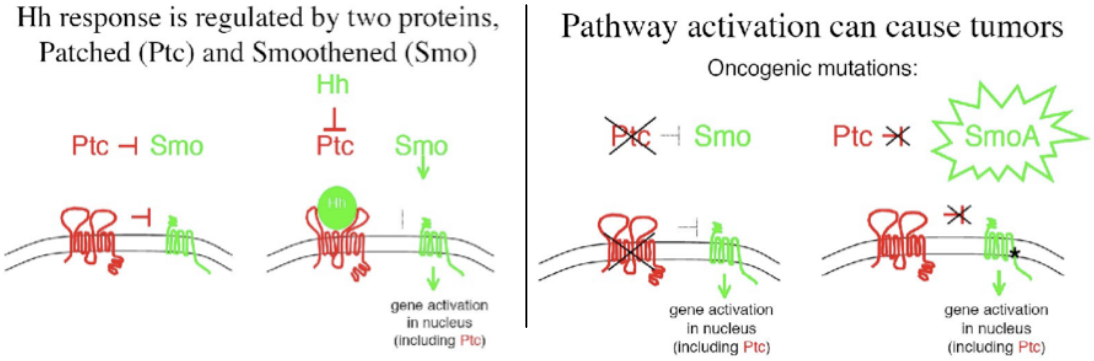
1. Clevers, H Wnt/beta-Catenin Signaling in Development and Disease. Cell 2006, 469 (2006)
2. van Amerongen R, Nusse R.Towards an integrated view of Wnt signaling in development. Development. 2009 Oct;136(19):3205-14.
3. Reya, T., and Clevers, H. (2005). Wnt signalling in stem cells and cancer. Nature 434, 843.
4. Cadigan KM, Peifer M.Cold Spring Harb Perspect Biol. 2009 Aug;1 Wnt signaling from development to disease: insights from model systems.
5. The Wnt homepage: http://www.stanford.edu/group/nusselab/cgi-bin/wnt/

Hedgehog Signaling in Regeneration and Neoplasia

Philip Beachy, Ph.D.
Howard Hughes Medical Institute
Institute for Stem Cell Biology and Regenerative Medicine, Stanford Cancer Institute

During the past twenty years the genetics of development and cancer have converged in the identification of extracellular signaling pathways, such as those headed by the Wnt and Hedgehog (Hh) signaling proteins, that play central roles in embryonic patterning and are also aberrantly regulated in cancer. In tissues where these pathways normally operate, often with roles in development and regeneration, tumors can result when these pathways become inappropriately active. Thus, for example, the Wnt pathway is inappropriately active in all or nearly all cases of colon cancer, and activation of the Hh signaling pathway is inappropriately activated in all or nearly all cases of basal cell carcinoma (BCC).

Genetic studies in *Drosophila* demonstrated that the Hh pathway is activated at high levels in *ptc* mutants. The Ptc protein thus appears to function in negatively regulating activity of the Hh pathway. Other genetic studies indicated that it does so by blocking the positive action of the seven-transmembrane protein encoded by the gene *Smoothened* (*Smo*). Stimulation by Hh protein inactivates Ptc, thus releasing the Smo protein for positive action within the pathway. Isolation of mouse and human genes for *Patched* permitted chromosomal mapping of these genes and identification of a disease, Basal Cell Nevus Syndrome, (BCNS, also called Gorlin Syndrome), associated with heritable, heterozygous mutations in the human *Patched* gene. BCNS patients (*Ptch* mutant heterozygotes) are prone to a high incidence of basal cell carcinoma (BCC) as well as other tumors such as medulloblastoma with loss-of function of the wild type *Ptch* allele. *Ptch* mutations are also found in sporadically occurring BCC and medulloblastoma in patients without BCNS, as are mutations that render Smoothened constitutively active, that is, insensitive to regulation by Ptch. The *Ptch* gene thus formally behaves as a tumor suppressor and *Smoothened* as a proto-oncogene.



The Hh pathway can also play a role within tumor cells without mutation of pathway components when pathway activity is induced by the Hh protein signal. Such tumors may include certain neural, or hematologic tumors. In addition, the Hh pathway can play a supporting role in tumor growth when it becomes activated in the stromal cells that support the growth of endodermal epithelia. One example of such a tissue where we understand the normal role of Hh signaling is the mammalian urinary bladder, in which epithelial stem or progenitor cells normally produce Sonic hedgehog (Shh) protein but are themselves not responsive. Shh production increases during regeneration after injury, and underlying stromal cells respond by expressing secreted factors such as Wnt and FGF that in turn support proliferation This epithelial/stromal interaction represents a critical new component in our understanding of normal tissue homeostasis and control of cellular proliferation, with significant implications for tumor growth and metastasis.

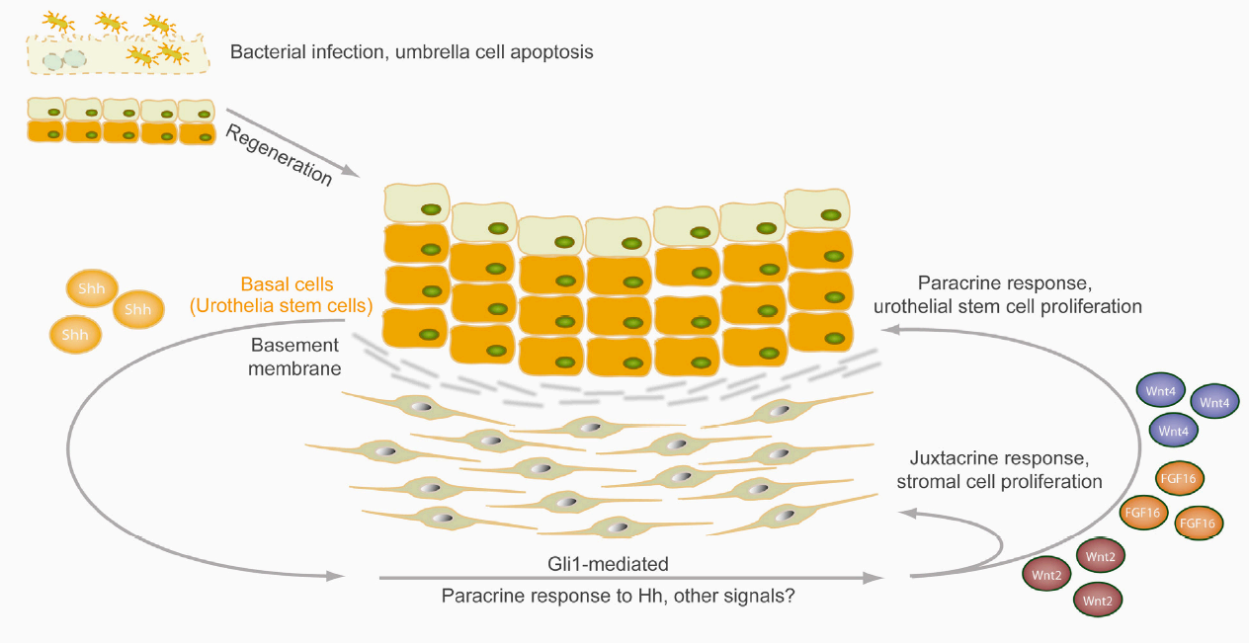


Figure 2. Epithelial/Stromal Interaction in Bladder Drives Regenerative Response to Bacterial Injury. Following bacterial infection and exfoliation of the facet cell layer, increased Shh expression in epithelial cells triggers increased expression of Wnt2, Wnt4, ad FGF16 via Gli1 in the stroma, and these factors in turn stimulate increased proliferation of both stromal and epithelial cells.

Drugs that inhibit Hh pathway activity have entered clinical trials in cancer patients, and were initially identified as plant-derived teratogens such as cyclopamine, which was identified from its ability to induce cyclopia and other malformations characteristic of Hh pathway loss in livestock. The drugs in patient trials include a cyclopamine derivative, synthetic compounds, and drugs approved by the FDA for other purposes. Most of the synthetic compounds act in the same way as cyclopamine, as antagonists of Smoothened. A few of these drugs act by mechanisms distinct from cyclopamine, and may be particularly useful for tumors in which the Hh pathway is activated by mechanisms that are not affected by cyclopamine mimics.

Recommended reading:

1. Kim, J., Lee, J.J., Kim, J., Gardner, D., Beachy, P.A. Arsenic antagonizes the Hedgehog pathway by preventing ciliary accumulation and reducing stability of the Gli2 transcriptional effector. Proc. Natl. Acad. Sci. 107:13432-13437 (2010).

2. Kim, J., Tang, J., Gong, R., Kim, J., Lee, J.J., Clemons, K.V., Chong, C.R., Chang, K.S., Fereshteh, M., Reya, T., Liu, J.O., Epstein, E.H., Stevens, D.A., Beachy, P.A. Itraconazole, a commonly used anti-fungal that inhibits Hedgehog pathway activity and cancer growth. Cancer Cell 17: 388-399 (2010). PMCID, in process.

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4. Taipale, J., Chen, J.K., Cooper, M.K., Wang, B., Mann, R.K., Milenkovic, L., Scott, M.P., and Beachy, P.A. (2000). Effects of oncogenic mutations in Smoothened and Patched can be reversed by cyclopamine. Nature 406:1005-9.

5. Teglund, S. & Toftgard, R. Hedgehog beyond medulloblastoma and basal cell carcinoma. Biochim Biophys Acta 1805, 181-208 (2010).

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Translational-Clinical Research Based on Signaling Studies

Jean Y. Tang, M.D., Ph.D.
Assistant Professor, Dermatology
Stanford University

This lecture will focus on targeting the Hedgehog (HH) signaling pathway for treatment and prevention of BCC tumors. The rapid translation of HH inhibitors into clinical trial testing can be attributed to accurate mouse models, biomarkers, and patients with many tumors.

Basal cell carcinomas (BCCs), is the most common cancer in light-skinned populations, with at least 800,000 new cases diagnosed in the United States each year. More recently the identification of aberrant activation of the hedgehog signaling pathway as the pivotal molecular abnormality in basal cell carcinomas (BCCs) has suggested the possibility of treating these locally advanced or metastatic BCCs with drugs that act specifically to block that signaling pathway. In the Phase 1 trial of the first of these specific hedgehog inhibitors, GDC-0449, 33 patients with locally advanced or metastatic BCCs were treated. The results were extremely promising in that there was significant shrinkage of metastatic and of advanced cutaneous BCCs with relatively mild systemic toxicity and excellent improvement in the subjects' clinical well being (16). Unfortunately, as been the case with several other such molecularly targeted anti-cancer drugs, at least some of these advanced tumors eventually developed resistance to the targeted therapy. The molecular mechanism(s) by which the tumors acquired resistance is not known. Several other systemic hedgehog inhibitors and at least one topical hedgehog inhibitor are in clinical trials but the results of their use have not yet been published.

In the general population, BCCs arise from the fifth decade of life and in small numbers (i.e. 1-2 tumors). However, approximately 1 in 60,000 Americans have an autosomal dominant genetic condition, basal cell nevus syndrome (BCNS), also known as nevoid basal cell carcinoma syndrome (NBCCS) or Gorlin syndrome (OMIM #109400). These individuals are predisposed to develop tens to hundreds of BCCs from puberty and throughout their lifetime [1, 2]. The discovery that these patients carry a germline mutation in the *PATCHED1 (PTCH1)* gene, rendering them constitutively heterozygous (+/-) for *PTCH1* pointed to the Hedgehog (Hh) signaling pathway as the pivotal cause of BCC [3, 4].

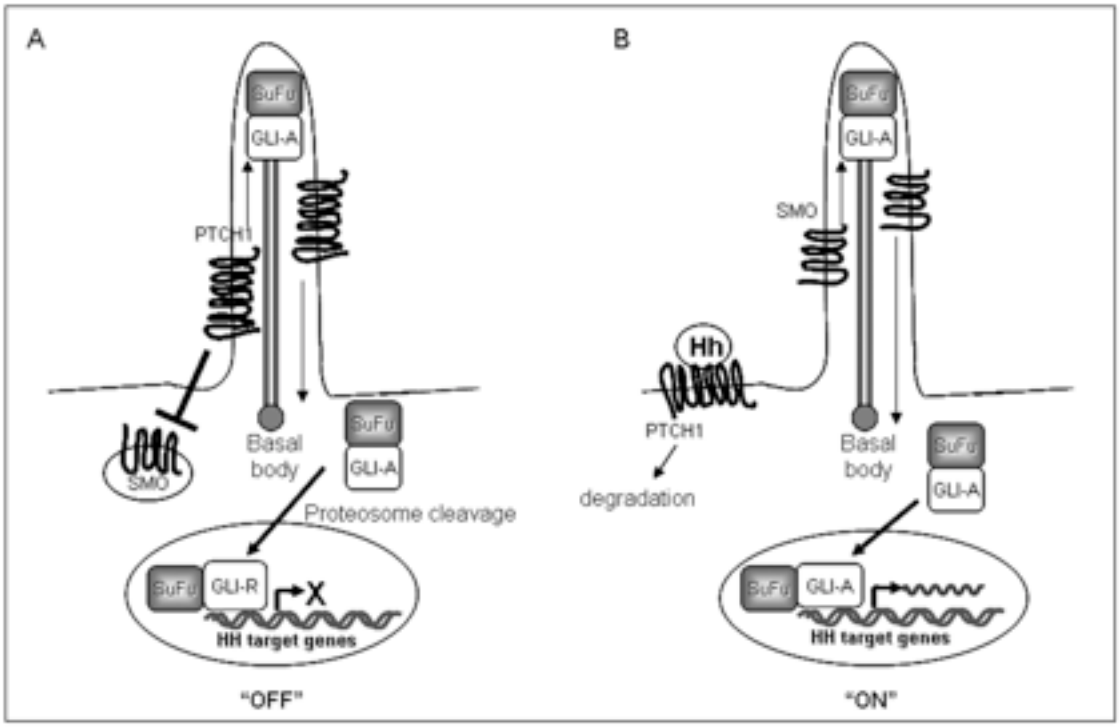
The Hh pathway is an important developmental pathway that is essential for embryogenesis. In adults, the pathway is generally dormant except in hair follicle cycling and in maintenance of some stem cell populations [5]. PTCH1 protein, a 12-transmembrane receptor, is a negative regulator of the Hh pathway (Figure 1). In the absence of Hh protein, PTCH1 inhibits the function of another transmembrane protein, SMOOTHENED (SMO), a key, positive regulator of HH signaling. Hh binding to PTCH1 alleviates repression of SMO to allow the latter to activate the Hh pathway via protein kinases, culminating in the transcriptional activation by GLI transcription factors of Hh pathway target genes, such as *Bcl2, FoxM1, Ptch1*, and *Gli1.* There are three GLI proteins - GLI1, GLI2, and GLI3.

In BCNS patients, BCCs commonly develop after somatic inactivation of the remaining *PTCH1* allele. Therefore PTCH1 acts as a classical tumor suppressor which inhibits Hh signaling and thereby prevents BCC carcinogenesis. Many studies confirm the pivotal role of aberrant Hh signaling in BCC carcinogenesis: all human and murine, sporadic and germline BCCs analyzed have abnormal activation of Hedgehog (HH) signaling, commonly due to *PTCH1* inactivating mutations and *SMO* activating mutations (approximately 90% and 10% respectively) [6-8]. In addition, murine transgenic models that over-express Sonic hedgehog (Shh) [9]; Gli1 [10] or Gli2 [11]; express constitutively activated Smo [12]; 'knock-out' Ptch1 [4, 13] or Suppressor of Fused (SuFu), a negative Hh pathway regulator [14, 15] result in BCC carcinogenesis, indicating that hyperactivation of Hh signaling initiates BCC development.

Understanding the etiology and molecular pathogenesis of this cancer and testing potentially viable drugs in preclinical systems are important approaches to the identification of novel therapeutic and preventive treatments for patients with BCC. For example, the irradiated $Ptch1^{+/-}$ mice provides an accurate, practical model for studying human BCC since it phenocopies both the familial (BCNS) and

somatic disease [16] therefore providing an invaluable preclinical 'tool' for the identification and implementation of novel, effective chemopreventive and therapeutic strategies for treating human BCC.

Figure 1. The Hedgehog (Hh) signaling pathway.
A) In the absence of Hh ligand, Ptch1 in the primary cilium represses Smo function, resulting in the proteolytic processing of Gli-Activator (Gli1-A) (bound to SuFu, a negative regulator of Hh signaling) to Gli-repressor (Gli-R). The latter then binds to the promoters of Hh target genes to repress transcription. B) In the presence of Hh, Ptch1 translocates out of the cilium and is degraded, allowing Smo to enter the cilium and activate the Hh pathway by preventing cleavage of Gli proteins to its repressor form. Gli-A enters the nucleus and activates Hh-target gene (e.g. *Gli1, Ptch1, Bcl2, FoxM1*) transcription.



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Cancer Genome Sequencing

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Introduction

Mutations and genomic aberrations in cancer genomes contribute to the initial development of cancer (Kinzler, Nilbert et al. 1991) and tumor behavior such as metastatic spread (Vogelstein and Kinzler 1992). These same mutations and aberrations may be useful as genetic prognostic and predictive biomarkers with clinical application (Mardis 2010). Full genome sequences of a number of tumors as well as surveys of the coding regions of cancer genomes have been recently been published, pointing to a new way of understanding the genetic and genomic basis of cancer (Meyerson, Gabriel et al. 2010). These systematic, large-scale DNA sequencing surveys have revealed that the genetic spectrum of mutations in cancers appears to be highly complex with numerous low frequency bystander somatic variations, and a limited number of common, frequently mutated genes (Stratton, Campbell et al. 2009).

Early examples of cancer genome sequencing analysis

Citing some examples, the earliest surveys of cancer genomes by sequencing involved analysis of the exome, the compilation of the exons in the human genome. These early studies used traditional Sanger DNA sequencing. For example, Wood et al. (2008) sequenced exons of 20,857 transcripts, representing 18,191 genes from 11 breast and 11 colorectal cancers and reported 1,718 genes with somatic non-silent mutations (Wood, Parsons et al. 2007). By evaluating the mutation data they identified 140 candidate cancer genes in both tumor types that are significantly represented above background mutations (Wood, Parsons et al. 2007). Their study revealed only few frequently mutated genes among abundance of rare variants and the identified gene mutations clustered key biological pathways such as PI3K-mediated signal transduction (Wood, Parsons et al. 2007). Likewise, Jones et al. (2008) sequenced 20,661 protein-coding genes in 24 pancreatic cancers and identified 1,327 genes with one mutation and 148 genes with two or more mutations (Jones, Zhang et al. 2008). An average of 63 genetic alterations was identified in the 24 pancreatic cancers surveyed that clustered in 12 generally defined cellular signaling pathways and biological processes. Similarly, Parsons et al. (2008) sequenced 20,661 protein coding genes in 22 glioblastoma multiforme samples (Parsons, Jones et al. 2008). Importantly, they identified frequent (12%) mutations in the *IDH1* (isocitrate dehydrogenase enzyme) gene that were associated with young patients with secondary disease and increased overall survival. Similarly, the Cancer Genome Atlas consortium, a National of Institute project geared towards the genetic characterization of cancer, sequenced 601 genes in 91 glioblastoma multiforme samples. Out of the total, 224 of these genes contained mutations in at least one sample. However, only eight genes and their mutations were identified to be statistically overrepresented compared to background mutations (2008). Noteworthy, the *IDH1* gene was not among the list of candidates in the Cancer Genome Atlas effort - underlining the importance of the comprehensive genome sequencing.

Sequencing Cancer Genomes with Next Generation Technology

Currently, next generation DNA sequencing technology is the platform of choice for characterizing the DNA of cancer genomes. These sequencers produce Gigabases worth of DNA sequence data and have enabled the comprehensive resequencing of cancer genomes. All of these new next generation sequencing methods generally require that the matched normal genomic DNA from the same individual be sequenced simultaneously to discriminate germline variants from somatic mutations. With multi-dimensional genomic characterization involving transcriptome and copy number variation surveys carried through DNA sequencing, new biological and clinical relevant genetic and genomic changes can be revealed (Network 2008).

To sequence a cancer genome with next generation sequencers, most research groups use "shotgun" methods(Koboldt, Ding et al. 2010). This typically involves the random fragmentation using physical or chemical breakage of cancer genomic DNA from a given sample with subsequent ligation of a sequencing "adaptor" oligonucleotide sequences to the ends of the DNA fragments. This preparation is frequently referred to as a "sequencing library". Physical shearing, chemical or enzyme-based methods have been utilized in fragmentation of the genomic DNA sample. The ide3ntification of point mutations is highly dependent on the overall sequencing fold coverage, which is a general term used to describe the extent of sequencing that can cover a complete human genome. The general threshold that is typically cited is a fold coverage cutoff of 30 where the genome has been sequenced redundantly 30 times. For cancer genomes, typically 60 fold coverage is considered as a minimum to accurately identify mutations.

Next generation sequencers can analyze paired ends of a DNA fragment from a given cancer genome (Korbel, Urban et al. 2007; Campbell, Stephens et al. 2008). This paired-end approach results in mate pairs of sequences derived from the same DNA molecule. As a result, one can detect a variety of structural variation and rearrangements. This includes insertions, deletions, and translocations from a cancer genome.

An example of this whole genome approach and the entailed cost is the recent publication of a cancer genome from an acute myelogeneous leukemia (AML) sample with normal cytogenetics (e.g. diploid chromosome complement without aneuploidy) (Ley, Mardis et al. 2008). For this cancer genome, the leukemia sample required 32.7-fold sequencing coverage, equivalent to 98 billion bases, and 13.9-fold coverage (41.8 billion bases) for the normal skin sample. The majority of mutations (5) were bystanders and not confirmed in other AML samples. Two mutations were known AML mutations that had previously been discovered. The cost was described as being over $150,000 for the two samples, but over the last year we have some a dramatic drop in the cost where sequencing genomes is 10 fold less than it was a year ago.

Other methods to characterize cancer genomes through DNA sequencing involves using RNA from cancer samples and sequencing the transcriptome to obtain information about the expressed subset of a cancer genome to find mutations and rearrangements. However, even with the next generation sequencing technologies, the overwhelming size of the human genome and need for very high fold coverage represents a major challenge for up-scaling cancer genome sequencing projects. Assays to target, capture, enrich or partition disease-specific regions of the genome are also being employed for reducing the complexity of sequencing cancer genomes. These "targeted resequencing" method enable the interrogation of larger sample sizes and deeper resequencing without some of the current bottlenecks for complete genome sequence analysis. Population studies will be needed to resolve clinical and biological relevance of the mutations as well as in detecting somatic variants in heterogeneous samples and cancer cell sub-populations.

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Genome Regulation in Cancer

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Large-scale transcriptional signatures in cancer

Global gene expression patterns can provide comprehensive molecular portraits of biologic diversity and complex disease states. Cancer samples in particular show large scale changes--in comparison to normal tissue of the same histologic type and amongst cancer samples that carry the same traditional diagnoses. Unsupervised analysis where similarities in the overall gene expression pattern are allowed to drive the grouping of tumor specimens has revealed the molecular heterogeneity of cancer. Many human cancers that carry the same pathologic diagnosis—e.g. breast cancer—are actually comprised of several distinct molecular subtypes that are distinguished by the variation in expression of dozens or hundreds of genes. These subtypes are stable through time in individual patients, show different propensities for disease progression, and are now known to be driven by different oncogenic pathways.

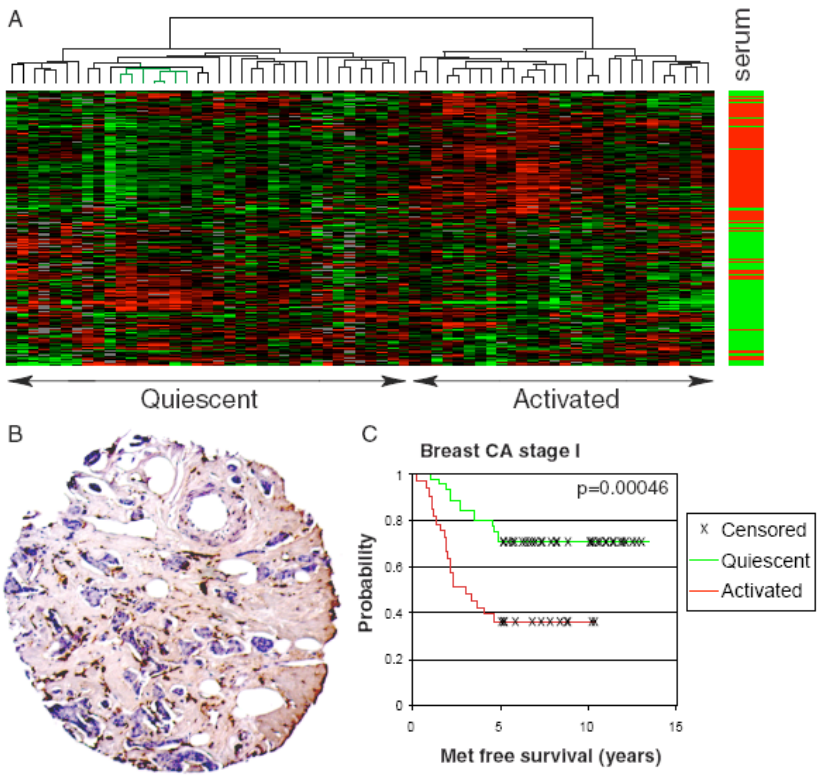

Figure 1. A gene signature predicts breast cancer progression. (A) Expression of genes induced by serum—as a model of wound healing—occurs in a biphasic pattern among human breast tumors. The activated group coordinately up regulates the serum-induced genes whereas the quiescent group expresses serum-repressed genes. The 7 benign samples are indicated by green branches. The status of serum regulation of each gene is shown on the right bar: Red indicates serum-induced; green indicates serum-repressed. (B) Validation of gene expression by in situ hybridization on tissue microarrays. The gene in question is expressed in stromal fibroblasts (stained brown) but not in tumor cells (counterstained purple by hematoxylin). (C) Prognostic value of fibroblast serum response. Kaplan-Meier survival curves of 78 breast cancer patients prospectively classified as having activated or quiescent stroma. Patients were matched for age, histologic diagnosis, and tumor stage and underwent the same treatment protocol. Tumors with activated phenotype had significantly increased risk of metastasis and mortality.

Top down and bottom up approaches to gene expression analysis

Understanding the physiologic meaning and genetic basis of the myriad gene expression changes have been a challenge. In the "top-down" approach, known clinical variables, such as tumor grade or eventual outcome, can guide the identification of specific gene signatures that act as biomarkers for cancer diagnosis and prognosis. In the "bottom-up" approach, investigators turn to nature for guidance about which gene expression patterns should be prioritized: By generating extensive compendia of gene signatures of different types of cells, cells engaged by different signaling pathways, and cells treated with different agents, investigators can then ask whether those signatures show up in the gene expression programs of authentic human tumors. The presence of specific gene signatures can thus suggest the presence of specific cell types and signaling events in those tumors. Various methods that formalize this concept include *gene module map*, and *gene set enrichment analysis* (GSEA).

Several new analytic strategies have now been developed to improve the interpretation of gene expression data. Because genes work together in groups to carry out specific functions, another strategy to refine gene set based analysis is to use knowledge about biological pathways-so called bibliomic approach. Here, the gene set is not defined by coordinate change in expression in an alternative experiment, but by knowledge of the function. Such pathways can even be organized into networks, where the genes are nodes and functional relationships define the edges between the nodes. Regulatory relationships can also be directly learned from cancer samples. For instance, by integration of DNA copy number variation (CNV) and gene expression, one can gain insight into how gene amplifications or deletions affect gene expression. Such relationships can be in cis, where the CNV affects genes within or nearby the CNV, or in trans, where the CNV affects a factor that then impacts gene expression on a global level.

Epigenomic profiling of cancer

Large-scale measurements of chromatin states can provide additional views of cancer. Instead of measurement of genes that are expressed, chromatin state measurements suggest mechanisms for gene control and reveal future potential (or lack thereof) of specific transcriptional programs. Epigenomic profiling include measurements of DNA cytosine methylation, nucleosome occupancy, histone modifications, and occupancies of chromatin modification enzymes, as well as long noncoding RNAs that recruit such modification enzymes. Although features of chromatin states, rather than mRNA levels, are measured, the strategies to associate chromatin states with behaviors of cancer or the use of gene set level analysis are very similar to mRNA analysis.

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Genome and Immunome Sequencing in Hematologic Malignancies

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Advances in DNA sequencing technology have presented new opportunities for studying the biology of B cell and T cell malignancies, as well as the complex populations of B cells and T cells in healthy human beings. Within the next few years, genome sequencing of lymphomas and lymphoid leukemias is likely to identify comprehensive lists of somatic mutations that may play important roles in oncogenesis. It remains to be seen what proportion of pathogenic mutations in any given patient's tumor or leukemia will belong to shared recurrent classes seen in many patients' malignancies, and what proportion will be very rare. Correlating the presence of relatively common gene mutations with the clinical behavior of the patient's disease, or its response to therapeutic agents, is certainly feasible, and is analogous to the way that karyotype analysis and detection of a few recurrent mutations and candidate molecular targets for drugs have been incorporated into the current World Health Organization classification of hematologic malignancies, last revised in 2008[1]. Assessing the impact of combinations of rare mutations will be a more difficult challenge for both basic and translational research.

There are additional hurdles to comprehensive understanding of a patient's tumor, namely the possibility of genetic and phenotypic heterogeneity within tumor cell populations. Most genetic analysis and essentially all clinical interventions directed at lymphomas or leukemias have treated these lesions as though they are homogenous populations of neoplastic cells [2-4]. While gene expression analyses and other studies of bulk tumor cell populations have been fruitful in revealing novel categories of disease and information about pathways of pathogenesis, the more challenge of assessing intra-tumor heterogeneity, and relating specific subpopulations to clinically meaningful outcomes, has only begun to be explored [5-7]. This is an important area for investigation, as recent literature from a variety of human cancer studies has indicated that minority populations within lesions can be of key biological and clinical significance [3,8].

B cell lymphomas with ongoing somatic hypermutation of the rearranged VDJ gene segments at the immunoglobulin heavy chain (IGH) locus effectively contain a 'molecular clock' marker that accumulates mutations over time, generating a tree-like record of cell divisions and clonal relationships (Figure 1). We are using high-throughput DNA sequencing methods to study rearranged IGH genes in such B cell malignancies to track sub-clone populations and to attempt to determine how they relate to the accumulation of other somatic mutations elsewhere in the genome, particularly in cases that progress from low-grade to aggressive high-grade disease[9].

An additional application of deep-sequencing of IGH V(D)J rearrangements that we are evaluating is the use of this approach to detect small amounts of residual disease after a patient's lymphoma or leukemia has been treated. The combinatorial segment and junctional base diversity of VDJ rearrangements makes them a highly specific marker of a patient's leukemia, and can be readily measured for disease tracking[9].

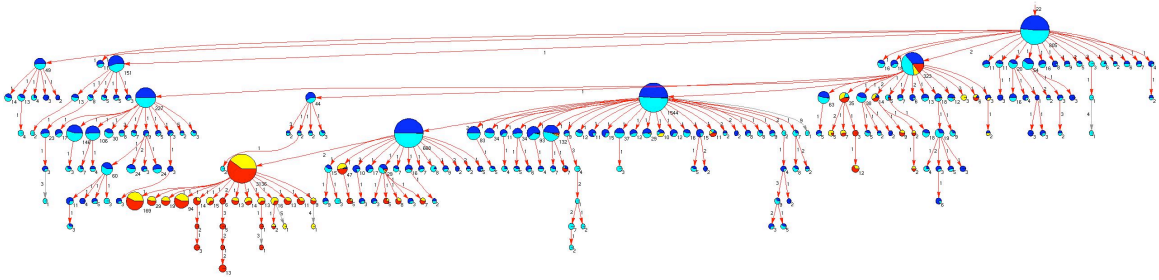

Figure 1. Tree diagram of lymphoma subclone relationships. IGH VDJ sequences from a biopsy of a patient's lymph node (blue/turquoise), and 6 months later, a skin sample (red/yellow), are shown. The

skin specimen shows a distinct sub-clonal relationship to the earlier lymph node sample. The two hues within each node of the tree represent sequences generated with two independent primer sets, in independent replicate amplifications of template from the specimens. Tree diagrams of the sequence populations were generated in collaboration of our lab and Andrew Fire's lab with Jonathan Laserson and Daphne Koller.

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Transplantation of Allogeneic Blood-Forming Cells: History, Achievements and Challenges

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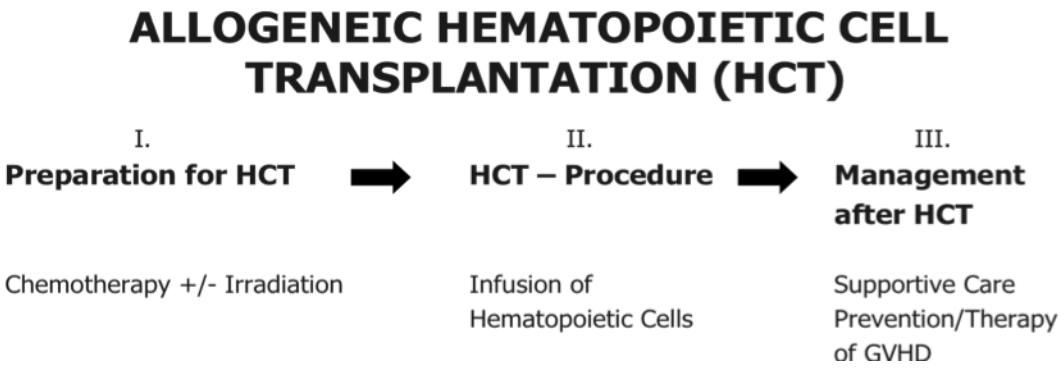
In 1886, two experimental pathologists reported that it is the bone marrow which produces all cellular elements of the blood. This observation stimulated an interest to cure patients with diseases of the bone marrow (leukemia, aplastic anemia and other life-threatening diseases) by transplantation of bone marrow cells. However, it took 100 years until in 1968, the first transplant procedures were successfully performed. The entire progress made in this field builds on pre-clinical studies in murine and canine models which still contribute most important information necessary for further improvements in allogeneic hematopoietic cell transplantation (HCT).

Table I - HISTORY OF TRANSPLANTATION OF BLOOD FORMING CELLS

1868	Neumann (Königsberg), Bizzozero (Pavia) **The bone marrow is the source of our blood cells**
1939	Osgood (Portland) **First documented clinical marrow transplant attempt**
1945	Nuclear bombs on Hiroshima and Nagasaki **Death due to bone marrow failure in people who survived the immediate impact**
1945-present	**Advances in marrow transplantation through animal studies in mice and dogs**
1949, 1951	Jacobson (Chicago), Lorenz (NCI) **Administration of marrow cells protects lethally irradiated mice from death due to marrow injury**
1954	Dausset (Paris) **Detection of the histocompatibility system in mice**
1957 - 1967	**Efforts by hematologists to save patients with leukemia by transplantation of marrow cells failed**
1968, 1969	Gatti (Minneapolis), Bach (Milwaukee), de Koning (Leiden) **First successful human transplants for children with inherited deficiency disorders of the immune system**
1975	Thomas (Seattle) **First large series of transplants for patients with leukemia**
1988	Weissman (Stanford) **Isolation of the blood forming stem cell (marrow/blood)**
1990	Thomas (Seattle) **Nobel Prize in Medicine and Physiology**
2011	**Number of patients transplanted over 4 decades worldwide approaching 1 million**

The main factors which made allogeneic HCT possible were the understanding of the major histocompatibility complex for the identification of suitably matched donors, the development of immunosuppressive drugs for the prevention or – if needed – therapy of graft-versus-host disease (GVHD) and the introduction of potent antibiotics, as well as other important clinical measures like transfusional support, fluid and electrolyte replacement, and parenteral nutrition. Currently, the cumulative number of patients transplanted world-wide is approaching one million of whom ~40% received grafts from related or unrelated donors while the other 60% of patients were autografted. Milestones of the history of HCT are listed in Table I. There are now patients alive and well who were transplanted 10, 20 and even over 30 years ago.

The technical approach to allogeneic HCT is illustrated in Figure 1. It consists of three consecutive steps:



During Step I, the patient is exposed to moderate or high doses of chemotherapy with or without irradiation. The purpose of this maneuver is to eradicate the underlying malignancy, to condition the patient so that durable engraftment becomes possible and to provide the incoming graft with space to grow. The introduction of reduced-intensity conditioning (RIC) has greatly expanded the list of candidates for allogeneic HCT.

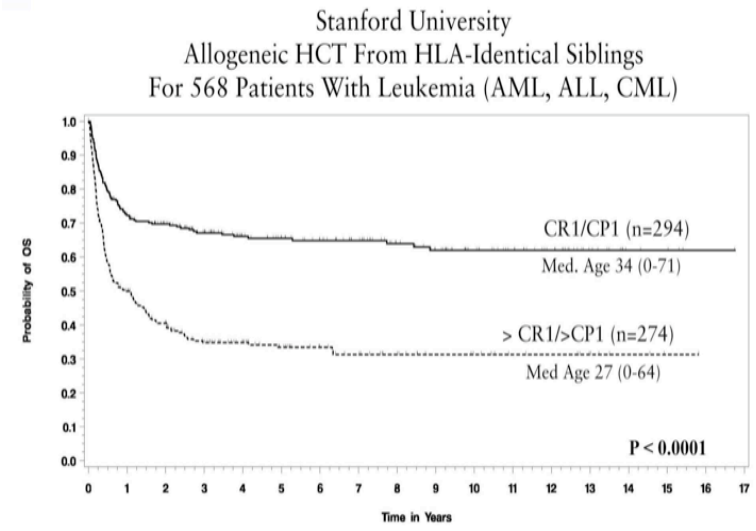
The sources of the donor graft for Step II, the HCT procedure, are either the bone marrow or circulating hematopoietic cells collected by apheresis or cord blood obtained following delivery. The graft, regardless of its origin, is infused intravenously and the stem cells find their way to receptors in niches in the marrow from where the marrow cavity is repopulated with donor cells from which donor-derived blood cells originate.

Step III consists of the skilled use of supportive care measures and the prevention or therapy of GVHD. Durable engraftment with complete or partial chimerism is generally (99%) attained. The graft and the transplanted immune system – although the cause of complicating GVHD – also contributes the major benefit, namely graft-versus-tumor effect (GVTE) which enhances the chances for long-term disease-free survival (DFS) and the cure of the underlying disease.

A list of conditions for which allogeneic HCT is clinically used is shown in Table II. The majority of patients transplanted (over 90%) are suffering from hematologic malignancies (leukemia, lymphoma, myeloma, as well as myelodysplastic or myeloproliferative disorders). Further indications are the different variants of bone marrow failure conditions, especially severe aplastic anemia, hereditary hemoglobin disorders, immunodeficiencies, cellular (leukocyte, macrophage or platelet) dysfunction syndromes, glycolipid storage disorders, and selected acquired autoimmune diseases.

Table II - Clinical Use of Allogeneic HCT
Acute Leukemias (AML, ALL)
Chronic Leukemias (CML, CLL)
Myelodysplastic Syndromes
Myeloproliferative Syndromes
Lymphomas (HL, NHL)
Multiple Myeloma/Amyloidosis
Selected Solid Tumors (Children, Adults)
Bone Marrow Failure Conditions (SAA, FA)
Hemoglobin Disorders (SS, Thal)
Severe Combined Immunodeficiencies
Leukocyte Dysfunction Syndromes
Hereditary Glycolipid Storage Disorders
Osteopetrosis
Erythrophagocytic Lymphohistiocytosis
Glanzmann Disease
Selected Autoimmune Diseases (SLE, MS)

Figure 2



R.S. Negrin and K.G. Blume, 2004

The success of allogeneic HCT greatly depends on the remission status (tumor burden) of the recipient, the number of prior exposures to chemotherapeutic regimens, and thus potential drug resistance of the malignant clone and the patients' performance status at the time when preparation for allogeneic HCT is begun. Figure 2 illustrates long-term outcomes data for 568 patients with various types of leukemia treated at Stanford University between 1986 and 2004. The grafts of all these patients stemmed from histocompatible sibling donors. Survival is significantly superior in patients transplanted in first complete remission (CR1) or first chronic phase (CP1) of their respective leukemias as compared to those patients whose conditions were beyond CR1 or CP1, respectively.

During the past four decades, major progress has been made in all areas of allogeneic HCT and the negative impact of serious complications and obstacles for success have been markedly reduced. The achievements which have made a wider use of this procedure possible are described in Table III. These achievements will be presented and discussed in detail during the presentation. It needs to be emphasized that further intense research in the pre-clinical and clinical arena is needed to overcome the still existing and often daunting challenges to make allogeneic HCT even more successful and more widely applicable.

Table III - Allogeneic Hematopoietic Cell Transplantation Achievements and Challenges in 2011	
1.	Recipient Age
2.	Availability of a Suitable Donor
3.	Regimen Related Toxicity
4.	Graft Failure/Rejection
5.	Opportunistic Infections
6.	Graft-versus-Host Disease
7.	Relapse
8.	Second/Secondary Malignancies
9.	Quality of Life Issues
10.	Cost/Charges

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Human Pluripotent Stem Cells

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Since the derivation of human embryonic stem cells (hESCs) in 1998, numerous lines have been developed, many with superior properties[1, 2]. Moreover, in recent years, alternative strategies to derive induced pluripotent stem cell (iPSC) lines with hESC-like properties have been introduced. Here we review human embryo development, hESC derivation from the inner cell mass (ICM) and alternative strategies to produce iPSCs. We discuss utility of hESCs and iPSCs for basic and clinical studies.

Human embryogenesis

Human development begins with the fusion of egg and sperm on Day 0 followed by genome-wide demethylation of maternal and paternal pronuclei on Day 1. During this phase, the nascent embryo is in a state of transcriptional silence, depending on maternal RNAs and post-transcriptional mechanisms, for nuclear reprogramming of the fused gametic nucleus to that of an embryo[3]. Cell divisions are termed "cleavage divisions" as the embryonic cells divide in the absence of overall embryo growth. Following a massive wave of RNA degradation through the first three days of development, the embryo begins to transcribe a distinctive cluster of 400 genes (including those reported for reprogramming of somatic cells) for the first time on Day 3[3]. By Day 5, formation of the blastocyst is observed; by Days 7 to 9, the cells of the inner cell mass (ICM) delaminate, migrate and form the distinctive epiblast and hypoblast[4]. Shortly thereafter, the first cell fate decisions are made with the segregation of cells destined to form the germ cell lineage from those that will form the somatic lineages and the remarkable differentiation events of gastrulation ensue[4]. Traditionally, development has been considered to be a progression towards the irreversible reduction of pluripotency with the exception of germ cell development. With reprogramming of somatic cells, this view has been altered.

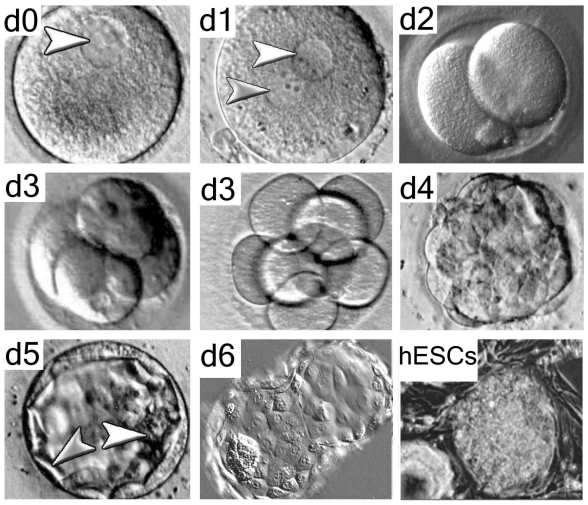

Figure (left). Images of human development from Day 0 (d0) to Day 6 (d6); human embryonic stem cells (hESCs) are derived from the blastocyst (d5 to d6).

Derivation of hESCs.

hESCs are derived from the ICM, are capable of contributing to all three germ layers, and the germ line, and are characterized by specific cell markers, epigenetic and genetic status, and growth requirements[2]. Recent studies indicate that mouse epiblast stem cells (mEpiSCs) are more similar to hESCs in terms of many characteristics, than mESCs derived from the ICM[5, 6]. These and other data suggest that hESCs derive from a point in human development selected by our culture conditions that represents a population of cells capable of extended self-renewal and differentiation.

Reprogramming

The developmental programs of somatic cells are characterized by stable patterns of gene expression and repression. Nonetheless, through nuclear reprogramming, the developmental programs of somatic cells may be erased and redirected. In recent years, much attention has been given to nuclear reprogramming of somatic cells in hopes of generating patient-specific embryonic stem cells (ESCs) that might provide valuable tools for basic science studies and potential novel therapeutics. Nuclear reprogramming was first demonstrated as an integral part of mammalian development; following fusion of the egg and sperm, the fused gametic nucleus must be reprogrammed, through a series of changes that include DNA demethylation and chromatin remodeling, to that of an embryonic cell if development is to be successful. In methods such as somatic cell nuclear transfer (SCNT), the nucleus of a somatic cell is transferred to an enucleated oocyte for reprogramming, through the use of the endogenous machinery[7, 8].

Derivation of IPSCs

Recently, cells with extensive similarities to mESCs and hESCs have been derived via reprogramming of fetal and adult fibroblasts[9-14]. By introducing a cocktail of 4 to 6 factors, somatic cells can be reprogrammed to an ESC or ESC-like fate distinguished by classic criteria of hESC identity. Although molecular details of acquisition of hESC fate have not been elucidated, mouse and human IPSCs possess the ability to differentiate to cells of all three germ layers; moreover, in the mouse contribution to the germ line has been observed[9-14]. Although initial studies included cMYC as one of the factors for reprogramming, subsequent efforts have excluded this factor to improve safety and have also moved from integrated lentiviral or retroviral vectors to excisable vectors and mRNA-based reprogramming[15,16].

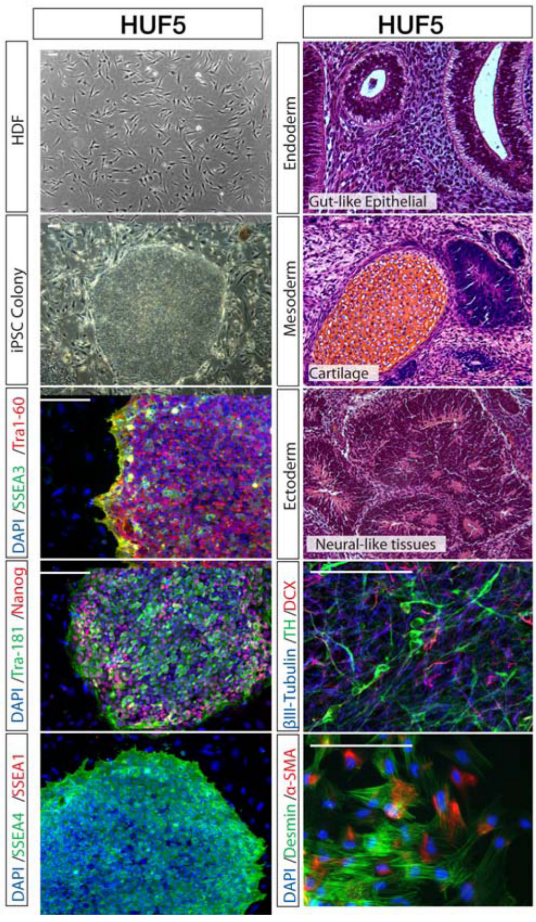

Figure (right). The derivation of iPSCs from somatic cells is shown. Cells are pluripotent as shown by pluripotency markers and can differentiate to all three germ layers.

Summary

Embryonic stem cells offer unique opportunities to probe the fundamentals of human development; moreover, the ability to differentiate to all the major cell lineages suggests potential applications in novel cell-based therapies. Several methods of deriving human embryonic stem cells, and alternative iPSCs with extensive similarity to hESCs are available and provide the foundation for further studies. The applications of hESCs and iPSCs are complementary with the later, perhaps, most ideal for studies of human disease and pathology.

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Normal and Cancer Stem Cells

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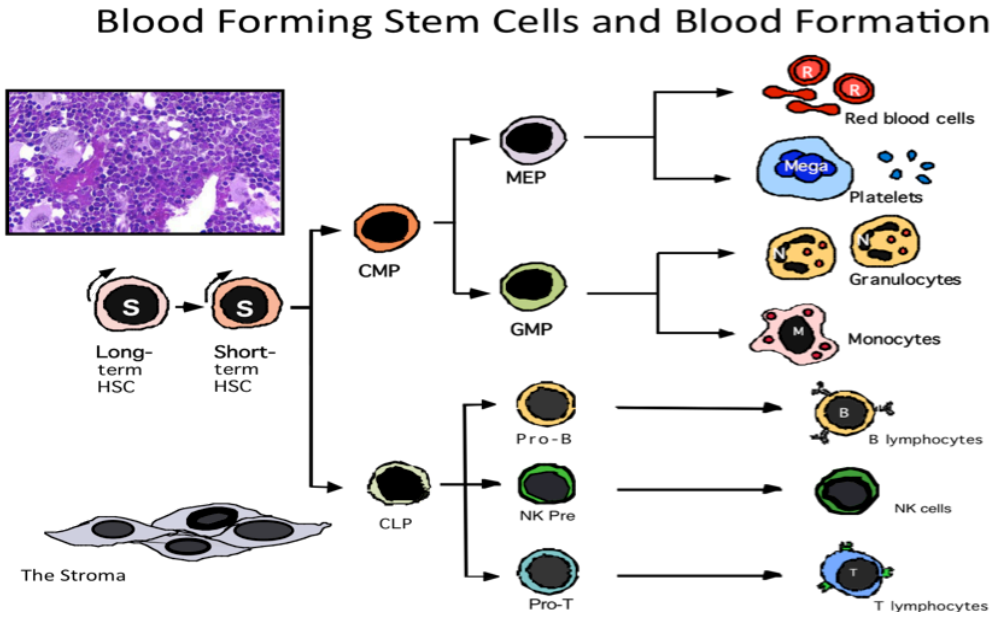
The classical definition of an adult stem cell is a multipotent cell that has both the capacity to self-renew and the potential to regenerate tissue over a lifetime. The property of self-renewal means that the stem cell can go through numerous cycles of cell division while maintaining an undifferentiated state. Without self-renewal, multipotent cells could give rise to a tissue, but could not maintain and replenish it over time. Tissue-specific or adult stem cells exist in most, if not all tissues, including skin, liver, and the gastro-intestinal tract, but the most well-characterized stem cell to date is from the blood system. Indeed, the "stem cell" concept was first proposed by Till and McCulloch following their pioneering studies of blood system regeneration *in vivo*[1-3]. Using sub-lethal irradiation, they induced clonal markers in donor marrow and then transplanted the cells in numbers that generated visible spleen colonies in 10 days. Each colony was identifiable by the chromosomal marker which was distinctive for that colony and that existed in all dividing cells of the colony, proving definitively that the spleen colony forming unit (CFU-S) arose from a single clonogenic cell. The colonies made the spectrum of blood types and the day 10 CFU-S cells could be transferred into lethally irradiated hosts giving rise to even more blood forming colonies. These experiments led Till and McCullough to propose the existence of hematopoietic stem cells (HSC) which could regenerate blood cells indefinitely, and opened the way to the subsequent identification and isolation of HSC. As shown in Figure 1, histologic sections taken through the bone marrow show a heterogeneous mixture of cells. HSC comprise ~1 in 2000 to 1 in 10,000 cells in the marrow and can now be isolated using fluorescently tagged monoclonal antibodies that bind to markers found on HSC[4,5]. A similar approach can be used to isolate the down-stream progenitors in the hematopoietic lineage.



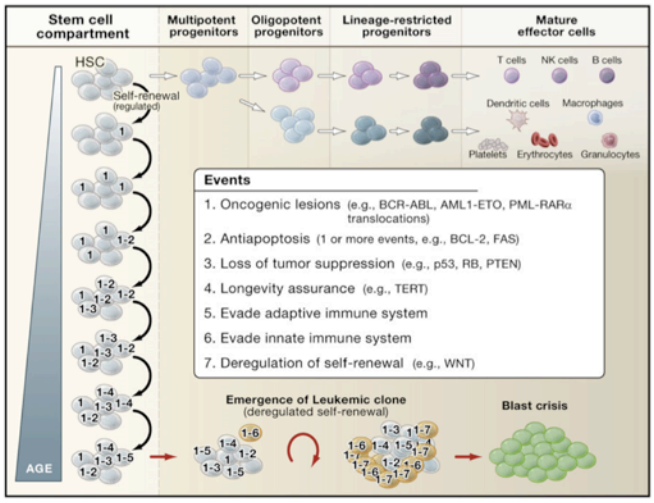
Similar methods have been used to isolate stem cells from other tissues. Critical to the identification of a stem cell is the method used to assay a candidate cell's self-renewal and pluri-potent capacity. In the hematopoeitic system, experimental techniques included the use of *in vitro* assays which support the growth of primitive blood cells, as well as *in vivo* studies such as the development of CFU-S. In other tissues, such as the alveolae of the lung, development of assays that can read out stem cell activity is much more challenging, thus making the rapid identification of stem and progenitors in this tissue difficult.

The existence of a cancer stem cell that is a cancer cell that possesses the property of self-renewal remains a subject of debate.[6] The cancer stem cell's (CSC) hypothesis suggests that contained within a cancer are CSC that have the capacity to self-renew and generate and re-generate tumors in the same way that normal stem cells do for tissues. This CSC model implies that not all cells contained within a tumor are malignant, and only the minority which comprise the CSCs perpetuate growth in an unregulated manner. The first evidence for CSC was described by Bonnet and Dick[7] who isolated a sub-population of cells from acute myelogenous leukemia that express the specific surface marker CD34 but lacked expression of CD38. Their assay for stem cell activity was the ability to perpetuate the $CD34^{+}/CD38^{-}$ sub-population in immune-deficient mice.

The link between normal stem cells and CSC is in the genetic pathways that regulate differentiation. Developmental pathways such as sonic Hedgehog and WNT[8]are used by stem cells to control their self-renewal and differentiation. CSC are thought to aberrantly turn on these pathways which in normal stem cells are tightly regulated. The accumulation of abnormal genetic and epigenetic events results in a CSC or leukemic stem cells (LSCs) that self-renew in a poorly regulated fashion and include nonmalignant cells in their progeny (Figure 2).

The implications of CSC for the treatment of cancer are many. Because CSC are the cells capable of perpetuating and spreading the cancer, they should be the targets for cancer therapy[6]. Thus, identifying the surface markers and pathways utilized by these cells which make them uniquely vulnerable to treatments are being pursued.

Model of Progression to Leukemia



In this model, HSCs serve as the reservoir for the accumulation of the genetic and epigenetic events that eventually lead to blast crisis and leukemia. Stem cell self-renewal and differentiation enable heritable mutations acquired in the stem cell compartment to be propagated to both self-renewing progeny and downstream progenitors over the lifetime of the organism. Stem cells with heritable lesions act as substrates for additional hits, which in turn can promote selection of preleukemic clones if lesions imparting a growth or survival advantage are acquired. In this model, seven events are listed as the full complement of events required for the progression of preleukemic clones to frank leukemia, but the actual number of events may vary depending upon the type of cancer and the nature of the lesions involved as it is possible that multiple oncogenic properties could be conferred upon a preleukemic clone through the acquisition of a single hit. Although the mutagenic events accrue in stem cells, the eventual emergence of leukemic clones occurs at the stage of progenitor cells downstream of HSCs that acquire the capacity for unlimited self-renewal. In chronic myeloid leukemia (CML), this can be the granulocyte/macrophage progenitor, whereas in acute myeloid leukemia, the leukemic clone can emerge at the level of a multipotent progenitor or at a stage further downstream depending upon the nature of the oncogenic lesions involved.

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Cellular Immunotherapy

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The concept of using cells for therapeutic purposes is an extremely attractive concept that has stimulated researchers for many years. Many important biological functions are controlled at the cellular level and therefore, the concept of isolating specific cellular populations separating or activating them *in vitro* and reinfusing them into patients with a broad variety of different disorders is an attractive concept. Despite significant effort and much progress the full value of cellular therapeutics has not yet been reached. The concept that immune-based cellular mechanisms can be utilized for the eradication of a complex malignant disease, for example acute leukemia, is well documented following allogeneic hematopoietic cell transplantation. Therefore, the question of whether or not cellular therapies can be effective has been answered, however, accomplishing this goal more reliably without complications such as other untoward immune reactions including graft-versus-host disease or other toxicities has been an important challenge. There are several potential clinical settings in which cellular immunotherapy could be particularly useful. Clearly, treatment of cancer is a major goal. Other potential treatments include that of treating infections and organ dysfunction such as heart disease or stroke are also clearly important. Many of the same concepts could be effective for the treatment of other immune dysfunctional reactions such as severe autoimmune disorders or solid organ transplantation rejection.

In the field of cellular immunotherapy for the treatment of malignancy, there are a variety of different cell populations that have the potential for disease recognition either directly or through the activation of other biological processes. These include natural killer (NK) cells, T cells, as well as antigen presenting dendritic cell populations. A critical question in these studies is how to direct the immune response towards antigens specifically presented by the tumor. Much effort has been directed into understanding what potential target structures could be presented by tumor cells and a number have been identified. These potential targets include lineage specific, cell type specific and even tumor specific antigens. Utilizing these concepts T cell-based immunotherapies directed against tumor cells has been attempted in a variety of different clinical situations. To enhance recognition of the tumor cells T cell receptors (TCRs) have been introduced into effector T cells directed against targeted antigens such as CD19 found on B cell acute leukemia or CD20 expressed by lymphoma cells. The technical approaches to accomplishing these types of cellular manipulations have been addressed such that this concept has been utilized in the clinic. Another approach has been to expand T cells against antigens such as minor histocompatibility antigens which are selectively expressed on tumor cells versus normal counterparts. This approach although complex has merit and has also been extended to the clinic.

An alternative approach is to utilize cells with so-called natural cytotoxicity such that these cells are capable of recognizing malignant cells without prior exposure such as either NK cells or cells with NK-like function. We have utilized one such cell population termed cytokine induced killer (CIK) cells which are activated *in vitro* and then expanded prior to reinfusing the activated cells back into patients. This approach has been explored elsewhere for the treatment of hepatocellular carcinoma following resection, where the use of this type of cellular immunotherapy has resulted in reduced risk of disease relapse and improved disease-free intervals. We have also utilized the expanded and activated cells to bring other potentially cytotoxic agents to the tumor site, for example, using CIK cells loaded with oncolytic viruses that can then be directed to the tumor whereby they release the oncolytic viruses within that tumor bed resulting in additional cellular cytotoxicity.

Another therapeutic concept has been to attempt to vaccinate patients against their particular tumor. One such approach involves pulsing antigen presenting cells (APCs) with different tumor antigens that are expressed on tumor cells. This is the basis of the Provenge© approach for the treatment of prostate cancer which has received FDA approval.

The concept of tumor vaccination has also been exploited by recognizing that certain tumor cells are capable of antigen presentation that can be improved with a variety of activators which result in the enhancement of these cells ability to stimulate an immune response towards a variety of different

antigens that may be expressed on those tumor cells. Activation of the APCs with toll-like receptor agonists such as CpG has been utilized in cancer patients to develop a vaccine. Here the tumor cells are activated with CpG to enhance their antigen presentation capabilities and then reinfused into patients to stimulate an immune response. One concept that has emerged from these studies is that T cell proliferation is likely required for more effective immunity and the utilization of lymphodepletion prior to the infusion of the activated T cells allows for creation of space for these T cells to proliferate in a process called homeostatic proliferation. These approaches are under active investigation by a variety of different groups with exciting preliminary results.

Along with the recognition that tumors can be specifically targeted has come the realization that the tumor microenvironment is a complex milieu of different cells and factors that can actively suppress an effective immune response. Therefore, efforts to target this tumor microenvironment has been suggested as an important part of enhancing an immunological reaction. In order for cellular immunotherapy to be effective, the effector cells must be able to traffic to the tumor sites following intravenous injection, and execute cytolytic function. One of the challenges in human clinical trials is that it is very difficult to visualize cellular migration at the different steps of this process and often surrogate assays are required. In murine models a variety of different imaging technologies have been developed to directly assess the ability of cells to infiltrate a particular tumor microenvironment and exert a biological effect. Typically model systems are developed in animals first which when promising can be extended to the clinic. Translation to the clinic involves a number of challenges that must be overcome including the biological challenges of whether the principles apply and how different the pre-clinical models are from the clinical situation. Further there are additional technical challenges such as the cell separation techniques, the availability of reagents for clinical translation and the requirements for specialized laboratories. Despite these challenges this area of research has enormous promise for future clinical application to provide an alternative approach to cancer therapeutics that could potentially be more specific, efficacious and less toxic.

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Social Adaptation after Cancer

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More than half of all newly diagnosed cancer patients will become long-term survivors (five or more years) and it is estimated that there are over 10 million long-term cancer survivors in the US. Most long term cancer survivors score well on quality of life instruments and report levels of psychological well being similar to the general population. However, despite these good indicators of well being, survivors continuously face physical, psychological and social challenges in their daily lives. As their ranks grow, a body of research focused on 'survivorship' has developed which investigates the continuing challenges facing long term survivors. ***Cancer survivorship can be defined as the experience of "living, with, through or beyond cancer."*** It is essential to recognize 'survivorship' as a distinct phase in the cancer trajectory and to examine it more closely to understand the different trajectories for patients and to identify which factors are associated with more positive outcomes.

Psychosocial Consequences of Cancer

Patients frequently discuss physical problems with healthcare professionals but only raise psychosocial concerns when specifically prompted. Most investigators have concluded that emotional quality of life and psychosocial adjustment are good for the majority of relapse-free, long-term cancer survivors. For most survivors, the emotional and psychosocial aspects improve during the first two years after treatment, stabilize, and then by five years, reach levels that are comparable with or better than those of individuals who have not had cancer. However, among the survivors who report overall emotional adjustment, there are areas of continuing disruption and difficulties. Understandably, ***fear of recurrence*** is the most commonly reported ongoing emotional problem as well as uncertainty concerning associated health problems that may emerge later on. Specific events that trigger feelings of vulnerability and worry continuously occur many years after treatment and include: news of other people's cancer, follow-up appointments and follow-up diagnostic tests. Cancer diagnosis and treatment causes tremendous disruption in patient's lives and the extent of these difficulties depends in part on the degree to which life roles, goals and expectations are disturbed. Thus, *age at the time of diagnosis* is a strong predictor of long-term fallout. In general, younger age in survivors of adult cancers is associated with greater ***emotional disturbances*** such as such as depression and anxiety whereas older cancer survivors are more likely to report difficulties associated with ***functional limitations.*** Additionally, younger survivors must contend with infertility and family planning. Often those affected in the mid-career stage also face major disruption in their life trajectories. Other factors associated with emotional and psychological fallout include prior psychological disorders, relationship dissolution, unemployment or a significant change in the nature of employment, and fewer economic resources in general. Additional financial/practical concerns facing cancer survivors include ***returning to employment, gaining credit or obtaining health and life insurance.***

In the survivorship literature, ***access to positive social support has consistently been associated with better emotional and psychosocial adjustment***. Virtually every study that examined the impact of social support or perceived access to support reported a significant advantage in emotional recovery. Low levels of social support predicted feelings of helpless-hopelessness and anxious pre-occupation whereas high levels of social support engendered a "fighting spirit" coping strategy. Thus, various levels of social support influence whether long-term survivors employ maladaptive coping versus more adaptive coping strategies. A secondary matter that merits more research is the nature of the stress placed on

primary caregivers who provide the social support for cancer patients during and after recovery from treatment.

Post Traumatic Growth after Cancer

Although cancer survivors commonly report symptoms of traumatic stress, they also report personal growth related to their diagnosis and treatment. Positive change in the aftermath of a crisis is not a new phenomenon. ***Post-traumatic growth*** has been defined as the "positive psychological change experienced as a result of the struggle with highly challenging life circumstances". Cancer survivors commonly report growth in 3 specific life domains: *enhanced social resources, enhanced personal resources and improved coping skills*. See Table 1.

Table 1: Commonly Reported Benefits of Cancer

Social Resources	Deeper love for family
	Improved relationships with family and friends
	Increased time and effort invested into relationships
Personal Resources	Better outlook on life
	Greater satisfaction with religion
	Increased compassion, sympathy and concern for others
Coping Skills	Greater acceptance of circumstances
	Learning to take things as they come
	More effective coping with stress

Predictors of posttraumatic growth include *younger age at diagnosis, greater processing of one's emotions, perceived social support and the use of 'positive reframing'* or finding something positive in their diagnosis. ***Younger age at diagnosis appears to be the most robust predictor of growth.***

Thriving after Cancer

Resilience and personal growth after suffering a crisis are innate human characteristics. Four possible consequences for an individual's level of functioning occur following a major adverse event and are depicted in Figure 1:

1) ***Continued Decline*** – recovery is impossible and death occurs
2) ***Survival with Impairment*** – previous level of functioning is never regained or survival with permanent impairment
3) ***Resilience*** – recovery to baseline functioning
4) ***Thriving*** – recovery beyond the level of functioning; positive changes can include increases in strength and /or psychological growth

Coping responses such as *problem-focused coping* and *positive reframing* are important determinants of 'thriving'. Environmental factors that contribute to 'thriving' include social support from family/friends as well as from health professionals. In contrast, 'maladaptive behavior' such as 'avoidance coping' and 'disengaging from the struggle' fosters poorer outcomes. Perhaps the most famous example of 'thriving' after cancer is Lance Armstrong, the seven-time winner of the *Tour de France*. In his book, "It's Not About the Bike", he details his personal story of his battle against testicular cancer, which included surgeries and chemotherapy, his journey of self-discovery and his period of survivorship, which also included his subsequent, multiple victories in the *Tour*.

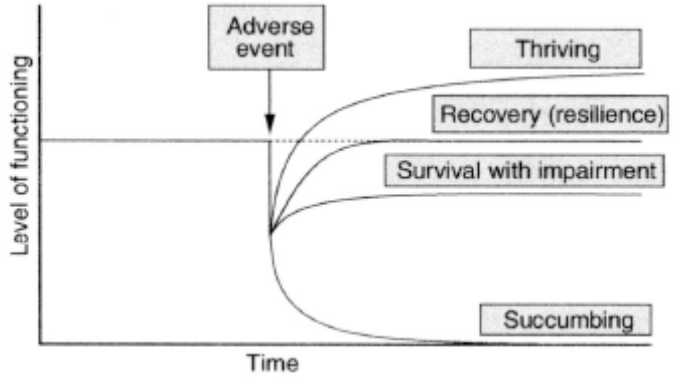

Figure 1. Model of Resilience and Thriving

Summary

Clearly having been treated for cancer continues to affect the lives of survivors, even years after treatment has ended. Access to social support is unequivocally the most valuable emotional resource for a patient living with and living after cancer. Other effective means of coping used by survivors include positive thinking, helping others, spirituality and confronting reality (facing the situation). Cognitive-behavioral stress management also facilitates posttraumatic growth. Providing optimal care for cancer survivors requires an understanding of the psychological and social, as well as the physical late effects of the disease and treatment. Understanding the likely long-term effects not only on health but also on the life trajectories of those who survive will help produce the types of support structures that facilitate positive coping.

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Quality of Life Studies in Cancer Patients: Focus on Sleep and Fatigue

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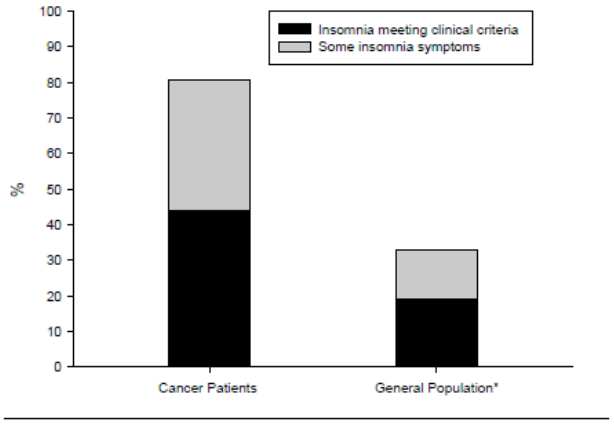
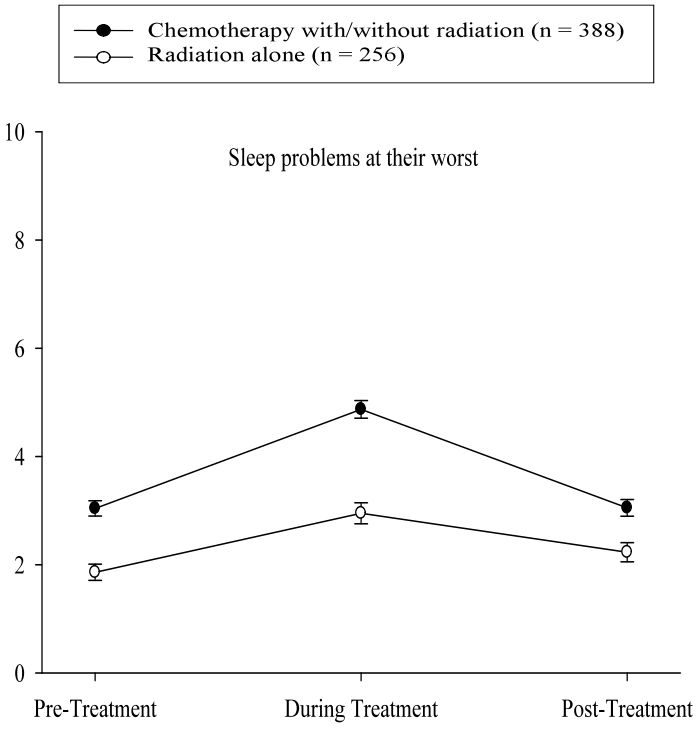
There are more than 11.7 million cancer survivors in the United States and the number is increasing rapidly.[1] Cancer survivors are living longer, but not necessarily better; many cancer patients experience disturbed sleep, sadness, and tiredness and overall reduced quality of life.[2-4] Insomnia, a particular type of sleep disturbance, is prevalent but under-recognized and undertreated in cancer patients and is reported by at least 30 to 50 percent of newly diagnosed cancer patients.[5] Recent data point to even higher prevalence of sleep disturbance (nearly 80%) in cancer patients undergoing chemotherapy (see Figure 1).[6]

Figure 1. Insomnia Symptoms in Patients Following First Cycle of Chemotherapy (N=823) vs. General Population



*From multiple epidemiological studies summarized in: Ohayon, Sleep Medicine Reviews, Vol. 6, No. 2, pp 97–11, 2002

Research suggests that sleep disturbance may be precipitated by cancer diagnosis and/or tumor biology, worsens during treatment, and continues even after the treatment is completed (see Figure 2). [7] Relatively little data are available on management of sleep disturbance in cancer patients receiving treatment.



In the general population, sleep disturbance (insomnia in particular) is associated with significant impairments in quality of life [8], almost a two-fold increase in the number of accidents[9], increased number of days spent in bed, and decreased activity. [10]. Moreover, some data show that sleep disruption and sleep loss are associated with dysregulated immune function.[11-13]

Emerging evidence also suggests that sleep disturbance negatively impacts health, quality of life, and psychiatric functioning in *cancer* patients.[6,14] Sleep disturbance rarely occurs in isolation and is frequently co-morbid with other psychiatric and psychological symptoms, with depression and fatigue being most frequent. Multiple studies report a significant positive correlation between sleep disturbance and depression (ranging from .30 to .57 depending on cancer diagnosis and treatment), suggesting that sleep problems and depression share approximately 25% of common variance.[15,16] Depression symptoms

are also very common among cancer patients. Nearly 25% of cancer patients meet criteria for major depression, with an additional 30% reporting symptoms.[17] Several epidemiological and experimental studies suggest that severe and chronic depression in cancer patients might influence disease progression.[17,18]

While sleep disturbance and depression are clearly linked, evidence is unclear whether depression precedes or follows sleep disturbance. Research evidence supports both hypotheses.[19,20] The close relationship between sleep disturbance (insomnia in particular) and depression suggests that perhaps the two conditions share a common etiology. Roth emphasizes that both depression and insomnia are associated with dysregulation of the hypothalamic- pituitary adrenal axis (HPA) and increased production of cortisol.[21] Serotonin (5-hydroxytryptamine, or 5-HT), a monoamine neurotransmitter that plays an important role in modulating mood, sleep wake regulation, metabolism, and body temperature, is low in both depressed patients and insomniacs. In a comprehensive review of the impact of antidepressants on sleep, Mayers and Baldwin [22] reported that in laboratory studies, patients and clinicians frequently report a positive impact of selective serotonin reuptake inhibitors (SSRIs) on sleep despite a disruption of REM sleep, especially in patients without the C variant in the 3111 T/C polymorphism in CLOCK gene.[23] This suggests that treating sleep disturbance might improve depression and treating depression might improve sleep.

Sleep disturbance frequently co-occurs with fatigue. Cancer related fatigue (CRF) is a very common and debilitating symptom experienced by the majority of cancer patients and survivors. Prevalence of CRF ranges from 60 to 100%, with over 41% of patients reporting severe levels of fatigue. [24-26] CRF is a multidimensional symptom that is different from the fatigue experienced by the general population. CRF is different from tiredness and it is not alleviated by either sleep or rest. CRF significantly interferes with patients' quality of life (QoL).[24-26] The etiology of CRF is not entirely understood, but it might be caused by tumor biology, cancer treatments or a combination of both. [27] CRF might contribute to developing sleep disturbance by causing patients to spend more time in bed not sleeping and it also might be worsened by poor sleep.

Chang et al. (2000) found that on average cancer patients experience 8 symptoms concurrently, and those who are fatigued report 13 concurrent symptoms. [28] Given the high rates of cancer-related symptoms and their impact on QoL, more investigation into their precipitating and perpetuating factors is needed to design the most effective interventions. While these symptoms are distressing, within the general population they are highly treatable with pharmacological, behavioral and psychological interventions (i.e., cognitive-behavioral therapy, exercise). Preliminary studies conducted in cancer patients and survivors suggest that pharmacological and behavioral interventions are effective in enhancing QoL of cancer patients and survivors who are experiencing these symptoms.[26,29,30] Additional research is needed to uncover physiological, psychological and behavioral factors that contribute to the development of these side effects/ symptoms in cancer patients and survivors so that their high prevalence could be ameliorated.

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Palliative Care Research

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Hospice and Palliative Medicine (HPM) is a sub-specialty in Medicine that has seen tremendous growth in the past decade. HPM was ACGME accredited in 2009.There are strong clinical palliative care programs in most hospitals in the country including Stanford Hospital. Palliative care education has seen strong advances with increasing penetration of palliative care content in our medical school, internal medicine residency curricula as well as the Hematology and Oncology fellowship program. However, palliative care research still is underdeveloped at Stanford. The reasons for this are two fold.

Researchers lack of understanding of:

1. The scope of palliative care how it can enhance hematology-oncology research
2. The science of the interplay and influence of the bio-psycho-socio-spiritual and cultural paradigm and how this can enhance oncology research
3. The special palliative care funding sources that are available.

Research on a palliative care patient population is complex:

1. Research in pain and other physical and psychological symptoms relies on patient self-report as the gold standard of assessment. Overwhelming disease burden in patients who are referred too late to palliative care precludes to detailed assessment and long-term follow up.
2. Researchers face complexities in studying patients with multiple symptoms that may interact with each other and because it may be difficult to distinguish symptoms caused by the patient's illness from those resulting from treatments.
3. RCTs are challenging due to ethical reasons, high rate of attrition

The solution:

The inherent challenges and issues in palliative care research will require active and ongoing interdisciplinary collaboration of clinician scientists, health services researchers, and statisticians, each of whom brings specific and targeted expertise to the complex and multifaceted problems faced by patients with serious and life limiting illness. Here are some examples of such palliative care research efforts at our institution:

1. Cancer Stem-cell with Research and Patient Reported Outcomes (CanSR-PRO)
2. Concurrent palliative care in GI Oncology
3. Differentiating between preparatory grief and depression in hematology and palliative care clinic patient
4. Understanding how cultural background affects perceptions of a dignified death in community dwelling persons.
5. Multi-site RCT on effects of citalopram on agitation in dementia patients
6. Effects of PTSD on mortality in Veterans
7. Phase 3 RCT on Methyl naltrexone in chronic opioid induced constipation

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Emerging Plasma Accelerator Technologies for Future Particle Therapies

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The linear accelerator has been in clinical use for over 50 years, and x-ray based radiation therapy since 1896. Over 40 million patients have been treated with this technology. Proton (or in general, particle) therapy has been used clinically in the US for 35 years. There are key advantages in the use of protons over photons in treating cancer. The rationale is that dose distributions are superior to x-rays for all anatomic sites [1]. Decreasing normal tissue dose facilitates increased treatment tolerance, reduced complications, dose intensification and increased tumor control. According to the Particle Therapy Co-Operative Group (PTCOG http://ptcog.web.psi.ch/), 55 000 people have been treated with proton therapy. This is a small fraction of the patients treated with x-ray based therapy. While many new proton centers are being built in the US and worldwide, limiting the widespread application of proton radiotherapy to the general community is the capital, building and operating costs associated with typical proton radiotherapy. These can greatly exceed $100 million per site, a substantial cost differential with x-ray facilities.

The existing commercial solutions for proton therapy rely on large, rather expensive cyclotron or synchrotron accelerators. Both operate by accelerating ions with an electric field, and confining them into a circular orbit with large magnetic fields. Repulsive forces between the ions and/or the need for powerful magnets to send the high energy ions on circular paths ultimately determine the minimum achievable device size (and hence cost). A compelling approach to the widespread expansion in the use of protons for cancer therapy is to obtain the physical advantages of proton therapy in a smaller, cheaper, preferably gantry-mounted system.

Four proposed technologies have emerged over recent years which aim to displace the use of these large, relatively expensive cyclotrons and synchrotrons. These include dielectric wall accelerators (DWA) [2], compact cyclotrons [3], laser ion acceleration [4], and compact plasma acceleration.

The DWA is an accelerator concept built around the development of high gradient insulators (HGI), which are conventional dielectric materials sandwiched between electrically floating metallic sheets, to form periodic structures (sub millimeters in wavelength) that appear to be able to withstand very high electrostatic gradients (MV/cm) without surface breakdown. Unlike conventional surface breakdown in dielectrics, where subsequent collisions of field-emitted electrons give rise to avalanche secondary emission and plasma formation, the nature of this structure in the HGI leads to the sweeping of the secondary electrons away from the dielectric, and does not lead to avalanche ionization. The DWA is inherently a pulsed accelerator device, and theoretical simulations suggest that a 1 MeV proton bunch can be accelerated to 10 MeV over 10 cm [5].

The compact cyclotron approach promoted by Still Rivers Systems [6] replaces conventional magnets used in large cyclotrons with superconducting high field magnets in order to reduce the accelerator size. At a targeted weight of about 20 tons, it is 90% less than the magnet weight of a conventional cyclotron, but still quite sizeable (requiring a gantry that is 2-3 stories tall). The magnet technology was developed by MIT, and a press release [7] in 2006 indicated that the first hospital system was expected to be running in late 2007 (of course, this target was not met). While important, the compact cyclotron approach is not seen to be revolutionary insofar as introducing new accelerator physics, and so such as approach to introduce new therapy solutions will not be discussed in this presentation.

Laser ion acceleration involves the use of ultra-high intensity ($>10^{18}$ W/cm^2) lasers to irradiate a thin foil generating a nonequilibrium plasma with high electron temperature. The tendency for electrons to migrate out of the formed plasma produces a strong space charge electric field ($>10^3$ MeV/mm), which

accelerates ions to energies as high as 60 MeV [8]. Unlike cyclotrons and DWA, laser ion acceleration shares the same ion energy features that plasma acceleration has (discussed below), with relatively broad ion energy distributions requiring energy selection and filtering.

The fourth approach uses the benefits of adding electrons to the ions (thereby creating a plasma) thereby shielding the repulsive forces between ions, which allows for much higher ion density in a compact and linear device that does not require confinement magnets. However, plasma-based accelerators relying on the Lorentz force for acceleration have traditionally delivered output beam energies that are too low to be useful for applications performed by cyclotron ion accelerators. Recent breakthroughs, however, in the stabilization of a special high-energy mode of operation have lead to an accelerator concept with the potential to make affordable, compact proton therapy possible. The breakthrough involves the harnessing and control of the transfer of inductive energy to electrostatic energy during the plasma detachment from the source. During this process, very high (MeV/mm) fields can be generated, which can be used to accelerate a significant portion of the particles to high energy.

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Small and Large Molecule Therapeutics and Drug Delivery

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Chemotherapy, diagnostics, imaging and research on living systems often depend on the translocation of molecules, whether small or large, across biological barriers. Because barriers such as cellular membranes are relatively non-polar while the extra and intracellular milieu is polar, the selection or design of drugs and probes that target intracellular receptors has historically been limited to molecules with very restricted physical properties. Agents that are either too non-polar or too polar are generally found to encounter problems. Non-polar molecules like taxol, for example, are difficult to formulate in water and often due to their membrane solubility are substrates for Pgp export, the latter a major contributor to multi-drug resistant disease. At the other end of the polarity scale, polar molecules like siRNA and many peptides and proteins while easy to formulate in water do not readily pass through the non-polar membrane of a cell. These factors have thus limited the universe of potential therapeutics and probes to a very small number, making the development of new strategies to enable or enhance the passage of drugs and probes through biological barriers one of the grand challenges as well as grand opportunities in science.

Nature's library, the product of 3.8 billion years of chemical evolution, provides examples of molecules that, despite their physical properties, readily cross biological barriers. One important example is the nuclear transcription activator protein (Tat) encoded by HIV type 1 (HIV-1), a 101-aa protein that is required for viral replication. Of particular interest for drug delivery is that HIV-1 Tat efficiently crosses the plasma membranes of cells, localizes to the nucleus, and is functional, stimulating HIV-long terminal repeat-driven RNA synthesis. The sequence responsible for the cellular uptake of HIV-1 Tat consists of the highly basic region, amino acid residues 49–57 (RKKRRQRRR). What is profoundly striking about this observation and attracted our interest is that this so called Tat-9-mer is a poly-cation and thus highly polar and soluble in water, yet it readily enters cells apparently not blocked by the otherwise difficult to penetrate non-polar lipid membrane.

Starting in the late 90s, we (the laboratories of Engleman, Fathman, Rothbard and Wender) initiated a program directed at understanding the molecular basis for Tat uptake into cells. Through a process that involved reverse engineering of the poly-cation sequence, we were able to arrive at an understanding of the molecular requirements for cell entry. This in turn led to the realization that many molecules could be designed in such a way that, even though they are polar poly-cationic systems, they would enter cells rapidly. Significantly a peptide linkage was shown not to be required for uptake, leading to the creation on many families of structural classes that exhibit passage across biological barriers. The original terminology of "cell penetrating peptides" associated initially with the Tat-9-mer was thus replaced by the more general classification of "molecular transporters" and those more specifically inspired by Tat as guanidinium-rich (GR) molecular transporters. Molecular transporters (MoTrs) have since been defined as agents which upon covalent or non-covalent association with a cargo will carry that cargo into cells. We have now reported on arginine-rich MoTrs, arginine-spaced MoTrs, peptoid MoTrs, GR dendrimer MoTrs, GR oligocarbamate MoTrs, and GR oligocarbonate MoTrs. GR MoTrs enable or enhance cellular uptake of a variety of cargos including small molecules, imaging agents, peptides, proteins, metals, plasmids, siRNA, quantum dots, and liposomes. We have also shown that the MoTr-cargo conjugates can release if needed free cargo once inside cells. We have also shown that the uptake is general for a variety of cell types. Moreover MoTr uptake works for passage across other barriers including skin, ocular, buccal, lung, GI, and blood brain barriers. A small molecule conjugate has advanced to phase II trials for dermatological applications and another for treating ischemic indications. We and Tsien have independently also shown that MoTr conjugates can be used to target drug delivery into cells and tissue in a way that is complementary to monoclonal antibodies. We have also shown that known anticancer drugs that by themselves fail due to Pgp-based resistance can upon conjugation to

MoTrs be used to treat resistant disease. In collaboration with Nelson Teng (Director, Gynecologic Oncology), we have more recently shown that this is effective ex vivo in treating malignant ascites from ovarian cancer patients.

This lecture will focus in part on MoTrs including GR and non-GR (nanotubes) systems, their mechanisms for crossing biological barriers, cargo types, release strategies, imaging applications, diagnostic applications, cell and tissue targeting strategies, and the use of MoTrs for treating Pgp-resistant cancer.

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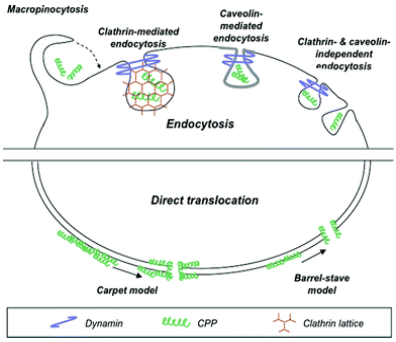
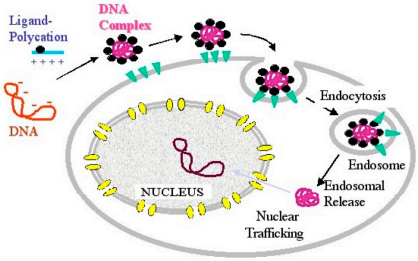
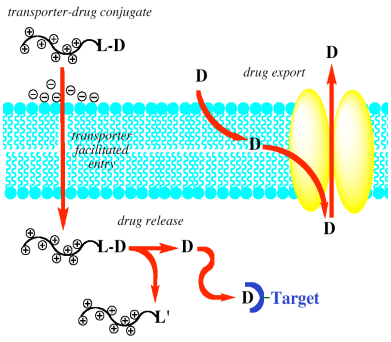
a) Mechanisms of cellular entry



b) Transfection with GR-MoTrs



c) Overcoming resistance with MoTr-Drugs



Systems Biology of Cancer

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Systems biology is a scientific approach that aims to unravel complex intra- and inter-cellular behavior, in normal and pathological processes, by integrating various experimental datasets with computational models. The result of this approach are often computationally-derived regulatory networks that are used to identify critical regulators, gene regulatory networks, signaling pathways and cross-talk among pathways that drive molecular processes. Because high throughput (HTP) assays for gene and protein expression generate a comprehensive view of molecular behavior, they provide a wealth of information well-suited for a systems biology approach. One of the common challenges when working with HTP data is deriving biological and statistically significant meaning from tens of thousands of simultaneous measurements. Systems biology addresses this challenge by integrating HTP datasets with computational analyses that often produce network based reconstructions, which yield insights that would not be evident from traditional experimental methods which focus on single gene or protein function. An overview of the principles and paradigms for systems biology is given in Reference 1. Here, we will discuss four widely used computational methods for analyzing high-throughput data to infer critical regulators and their regulatory behavior.

Statistical Analysis of Microarrays

The most common approach for analyzing HTP data simply seeks to identify genes and/or proteins that are differentially expressed under multiple experimental conditions. In Reference 2, the authors introduce a now widely used method named Statistical Analysis of Microarrays (SAM) for analyzing gene expression microarrays. This approach evaluates the significance of the difference in individual genes one at a time, while taking into account the "multiple hypothesis testing" problem that arise when analyzing thousands of genes simultaneously. However, SAM, while well-suited for many problems, will not detect situations where the difference in a set of related genes is small but highly coordinated across the entire set.

For more information on SAM, go to www-stat.stanford.edu/~tibs/SAM/

Gene Set Enrichment Analysis

Approaches have been developed to detect changes in biological processes that are distributed across a network of genes, that may be too subtle to detect at the level of individual genes. In Reference 3, the authors introduce a method named Gene Set Enrichment Analysis (GSEA) to determine if a predefined set of genes is differentially expressed. The predefined gene sets may be from come from a variety of sources. The gene sets consists of genes in a known signaling or metabolic pathway, a gene ontology category, or a set of co-expressed genes observed in an experiment.

For more information on GSEA, go to www.broad.mit.edu/gsea

Gene Regulatory Networks

A substantial leap from identifying differentially expressed genes or gene sets involves inferring a network of interactions among individual genes or gene sets. An overview of statistical methods for inferring regulatory networks is provided in Reference 4. References 5 and 7 present two widely published methods for interfering gene regulatory networks. The most significant value of these and similar methods is that they can be used to generate novel hypotheses about regulation that can then be experimentally validated. Reference 5 describes an approach, first introduced as Genomica, that uses Bayesian reasoning to identify modules of coregulated genes, genes that regulate the modules, and conditions under which the regulation occurs. This approach generates testable hypotheses in the form "regulator X regulates module Y under conditions W." A recent extension of this work,

described in Reference 6, integrated gene expression and chromosomal copy number data for the purpose of inferring molecular drivers of melanoma progression. Reference 7 describes an alternative approach for "reverse engineering" transcriptional networks, called ARACNE (Algorithm for the Reconstruction of Accurate Cellular Networks). In contrast to Genomica, ARACNE identifies statistically significant coregulation between gene pairs by mutual information, which is an information-theoretic measure of relatedness. A recent application of this approach, described in Reference 8, identified master regulators of mesenchymal transformation of brain tumors.

For more information on Genomica, go to genomica.weizmann.ac.il

For more information on ACRANE, go to: amdec-bioinfo.cu-genome.org/html/ARACNE.htm

Protein Signaling Networks

Bayesian methods for inferring gene regulatory networks have also been applied to inferring protein signaling networks, as described in Reference 9. Here the data consisted of multiple phosphorylated protein and phospholipid components in thousands of individual primary human immune system cells, activated under different stimuli. Bayesian network computational methods were shown to automatically identify most of reported signaling relationships and predict novel inter-pathway network causalities, which were verified experimentally.

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Biocomputation in Genomics

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Comprehensive surveys of genomic data are now commonplace in cancer research. Genomics studies have detected novel subtypes of tumors and revealed basic biological processes that provide prognostic value in many cancers. While large-scale assays using genomics technologies such as microarrays and Next-Gen sequencing allow us to simultaneously survey tens of thousands of genes, they require special considerations with research to analyzing, annotating and understanding our data and using them to make biological conclusions. Bioinformatics, the application of computational methods to biological questions, is essential in genomics studies.

In this course, we will describe a few common questions cancer researchers ask using genomics technologies, specifically gene expression assays using microarrays and Next-Gen sequencing to get a bird's eye view of how they might be answered using bioinformatics techniques.

We will examine questions such as:
How can hierarchical clustering be used to find groups of genes with similar expression patterns?
How can two-way clustering help detect novel subtypes of a cancer?
What are some approaches to find genes that differentiate classes of tumors?
How can I determine the biological processes that are playing a role a cancer?
What can high-throughput sequencing tell me about the mutational spectra of cancer?

Resources:

Stanford Functional Genomics Facility
Protein and Nucleic Acid Facility
Stanford Microarray Database

Stanford offers several courses that will provide a formal framework if you want to learn more about genomics:
STAT 141 (Biostatistics)
HRP 259 (Introduction to Probability and Statistics for Epidemiology)
GENE 211 (Genomics)
GENE 214 (Representations and Algorithms for Computational Molecular Biology)
GENE 244 (Introduction to Statistical Genetics)
GENE 245 (Computational Algorithms for Statistical Genetics)
DBIO 273A (A Computational Tour of the Human Genome)
BIOC 218 (Computational Molecular Biology)
BIOC 228 (Computational Genomic Biology)

How to Write a Grant Application

Mildred Cho, Ph.D.
Associate Professor of Pediatrics
Stanford University

General principles and rookie mistakes

- Assure fit with funder
  - rookie mistake: not to get to know the program officer and get input from funding agency
- Know your application constraints and requirements
  - rookie mistake: not to read the program announcement, request for applications or grant application instructions carefully
- Don't go it alone
  - rookie mistake: not to find a grant mentor and not to get comments from external readers

Timeline of tasks

- Identify due dates
- Communicate with RPM
- Letter of intent
- Identify collaborators
- Outline major budget items
- Establish timeline for proposed project
- Draft grant
- Upload documents to SF424-RR form
- Submit to RPM
- RPM submits to NIH
- Confirm assignment to appropriate review group

Successful grant writing

- Understand the review process
- Understand the reviewer perspective
- Don't focus only on WHAT you're going to do
- Give compelling rationale for WHY you're proposing what you're proposing
- Give compelling rationale for WHY you're NOT proposing alternatives
- Show that you are aware of potential problems and challenges
- Provide solutions for potential problems and challenges
- Be realistic about what you can achieve in the timeline

Resources

1. NIH SF424 (R&R) Application and Electronic Submission Information
   http://grants.nih.gov/grants/funding/424/index.htm
2. NIAID New Sample R01 Applications and Summary Statements
   http://funding.niaid.nih.gov/researchfunding/grant/pages/appsamples.aspx
3. Grants
   http://funding.niaid.nih.gov/researchfunding/grant/pages/default.aspx

How to Design a Clinical Trial

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Today's standard cancer treatments were yesterday's clinical trials. Cancer clinical trials aim to develop new strategies for the prevention, detection, treatment, and overall improvement of the quality of life of people with cancer or people at high risk for developing cancer. Clinical trials differ by type and phase. The Food and Drug Administration sets the guidelines on the design and terminology used in clinical trials. The table below list the **PHASES** of clinical trials: from first applications in humans (Phase 1) through limited and broad clinical tests (Phase 3), to postmarketing studies (Phase 4). The process starts with small trials testing the safety of an intervention and moves to progressively larger trials. The larger trials compare the effectiveness of the new intervention given to the investigational group to the currently accepted standard care given to the control group.

	Phase 1	Phase 2	Phase 3	Phase 4
Number of participants	15-30 people	Less than 100 people	Generally, from 100 to thousands of people	Several hundred to several thousand people
Purpose	*To find a safe dosage *To decide how the agent should be given *To observe how the agent affects the human body	*To determine if the agent or intervention has an effect on a particular cancer *To see how the agent or intervention affects the human body	*To compare the new agent or intervention (or new use of a treatment) with the current standard	*To further evaluate the long-term safety and effectiveness of a new treatment

Each **TYPE** of clinical trial attempts to answer different research questions:

* **Prevention**
* **Screening**
* **Diagnostic**
* **Treatment**
* **Supportive Care/Quality of Life**
* **Genetics**

First, develop the hypothesis**. A hypothesis is a tentative construct to be proved or disproved according to the evidence.** A good hypothesis should be testable, convey nature of the relationship being tested, state exactly what variables form this relationship and reflect ALL variables of interest.

The **clinical protocol** should describe the scientific rationale, objective(s), design, methodology, statistical considerations, data collection and organization of the planned trial.. When designing a clinical trial, it is imperative that the statistical considerations are sound and that the trial design incorporates sufficient **"power"** which is defined as the ability of the study to reliably detect the size of the effect of the study intervention. The number of patients enrolled is a major determinant of the power of the trial.

The **Informed Consent** document explains trial details, such as its purpose, duration, required procedures, risks, potential benefits and key contacts. Patients have the option of withdrawing consent at any time during the trial and protection of human subjects is of utmost importance. The mandate of an Institutional Review Board is to ensure the ethical conduct of the trial while the Data Safety Monitoring Board is responsible for monitoring the results and reported adverse events of the study at specified timepoints during trial accrual.

The conduct of a clinical trial requires an enormous amount of resources iincluding financial support, proper facilities and dedicated time from investigators, nurses, data managers, oversight boards and of course, patients. **Thus, the success of a clinical protocol depends on such factors as funding, biostatistical support, realistic accrual goals and timeframes, easily measurable endpoints, thoughtful consideration of eligiblity criteria, feasible treatment plans and reliable data management support**. The Principal Investigator must prospectively address all of these factors to facilitate the successful completion of a protocol and to ensure the integrity of the data collected.

Common Mistakes in Clinical Research

- Failure to critically examine literature for similar, prior research
- Failure to carefully specify inclusion and exclusion criteria for your subjects
- Failure to specify exact statistical assumptions including power calculations
- Failure to perform sample size analysis before study begins
- Failure to have detailed, written and vetted protocol
- Failure to vigorously recruit and retain subjects
- Failure to write and adhere to projected time line
- Failure to report missing data, dropped subjects and use of an intention-to- treat analysis
- Failure to point out weakness or "potential pitfall" of study

Recommended reading:

1) Steven Piantadosi. Clinical Trials: A Methodologic Perspective.1997. John Wiley & Sons, New York NY

2) Lawrence R. Friedman et al. Fundamentals of Clinical Trials 1998. Springer Science+Business Media LLC, New York NY

3) www.cancer.gov/clinicaltrials/learning

4) www.clinicaltrials.gov

How to Write a Scientific Paper

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After several years of experiments, and no matter how spectacular the data, your work is not completed until the manuscript is published. Publishing an original research article is the avenue through which you will become part of the broader national and international community of scientific investigators.

Our education is overwhelmingly focused on the technical aspects of science and experimentation, whereas the communication arts of writing and publishing are neglected. Some scientists are excellent writers, most are good writers yet some are poor writers. The purpose of this session is to outline the basic principles that may help you prepare a manuscript for publication.

The structure of a scientific article is familiar to all and is standardized across disciplines and includes: a Title, an Abstract, an Introduction, a Material and Methods section, the Results, and a Discussion of the results. There is no need to re-invent the structure; rather an important goal is to become proficient in mastering the structure so that the value of your data becomes clear, understandable, and recognized.

When to publish:

Most often, first disclosure of your research takes place with an oral or poster presentation at a scientific meeting. The presentation will help you organize your data, and may allow you to better understand what your audience likes about your research, or potential weaknesses that require further attention. At this stage in your career, it is important to convert every poster or oral presentation into a scientific publication. Therefore, you should begin to write your manuscript soon after your presentation (while fresh in your mind) yet before you complete your last experiment. If you wait until after your last experiment, you will no doubt move on to other exciting projects generated from your research and risk losing enthusiasm for writing your work. Also as your manuscript acquires structure and direction, you will see holes in your narrative that may require an additional experiment or two. The worst time to learn this is after you think you are finished the work, because it is somewhat demoralizing to go back, let alone how difficult it may be to find old reagents, and restart the experiment working successfully again.

What to publish:

As a general rule, it is better to publish one solid full-length original peer-reviewed research paper than to split the same work into two or more brief reports (also called, short notes). Full-length contributed articles are ~5000 words and are usually derived from of a series of experiments that form a coherent story. Brief reports or Short Notes are ~2000 words and usually represent one or two experiments that are stand alone pieces of work.

Where to publish and know your audience:

Before starting to write, consider to which journal you will submit your manuscript. Scan the current contents of potential journals looking for and reading articles on topics matching your research, and discuss journal selection with your research supervisor or other experienced colleagues. Keep in mind there are two audiences: the reviewers and the readers. Presume the reviewers are the 'experts' in your field and be sure to cite (original publications and not review articles) those who have made significant contributions to the theme of your paper. Each year, most journals publish a list of reviewers from the previous year and I encourage you to consider perusing the list of possible reviewers. Ultimately, your audience is the readers who are colleagues, competitors and others with a general scientific interest. The clarity with which you write your manuscript will draw in this audience.

The order in which you write your manuscript:

The Methods section is generally the place to begin. This section is usually initiated with little difficulty. This section should be written in the past tense and the Methods are often common to your laboratory so previous publications from your lab should be used as a template and cited. Many of the readers will skip most of this section. Yet it is critical that it be written carefully and is sufficiently complete that someone trying to understand, replicate or critique your work understands how your experiments were performed.

The second portion to write is the Results section. A reasonable way to first approach this section is to outline your story and construct the figures and tables you wish to present. As general rules, not all data have to be presented using figures or tables, and sometimes a simple narrative is better (i.e. the maximum yield was obtained at 37°C and at pH 8.1: a table or figure showing all other experimental conditions is not required). Tables are often reserved for important repetitive data that is too cumbersome to write. Avoid the temptation to rewrite the entire contents of the Table in the results narrative, rather try to highlight in the text just the key elements in the table. If the data shows pronounced trends, using a graph or figure can be helpful. If the numbers just sit there and there is no dynamic trend than either write as text or put in a table. STRIVE FOR CLARITY.

The last sections to write are the Discussion and the Introduction, and then finally the Abstract and Title. These sections should be written last because their contents are dictated by the Methods and Results section. The Discussion is usually the most difficult section to write. The opening paragraph of the Discussion typically is a series of 4-5 sentences that restate what was found and what it means. The second paragraph often casts your data in context of the work of others, and possibly how your work extends what is already understood. The subsequent paragraphs explore the meaning and limitations of your results, or raise new questions. In each of these paragraphs, summarize your data (evidence) for each conclusion. The final paragraph should end with a brief summary and a fitting climax. Do NOT end with 'more studies are needed, do NOT recapitulate the results in the Discussion, do NOT overstate your interpretation of your data (this offends most reviewers and leads to rejection of a manuscript) and avoid priority claims ("we are the first to show...").

The Introduction has elements in common with the Discussion but its goal is to establish the rationale for the present study. Introductions are generally organized as 3-5 short paragraphs. With clarity, the nature of the scope of the problem should be framed in the opening paragraph of the introduction. The subsequent 2-4 paragraphs should review the pertinent literature to orient the reader to the problem. These paragraphs should highlight controversies or needed improvements. The final paragraph of the introduction should state the principal results of your investigations and the principal conclusion(s) suggested by the results. But probably don't give away the punch line, reserve this for the final paragraph in the Discussion.

Once the above mentioned sections are complete, the Abstract and Title can be written. Most people will only read the Title and perhaps the Abstract whereas those who reviewed your paper and who are closet to your work will read the entire publication. Therefore, aim to try and capture the reader with your Title and Abstract and entice them to at least have a look at the figures and tables, and maybe more. The Abstract is a mini-version of your paper and succinctly summarizes the issue, the methods, select pieces of data, and 1 or 2 major conclusions. Writing a Title is an art; you need the correct sound bites yet without overstating the contents of your work.

And finally, you must be receptive to constructive editorial feedback.

Recommended Reading

1. How to write and publish a scientific paper. 5th edition. Ed. Robert A. Day. Oryx Press, 1998.
2. Wells, W.A. Me write pretty one day: how to write a good scientific paper. Journal of Cell Biology, 165:757-758, 2004.
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Cancer Drug Development by the Pharmaceutical Industry

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Currently, nearly 900 novel cancer agents are undergoing investigation in more than 6,000 clinical trials[1,2]. A critical lack of financial and patient resources significantly reduces the chances of adequately developing this large number of investigational agents. Clinical drug developers must meet this challenge with innovation in their approaches to determine which drugs to develop further, selection of appropriate patient populations in which to test novel agents, and maximization of resources.

Cancer drug development is based on a fundamental understanding of cancer biology and the selection of appropriate targets for therapeutic intervention[3]. Large-scale initiatives to sequence cancer genomes across the range of tumors in the next 5-7 years will provide insight into the evolution of cancer and mechanisms of resistance and promise to lead to new therapeutic and prevention approaches[4]. Small biotechnology companies and large pharmaceutical companies differ in their scope and approach to oncology drug development based on proprietary intellectual property, but they are similar in that they must be based in outstanding science and have a viable business model to be successful. This overview will consider drug development from the decision to go forward with a new drug application (IND) to study a potential new product in humans. The Food and Drug Administration (FDA) regulates the IND process in the U.S. in adherence to international standards for pre-clinical safety and toxicology.

Entry of a new pharmaceutical into man is founded in the vision for the molecule (first in class, best in class) and a core strategy that takes into consideration the unmet medical need, competitive landscape, and potential market in concert with the distinctive attributes of the molecule. A thoughtful diagnostic strategy with a real or potential enrichment based on a biomarker(s) is now standard and, increasingly, a pharmacodynamic strategy to determine that the experimental agent is achieving the targeted biologic activity is integral to the early development program. Once an IND is granted, the molecule proceeds to phase I clinical assessment.

The traditional goals of phase I testing are to demonstrate safety and achieve target exposure, confirm pharmacodynamic modulation for proof of principle, and establish the recommended dose for further clinical development. Prior to this point, the development opportunity has been carefully considered, including the path to registrational trials (leading to a new drug or new biologic application and supplemental applications), and a target product profile (TPP) has been generated. The TPPs outline the proposed indication, the endpoints to be used, the relevant diagnostic, safety and toxicity profile, and the proposed labeled dosing and administration. Timelines are critical in a competitive and resource-constrained environment; prior to phase I, the timeline for phase I-III development, filing and approval, and the probability of technical success are outlined.

Phase I dose finding starts with a dose based on safety margins from the preclinical studies and proceeds to achieve the targeted effective dose and beyond, according to safety considerations. Although innovative phase I dose escalation schema have been proposed in the literature, the cohort escalation (3-6 patients) continues to be the most typical. Expansion at the maximal tolerated dose (MTD) proceeds to achieve a preliminary assessment of clinical activity, often in a number of tumor types. If safety is unacceptable, the MTD is less than predicted as necessary from the preclinical experience, or there is no pharmacodynamic or clinical activity, the investment may stop.

Hunsberger and colleagues proposed that a dose response relationship may differ for the molecularly targeted agents for which a plateau exists on the dose efficacy curve where higher doses may not necessarily improve benefit, especially if the patient population is properly selected[5]. However, and in contrast, recently reported outcome results of 1,908 patients treated on 53 eligible clinical trials of molecularly targeted agents at multiple institutions throughout North America sponsored by the National Cancer Institute's (NCI) Cancer Therapy Evaluation Program (CTEP), suggest an increasing

benefit for patients treated at doses at or near the MTD[6]. These data indicate that dose-ranging studies must still be considered for molecularly targeted agents.

The BRAF V600E kinase inhibitor (RG7204, PLX 4032) is a recent example of phase I success with a highly targeted agent[7]. Patients with V600E mutated metastatic melanoma were selected (enrichment) with a specific companion diagnostic and remarkable phase I activity was observed in the expansion cohort using traditional and PET imaging. Subsequent phase 2 testing confirmed the activity of this agent and the confirmatory randomized phase 3 trial was quickly conducted, achieving its co-primary endpoints of progression-free survival and overall survival. At the same time, the preliminary assessment of this agent in BRAF V600E-mutated colorectal cancer failed to demonstrate similar activity in the expansion cohort, showing that the existence of the mutation does not necessarily determine that it is a primary driver of proliferation and survival. Another example with a highly targeted agent in a "selected" population is the hedgehog pathway inhibitor GDC-0449, a small molecule inhibitor of Smoothened, which resulted in a high response rate in advanced basal cell cancer[8]. The unprecedented efficacy of such agents has led some to recommend early accelerated approval after phase I[9].

The complexity of cancer, with its genetic diversity, redundancy and plasticity, requires strategies to overcome endogenous or acquired resistance. The development of two unapproved drugs simultaneously presents a regulatory challenge and, if one comes from a competitor, a commercial concern. New draft FDA guidelines allow flexibility for testing new agent combinations. Preliminary data from a dual phase I dose escalation of a PIK3 inhibitor and a MEK inhibitor were recently presented[10]. One of the mechanisms of resistance of RG7204 in metastatic melanoma involves downstream activation of MEK and combination studies are currently underway.

Phase 2 studies are designed to definitively establish proof of concept and gate investment to large, confirmatory phase 3 studies. Notably, oncology trials have among the worst track records for phase 3 success, leading to a major reconsideration of the early development process. Data from 2002 to 2006 across the pharmaceutical industry show a high attrition rate among oncology drugs from preclinical to market approval, yielding only a 12% success rate[11]. Beginning with phase III, the success rate to market approval is only 50%.

A major concern in phase 2 studies is the choice of endpoint, where it is advantageous to select surrogate endpoints for timeline considerations, but where the surrogacy is uncertain or not well established. Recent examples of the lack of association of overall survival with progression-free survival are notable in this regard[12]. Appropriate patient selection with enrichment for the drug target is critical, as exemplified by the classic experience with gefitinib in lung cancer and the more recent experience, also in lung cancer, with metmab, a single armed antibody that selectively blocks c-met signaling. Because open-label single-arm phase II trials are subject to patient selection and data interpretation biases, there is increasing reliance on randomized phase 2 studies and the development of new ways of evaluating therapeutic effect, including assessment in the neoadjuvant setting, waterfall and spider plots as representative displays of individual patient tumor responses, and novel immune-related response to reflect the unique antitumor activity of immunotherapy. Principles for phase 2 design include, first and foremost, the context of the future phase 3 trial and predicting for its success, which includes appropriate endpoints and patient selection, a minimum threshold of efficacy, and a critical evaluation of the totality of the data in decision making.

Phase 3 trials represent large investments, are highly regulated, and subject to multiple risks to the probability of a new agent launch or supplemental indication. The challenges in phase 3 include the need to satisfy global regulatory authorities, which are often in disagreement with one another regarding appropriate endpoints, study design, degree of efficacy necessary, and requirements for additional studies (post-marketing commitments or requirements) if approval is granted. Phase 3 trials must be rigorously conducted, statistically persuasive and clinically meaningful. These aspects lead to the need for placebo controls, independent radiology review, and extensive analyses to evaluate potential systematic biases in the study conduct, measurement of effect and interpretation. Statistical designs and statistical analysis plans are critical aspects of phase 3 drug development. Studies are typically powered at 90% to reduce the probability of a false negative outcome, and the false positive threshold is usually assigned a two-sided value of 5%. The targeted treatment effect is described in the hazard ratio (HR), which can be translated into median differences in the primary study endpoint (e.g. overall survival). For example, assume a 2 month difference (6 to 8 months) is the smallest benefit of clinical significance in the disease setting of

interest. The trial will be powered to detect this minimal detectable difference with a true HR of 0.65, a 6 to 9.2 month difference, because a 2 month difference would be statistically significant with this design.

Increasingly, companion diagnostics (e.g. Hercep test. BRAFV v600 mutation) accompany drug development in phase 3. These diagnostics are also regulated and must be developed in parallel for synchronous approval. Biomarker science is an important aspect of phase 3 development that is complicated by interaction with the disease setting, differences in combination therapy that includes a targeted agent(s) in the investigational arm, and the comparator. Much of this work is done in phase 3, because it is at this time that the full development plan for a molecule is undertaken.

From the business perspective, phase 3 drug development often involves trade-offs between speed, cost and risk. The regulatory environment, competition, health care payers, patent expiry, development costs and projected value are all important considerations in decision making. However, following sound scientific principles and addressing unmet medical need are the driving forces for phase 3 success.

2011 is on of the most exciting times in the history of cancer therapeutics. Three advances are dramatically changing the landscape of oncology: 1) the large number of drugs (hundreds) that inhibit oncogenic targets, 2) the reclassification of malignant diseases as molecular diseases, and 3) the advent and implementation of high-throughput technologies to predict drug sensitivity and risk of relapse. Cancers are robust diseases that will require multi-targeted drug approaches (just like chemotherapy) to address redundancy and restore disrupted biologic networks. Much work is to be done; the potential rewards for cancer patients have never been greater.

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Targeting Mutant Ras

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Ras genes are frequently activated in human cancer. Of the Ra superfamily of small GTPases, K-Ras is by far the most frequently activated, followed by N-Ras and, rarely, H-Ras. Oncogenic mutant Ras proteins are resistant to negative regulation by GTPase Activating Proteins (GAPs) and therefore constitutively bound to GTP. Attempts to develop drugs that target mutant Ras have been unsuccessful, since these proteins are not enzymes and in the conventional sense, and do not have pockets in which a small molecule could bind. Likewise, major efforts to prevent Ras proteins localizing in the plasma membrane have failed. The reasons for these failures, and possible solutions, will be discussed.

More recent efforts to block activated Ras have focused on pathways downstream (Downward 2008). Ras proteins in their active states engage a large number of potential effector proteins. Of these, the Raf kinases, sub-types of PI 3' kinases and the RalGDS proteins are best known, but others may also play an important role in Ras signal transduction and in cancer. In addition, a number of Ras-related proteins have been characterized, and many of these are likely to play roles in cancer, though their functions may be less direct than the classical Ras proteins that are activated directly by point mutation.

Drugs that inhibit Raf kinase have shown clinical benefit in treatment of malignant melanoma, in which BRAF V600E is a major oncogenic driver (Smalley 2010). However, these drugs have failed to show clinical benefit in Ras-mutant tumors. In contrast, they paradoxically induce the Raf MAPK pathway in these cells, and in normal cells in which Ras is active (McCormick 2010). This is because Raf kinase is negatively regulated by an auto-phosphorylation event, so that Raf inhibitors relieve auto-phosphorylation before they inhibit MEK phosphorylation (Figure One). It therefore remains unclear to what extent Ras depends on Raf kinase for transforming activity, even Raf proteins bind directly to Ras and are certainly major effectors of Ras action in normal cells and in development.

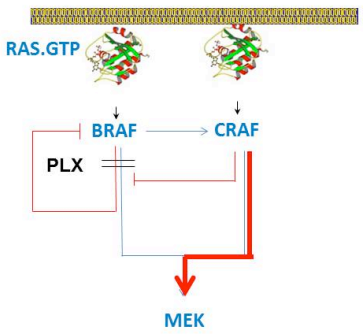

Figure One: an auto-phosphorylation site on BRAF is blocked by a Raf Inhibitor (PLX), resulting in up-regulation od C-Raf kinase

In contrast, MEK inhibitors inhibit the Ras-MAPK pathway in many tumor cells, but activate the PI 3' kinase (Mirzoeva, Das et al. 2009), and have, so far shown little clinical benefit. This activation event is mediated by EGF-R, and other receptor tyrosine kinases through relief of a negative feedback loop from ERK (Figure Two).

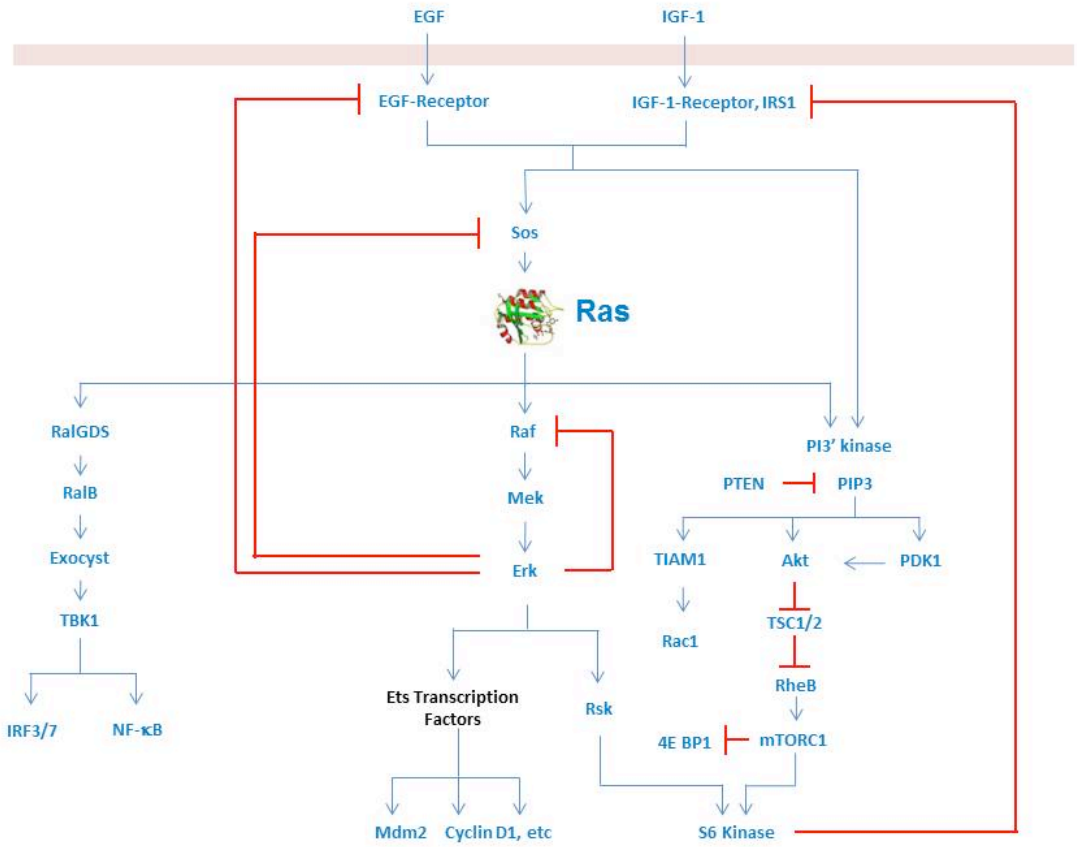

Figure Two: Feedback loops upstream of Ras

PI 3' kinase inhibitors are expected to block Ras transformation, and are being tested in the clinic in Ras mutant tumors, as well tumors with other genotypes, such as those lacking PTEN or expressing activated alleles of PI 3' kinase. It has already been established that drugs blocking the PI 3' kinase pathway further downstream lead to up-regulation of receptors upstream, by relieving a negative feedback loop from S6 kinase (O'Reilly, Rojo et al. 2006). Whether these types of feedback loop will defeat PI 3'kinase inhibitors remains to be seen. Surprisingly, the opposite outcome has been observed: PI 3' kinase inhibitors lead to inhibition of the MAPK pathway, though a cross-talk mechanism that has yet to be identified.

Because of these un-anticipated complexities, and the "un-druggable" nature of Ras proteins themselves, little progress has been made treating Ras mutant tumors to date. Future prospects will be discussed.

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Therapeutic Targeting of Human Acute Myeloid Leukemia Stem Cells

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Acute Myeloid Leukemia Is Organized as a Hierarchy Initiated by Leukemia Stem Cells

Acute myeloid leukemia (AML) is a clonal malignancy of the bone marrow characterized by the accumulation of immature myeloid cells defective in their maturation and function. AML affects 13,000 adults annually in the United States, most of them over the age of 65. Current standard of care includes treatment with several cycles of high dose chemotherapy and often includes allogeneic bone marrow transplantation. Even with these aggressive treatments, which have significant side effects, five-year overall survival is between 30-40%, and much lower for those over age 65 (Estey and Dohner 2006).

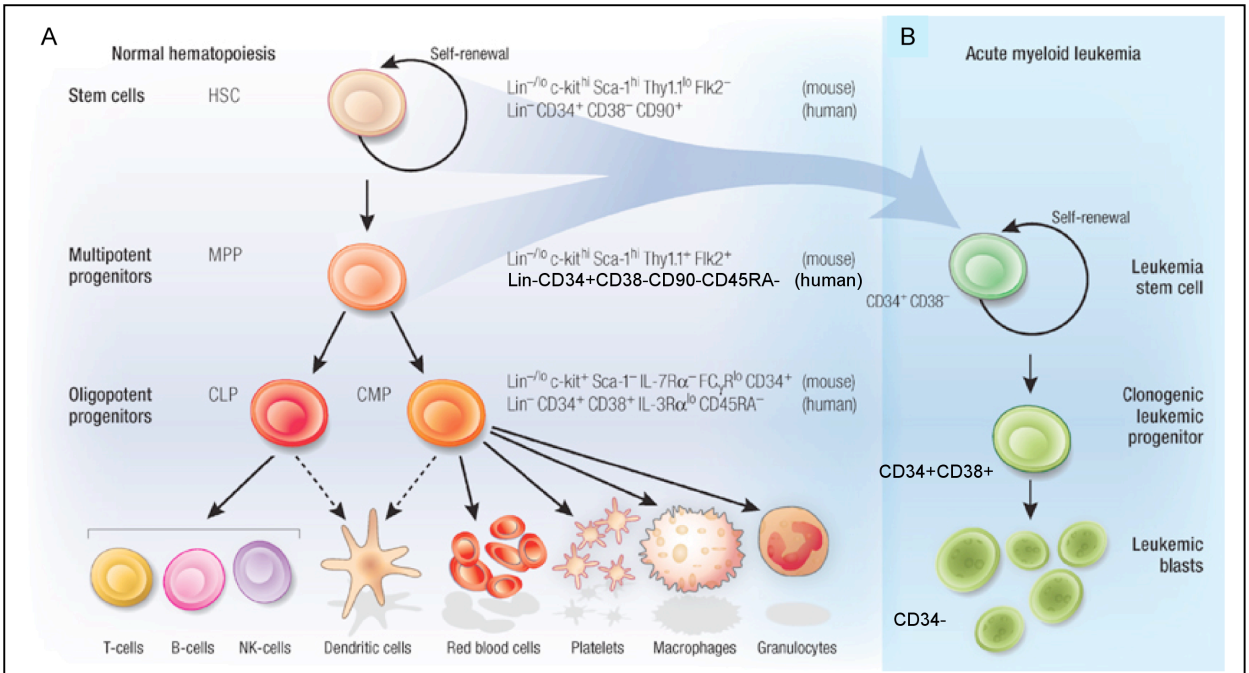

Figure 1: Hierarchical Model of Normal Hematopoiesis and Human Acute Myeloid Leukemia
(A) Self-renewing HSC reside at the top of the hierarchy, giving rise to multipotent progenitors that in turn give rise to lineage-committed progenitors that eventually produce terminally differentiated blood cells. (B) AML is organized as a hierarchy initiated by self-renewing leukemia stem cells that give rise to leukemic progenitors, which in turn give rise to the leukemia blasts.

Recent experiments have added to our fundamental knowledge by demonstrating that AML, like normal hematopoiesis, is composed of a hierarchy of cells that is maintained by a small pool of self-renewing leukemia stem cells (LSC) (Figure 1) (Dick 2008). Dick and colleagues were the first to establish xenotransplantation models for human AML, and used this assay to identify long-term engrafting AML subpopulations isolated by fluorescence-activated cell sorting (FACS). In published reports assaying a variety of clinical and biological subtypes of AML, LSC were found to be positive for expression of CD34 and negative for expression of CD38. Similar to the normal hematopoietic differentiation pathway, these LSC in turn give rise to leukemia progenitor cells with the surface immunophenotype of CD34+CD38+, which further differentiate into the bulk leukemic blast population defined as CD34- (Figure 1B).

AML Stem Cell Activity May Exist in Multiple Leukemia Subpopulations

A number of recent publications have begun to reassess the immunophenotypic identity of AML LSC using newer xenotransplantation models. These reports suggest that in some cases, leukemia stem cell activity may exist in additional subpopulations beyond the CD34+CD38- fraction. In the first such report, investigators determined that many anti-CD38 antibodies, including those utilized in the original isolation of LSC, inhibit engraftment of CD34+CD38+ leukemia cells through an Fc-dependent mechanism (Taussig et al 2008). When this mechanism was inhibited by immunosuppression, AML repopulating activity was detected in the CD34+CD38+ population. Investigators from the UK examined CD34 and CD38 expression on a large panel of patient samples, and found that in most AML patients, LSC were enriched in the CD34+CD38- fraction, but were also present in the CD34+CD38+ fraction (Goardon et al 2011). Moreover, these populations were hierarchically ordered with CD34+CD38- cells giving rise to CD34+CD38+ cells. Finally, a separate report identified LSC activity in CD34+CD38-, CD34+CD38+, and CD34- fractions of AML cells, although stem cell activity was enriched in the CD34+CD38- fraction (Sarry et al 2011). These results indicate that the surface immunophenotype of AML LSC remains an open question for investigation.

Therapeutic Targeting of Acute Myeloid Leukemia Stem Cells

A key implication of this cancer stem cell model is that in order to eradicate the tumor and cure AML patients, therapies must target and eliminate the LSC (Reya et al 2001). Investigation into the properties of AML LSC determined that the majority of the CD34+CD38- cells, unlike other fractions, were in the G_0 quiescent phase of the cell cycle, and moreover, that these cells were relatively resistant to chemotherapeutic agents (Ishikawa et al 2007). Recently, G-CSF was used to mobilize quiescent LSC into the active cell cycle, which in turn rendered these cells susceptible to killing by chemotherapeutics (Saito et al 2010). A second strategy to eliminate LSC is to use agents to differentiate them into more mature cells. Targeting of CD44 with a monoclonal antibody was shown to markedly reduce AML engraftment in mice, with evidence that it acted specifically on LSC to induce differentiation (Jin et al 2006). Recruitment of immune effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) is a third strategy to target LSC. Antibodies targeting the new marker TIM-3 on the surface of LSC demonstrated significant activity both *in vitro* and *in vivo* in pre-clinical xenotransplant models (Kikushige et al 2010). Finally, we have demonstrated that the innate immune system can be employed to phagocytose and eliminate AML LSC through the targeting of CD47 with a blocking monoclonal antibody (Majeti et al 2009).

CD47 is a cell surface molecule that serves as the ligand for SIRPa on the surface of phagocytes, which in turn transmits a dominant inhibitory signal for phagocytosis. In this way, CD47 essentially functions as a "don't eat me" signal. We found that blocking monoclonal antibodies directed against CD47 enabled phagocytosis of AML LSC, but not normal CD34+ human bone marrow progenitor cells, by human macrophages *in vitro*. Furthermore, analogous to a clinical therapy, treatment of human AML-engrafted mice with anti-CD47 antibody eliminated AML cells in the peripheral blood and bone marrow, and targeted LSC (Figure 2). On the basis of these results, we are currently developing a humanized anti-CD47 antibody as a novel therapeutic for AML.

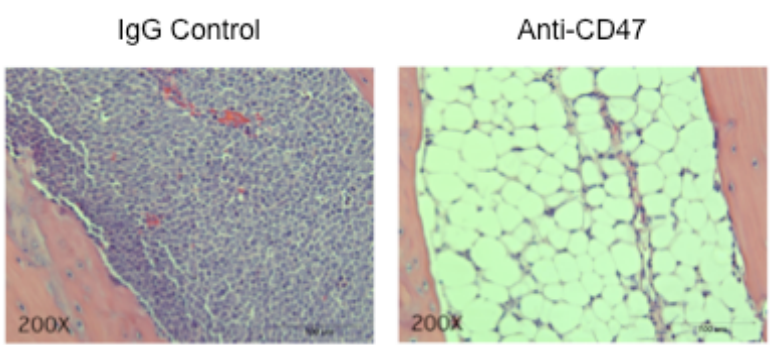

Figure 2: A Blocking Anti-CD47 Antibody Eliminates AML In Vivo
Immunodeficient mice were transplanted with human AML LSC. Several months later, mice were treated with either a control IgG or anti-CD47 antibody for 2 weeks. Histological examination of the bone marrow demonstrated elimination of AML cells in anti-CD47 treated mice.

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Molecular Risk Assessment for Lymphoma

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The most common subtype of non-Hodgkin lymphoma – diffuse large B-cell lymphoma (DLBCL) – is also among the most curable aggressive human cancers, yet it remains clinically heterogeneous. Presently, standard systemic chemotherapy regimens such as R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) induce complete remission rates in DLBCL exceeding 75%.[1, 2] Nevertheless, current long-term event-free survival ranges from 50-60%, and 30-40% of patients eventually succumb to their disease.[1-5] Predictive indices that capture such clinical heterogeneity might guide better therapeutic strategies. For instance, molecular risk assignment could be used to predict responses to specific therapies,[6] or to allow risk-adapted stratification within clinical trials thereby improving their statistical power.[7]

Traditional stratification schemes based on clinical characteristics such as the International Prognostic Index (IPI) have provided prognostic guidance in the management of patients with DLBCL.[8] Despite the ease in its implementation IPI does not fully represent disease heterogeneity.[9] Therefore, efforts have shifted to broad molecular profiles that model and stratify risks of adverse outcomes. Using genome-scale expression profiles, we previously defined two distinct subtypes of DLBCL with different normal counterparts and clinical outcomes.[10] With their distinct biological and clinical features independently validated,[10-14] these two DLBCL groupings – Germinal Center B-cell like (GCB-like) and Activated B-cell like (ABC-like) – are recognized as DLBCL subtypes in the current World Health Organization classification.[15] However, the current lack of standardized methods for routine clinical use of expression profiles and requirement for fresh or frozen tissues has limited their clinical utility.[5, 16, 17]

Therefore, to develop a practical clinical risk tool we examined associations between genome-wide expression profiles and outcomes at the single gene level (Alizadeh et al, in preparation). We aimed to construct prognostic models that integrate clinical and molecular indices in the current therapeutic era, then to test and validate them in a simple assay amenable to routine clinical practice. In studying 787 patients with DLBCL, we built and validated a simple model employing one gene expressed by tumor cells and another expressed by host immune cells, assessing added prognostic value to the clinical International Prognostic Index (IPI). We verified expression of *LMO2* as an independent predictor of survival and 'Germinal Center B-cell' subtype. We discovered expression of *TNFRSF9* from the DLBCL microenvironment, as the best in bivariate combination with *LMO2*. We studied distribution of *TNFRSF9* tissue expression in 95 patients with DLBCL, finding limited expression within T-cells from the tumor micro-environment. For patients with diverse non-hematolymphoid tumors, *TNFRSF9* expression similarly predicted clinical outcomes.

A *Two Gene Score (*TGS) risk model integrating these two genes for DLBCL was independent of 'cell of origin' classification, 'stromal signatures', IPI, and added to the predictive power of the IPI. This bivariate model and a composite score integrating the IPI performed well in three independent cohorts of 545 previously described DLBCL patients, as well as in a simple assay of routine formalin fixed specimens from 147 newly diagnosed patients with DLBCL for independent validation. Thus, measurement of a single gene expressed by tumor cells (*LMO2*) and a single gene expressed by the immune microenvironment (*TNFRSF9*) powerfully predicts overall survival in patients with DLBCL. A test integrating these two genes with the IPI can readily be used to select patients of different risk groups for clinical trials.

The newly described bivariate model and a composite index integrating the IPI were reproducible within validation studies, including in a simple assay employing routinely obtained diagnostic pathological specimens that were formalin fixed and paraffin embedded. While IPI captures a significant portion of attributable risk for adverse outcomes even in the current era,[5] the TGS carried independent value and obviated the need for more complex multi-gene indices. Both the TGS and the IPI added to each other within the TGS-IPI, capturing a larger fraction of patients with adverse outcomes.

Though originally devised prior to introduction of rituximab, the components of the IPI can be used to predict extremes of outcomes in the current therapeutic era.[4, 5] For instance, patients with zero IPI risk factors have 4-year survivals estimated at 95% in the current era but comprise only 10-15% of unselected cohorts.[4, 18] In comparison, the TGS-IPI identified over 30% of patients exhibiting similar outcomes after therapy with R-CHOP. Conversely, whereas patients with adverse risk as defined by TGS-IPI comprise at least 25% of the R-CHOP treated patients, the corresponding IPI High Risk group captures only ~10% of patients. Therefore, by capturing more patients at low and high risk for progression and death, this composite index allows better risk assignment than either the IPI or TGS alone.

Since less than half of patients within the high TGS-IPI risk group are cured, novel strategies to improve their outcomes are urgently needed, incorporating additional therapies or alternative regimens to R-CHOP. Conversely, given their highly favorable risk profile, patients with good risk features are unlikely to benefit from therapies adding to R-CHOP; similar strategies could be used to guide trials aiming to minimize toxicities from chemotherapy in this group.

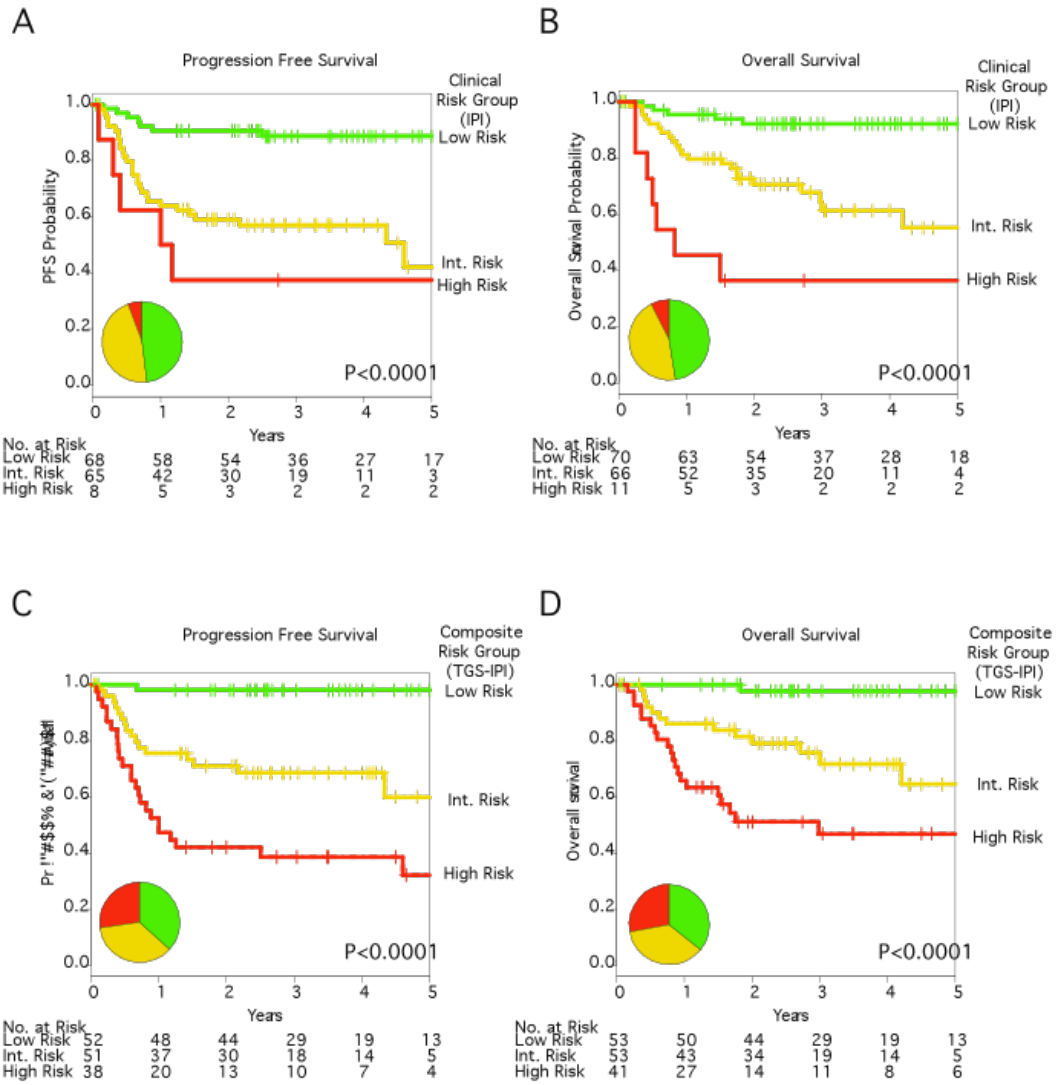

Figure 1. External validation of the composite predictor in fixed samples by quantitative real-time polymerase chain reaction. Kaplan-Meier estimates of PFS (panels A and C), and OS (panels B and D) for strata defined by the IPI (panels A and B) or by prospectively defined thresholds of a composite model integrating the two gene score and IPI (TGS-IPI; panels C and D). The three TGS-IPI strata correspond to a priori defined thresholds depicted in Figure 3E (High Risk, TGS-IPI > 4.51; Intermediate Risk, 4.51 ≥ TGS > 3.47; Low Risk, TGS ≤ 3.47). Depicted p-values reflect log-likelihood estimates. All depicted associations were also significant by log-rank product limit tests of Kaplan-Meier strata, including between TGS-IPI and PFS (p<0.0001, HR 3.9, 95%CI 2.4-6.3; Panel C), and TGS-IPI and OS (p<0.0001, HR 3.7, 95%CI 2.2-6.2; Panel D). Inset pie charts reflect distribution of risk groups as defined by the IPI or TGS-IPI, with color coding corresponding to Kaplan Meier curves.

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Prostate Cancer: The Challenge of Screening and Prognostication

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Prostate cancer as a model for cancer screening

One strategy for reducing cancer deaths is aggressive screening to identify cancers when they are small, localized (non-metastatic), and amenable to local therapies that will eradicate them. Over the past two decades prostate cancer has served as a model for the effects of wide-scale application of a cancer screening method to the population. Prostate Specific Antigen (PSA) is a serine protease made uniquely by the prostate gland and can be detected in the serum. Elevations of serum PSA levels have been used to screen for prostate cancer, and men with high PSA levels are counseled to undergo systematic biopsies to test for the presence of cancer. Although PSA has been used extensively to screen for prostate cancer over the past 25 years, many now question its utility and point out the consequences of large-scale screening.

In this talk we will review aspects of the PSA screening controversy and address challenges that have arisen as a result of PSA screening. Aggressive and repeated use of PSA as a screening tool has led to a decrease in diagnosis of locally advanced and metastatic prostate cancer from approximately 30% of cases in 1980 to 5% of cases in 2005. Furthermore, prostate cancer deaths have dropped from their peak of 40,000 in 1992 (approximately 5 years after PSA screening began) to an estimated 27,360 in 2010. Whether this drop is due to PSA screening is unknown, although some experts consider PSA screening to be largely responsible. However, 2 recent prospective randomized trials have called into question of PSA screening for reducing prostate cancer deaths. The PLCO trial in the US showed no difference in prostate cancer death rates after 10 years of PSA screening. The ERSPC trial based in Europe showed modest improvements in survival by 9 years, but at the expense of significant overtreatment of prostate cancer.

Consequences of PSA screening

The challenges arising from PSA screening are likely to be germane to most cancers as new screening tools are developed. As a screening test, PSA has relatively poor sensitivity and specificity. The poor specificity of PSA has meant that many men (about 2/3 of the 1 million men biopsied every year) have undergone an uncomfortable biopsy, with potential grave side effects, for no reason. Therefore, the next challenge in prostate cancer is to develop a screening test to improve on the specificity of PSA to reduce unnecessary biopsies. Furthermore, approximately 15% of men harboring prostate cancer are missed by routine PSA screening highlighting the need for a more sensitive test for prostate cancer. Whether early detection of these "low PSA" tumors impacts prostate cancer death rates is unknown and is deserving of further study. Recent data from the lung cancer screening trial show reduction of deaths at the cost of many unnecessary surgeries for screen detected lung nodules.

Perhaps the greatest challenged unearthed by PSA screening is over-diagnosis of prostate cancer. Since initiation of PSA screening, the gulf between the number of cases detected and the number of lethal cases has widened such that a man has a 1/6 lifetime risk of being diagnosed with prostate cancer and only a 1/35 chance of dying of his disease. This translates into many men being told they have prostate cancer who will never die of their disease, do not need to be treated, and indeed should never have been detected. Unfortunately, these men are faced with a decision to undergo life-altering treatments in the face of inadequate data on whether such treatments are necessary.

Strategies to address the unintended consequences of screening

The clinical challenges that arise from PSA screening present researchers with several translational research opportunities, particularly in the development and application of genomic and imaging technologies.

Markers of disease risk: Genome-wide scans have identified high-risk alleles for cancer susceptibility in individuals who can then be either screening more aggressively and at a younger age or treated with prophylactic removal of the organ at risk when possible. In the future, high risk individuals could be offered cancer preventive interventions.

New detection strategies: One of the challenges in cancer screening is poor test performance. For instance, PSA has only modest sensitivity for detecting localized prostate cancers and poor specificity since only 20-40% of men with an elevated PSA harbor cancer. The product is that some cancers are missed that should have been detected, while many men undergo unnecessary biopsies when the do not have cancer. Improvement in diagnostic tests, such as molecular imaging approaches or development of cancer specific biomarkers, would improve screening performance and likely translate into decreased morbidity and mortality. Examples of new screening biomarkers will be discussed.

New biomarkers of prognosis: Given the disparity in the number of cases discovered vs. the number of lethal cases of prostate cancer, considerable effort has been devoted to developing clinical and molecular classification schemes of low- and high-risk cancers. Genomic analysis of tumors can provide new classifications of tumors that correlate with biological (and clinical) differences in the behavior of tumors that had not been distinguishable using traditional clinical classifiers. In prostate cancer and kidney cancer, gene expression subclasses have been identified that that are correlated with treatment efficacy and with survival after therapy. Application of a variety of statistical tools has led to identification of genes that differ between normal tissues and cancer that are candidate diagnostic markers. Gene sets that correlate with clinical outcome (recurrence or death) have helped identify putative prognostic markers that could inform decisions regarding the necessity for and type of therapy. Some markers have already been incorporated into routine clinical care and many more are certain to follow. However, many challenges continue in evaluation and application of these biomarkers in the clinic.

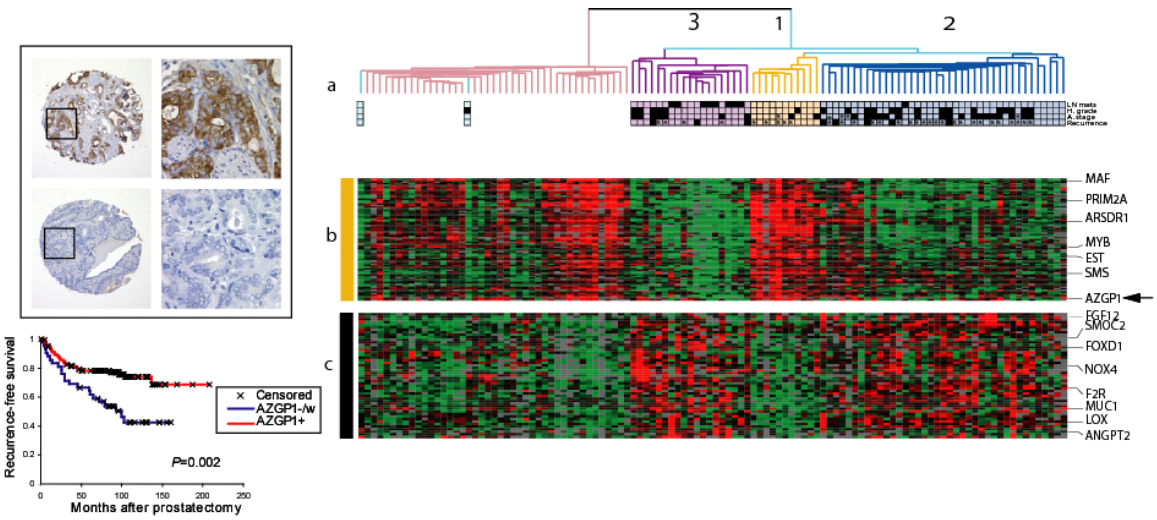

Figure: AZGP1 is differentially expressed between subclasses of prostate cancer and predicts outcome after surgery in an independent cohort of patients.

Clinical/translational research opportunities: The literature is replete with putative prognostic biomarkers for prostate cancer that have been discovered through genomic and hypothesis-based research. However, nearly all of these biomarkers have been tested as prognosticators on relatively small patient datasets derived from single institutions, usually from whatever samples were at hand - including old samples harvested prior to widespread PSA screening. These sample collections are often marred by selection biases, limited follow-up, limited sample sizes, unclear or widely differing definitions of endpoints, and

differences in patient populations that make it impossible to compare results from different institutions. Furthermore, very few markers have been validated on independent patient sample sets. Therefore, despite the plethora of candidate prognostic biomarkers, no markers or sets of markers are currently in use for the clinical management of prostate cancer. While the need for rigorously validated markers of prognosis is acute in prostate cancer, the steps toward validation of a tissue marker are not firmly established. Clinician-scientists will need to assemble bioreagents (blood, tissues, etc.) from affected patients that have been collected to minimize selection biases. These datasets will serve as a foundation for testing the performance of biomarkers in order to demonstrate their usefulness in patient care. These experiments are likely to be expensive and somewhat boring, but are essential to realizing personalized medicine in the context of cancer screening.

Additional reading

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Innovative Approaches to the Treatment of Non-Small Cell Lung Cancer

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In the United States, an estimated 222,520 cases of lung and bronchus cancers will be diagnosed in 2010, and 157,300 people will die of this disease. Therefore, there is an urgent need for safer and more effective therapies for lung cancer. Lung cancer falls into two major classifications, non-small cell lung cancer (NSCLC) which accounts for approximately 87%, and small cell lung cancer, which accounts for approximately 15%. While conventional chemotherapy is modestly effective in the treatment of advanced (metastatic) NSCLC, there is an increasing recognition that tumor histologic subtype, clinical features, and molecular alterations are important predictors of response to novel targeted therapies that are more specific and better tolerated than chemotherapy.

Small molecule tyrosine kinase inhibitors (TKI) of the epidermal growth factor receptor (EGFR) signaling pathway, such as erlotinib and gefitinib, improve survival in the second-line treatment of unselected patients with NSCLC. However, retrospective subgroup analysis of these clinical trials has revealed that patients with particular clinical features were more likely to benefit from therapy, such as those with tumors of adenocarcinoma histology, women, Asian ethnicity, and light or never smokers.[1,2] DNA sequencing of tumors from multiple series of patients that had dramatic responses to these drugs, as compared with patients without responses, revealed the presence of characteristic genetic mutations in the *EGFR* gene.[3] The previously identified clinical markers of response to EGFR TKIs were found to be commonly associated with the presence of these mutations; thus, the aforementioned clinical features are actually believed to be surrogates for a molecular biomarker known as an *EGFR* mutation.

Over 90% of EGFR tyrosine kinase domain mutations associated with sensitivity to EGFR TKI therapy fall into two categories, in-frame deletions in exon 19, and the L858R point mutation in exon 21. These mutations appear to specifically activate both cell proliferation, via activation of the MAP kinase pathway, and survival signals, via activation of the PI3 kinase pathway. Therefore, tumors with *EGFR* mutations are "oncogene addicted" to EGFR survival signals, relying exclusively upon the EGFR signaling cascade to maintain viability, which explains their exquisite sensitivity to TKI therapy. A number of recent large randomized studies have conclusively demonstrated that clinical selection of patients alone is inadequate, and instead establish *EGFR* mutation status as the single most important predictive marker of response to EGFR-TKI therapy.[4]

While patients with metastatic non-small cell lung cancer whose tumors contain EGFR mutations can have response rates of over 70% following treatment with erlotinib or gefitinib, most patients eventually have disease progression. While these responses can last for years, on average patients have recurrence and growth of their tumor in 9-14 months. Research into the development of resistance has yielded some insight into the molecular basis of the cell's ability to overcome inhibition of the EGFR signaling pathway. One mechanism of resistance is via the emergence of a secondary mutation in the EGFR gatekeeper residue, T790M. This amino acid substitution results in a large methionine residue that prevents drug binding to the kinase domain.[5] Another mechanism of resistance is via re-activation of critical signals downstream of EGFR. Despite inhibition of EGFR, cells can re-establish these survival signals by activation of an alternate signaling pathway. Amplification of the MET receptor tyrosine kinase in cell culture systems activates PI3K via an ErbB-3 dependent mechanism, allowing cell survival.[6]

Other mechanisms of resistance are likely to also occur, including the intriguing observation in some patients of a switch in tumor histological differentiation into small cell lung cancer.

In another emerging yet similar story, genetic fusion of the anaplastic lymphoma kinase (ALK) tyrosine kinase to a partner protein, EML4, appears to strongly predict sensitivity to the ALK TKI, crizotinib, with response rates of >70% and over 9 months of progression free survival.[7] As with EGFR inhibitors, resistance mutations have already been described both clinically and in model systems. Finally, there is evidence that less common mutations in NSCLC, such as *BRAF* mutations and *ERBB2* (e.g. *HER2*) mutations, may also predict response to targeted therapies. Therefore, identification of these genetic alterations in tumor samples is increasingly essential for individualizing treatments in patients with NSCLC.

Table: Common genetic alterations in NSCLC adenocarcinoma and corresponding targeted treatments

Genetic alteration	Frequency (smokers)	Frequency (nonsmokers)	Targeted therapy
KRAS mutation	40%	<5%	?PI3 Kinase and/or MEK inhibitors
EGFR mutation	<10%	40%	erlotinib, gefitinib
ALK translocation	<5%	15%	crizotinib

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Breast Cancer: Epidemiology and Risk Factors

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Breast cancer is the most common non-skin cancer of women in the United States (U.S.), and is the second-leading cause of cancer mortality. In 2010, there were approximately 207,180 new breast cancer cases and 40,540 breast cancer deaths; compared to other solid tumors, this ratio of deaths to diagnoses (20%) is a favorable one. Over the last several decades, there has been an increase in U.S. breast cancer incidence, coupled with a consistent decline in breast cancer mortality that has been driven by advances in early detection and treatment of early-stage disease (Figure 1). Whereas in past years developing countries have lagged developed countries in their breast cancer incidence, there is now evidence that breast cancer is on the rise in Asia and the Middle East, prompting concern that there could be a global epidemic as developing populations adopt an increasingly Western lifestyle. Within the last few years, reports of a decline in U.S. breast cancer incidence have prompted intense investigation as to the causes for this apparent reversal of a long-term rise.

There are several well-established risk factors associated with developing breast cancer, including hereditary, hormonal, and lifestyle exposures (Table 1). Gender and age remain the strongest predictors of breast cancer incidence, with women having substantially higher risk than men, and post-menopausal women having significantly higher risk than pre-menopausal women. The next strongest risk factors include family history of breast cancer in a first-degree relative, or an identified mutation in a breast cancer susceptibility gene. Highly penetrant genetic mutations account for 5-10% of U.S. breast cancer, with mutations in the *BRCA1* and *BRCA2* cancer susceptibility genes responsible for most hereditary cases. Such mutations convey 5 to 20-fold increases in the risks for breast and ovarian cancer; it is estimated that more than 500,000 Americans carry *BRCA1* or *BRCA2* mutations, and female mutation carriers are offered intensive strategies for early breast cancer detection and prevention. Other factors associated with elevated breast cancer risk include precursor lesions such as atypical hyperplasia, hormonal exposures such as nulliparity and use of menopausal hormone therapy, and lifestyle exposures such as chest wall radiation and tobacco. A number of statistical models, such as the Gail, Claus, and IBIS models, are used in the clinic to estimate a woman's breast cancer risk based on her reported risk factors; such risk estimates are used to target breast screening with mammography and magnetic resonance imaging, and cancer prevention with selective estrogen response modulators and surgical procedures.

Recently, the application of molecular profiling technologies has led to the discovery of distinct sub-types of breast cancer, which differ in their prognosis and response to standard therapies. Multiple different gene array platforms have reproduced the clustering of breast cancers into luminal, HER2/neu-positive, and basal categories. These molecularly defined sub-types overlap to some extent with sub-sets defined by patterns of three tumor markers routinely assessed in clinical practice: estrogen receptor (ER), progesterone receptor (PR), and the HER2/neu oncogene. Whereas the luminal sub-types are generally ER or PR-positive and responsive to endocrine therapies, the basal or "triple-negative" sub-types lack ER, PR and HER-2/neu expression, and carry a poor prognosis that may result from their lack of response to endocrine or HER2/neu-targeted therapy. The distribution of these breast cancer sub-types varies with age and race, and may partially explain some outcome disparities recognized prior to their identification: for example, younger African-American women are known to have worse breast cancer survival than other racial / ethnic groups, and they have recently been found to have a high incidence of poor-prognosis basal sub-type tumors. Although variation in access to care and exposure to known risk factors undoubtedly contributes to breast cancer outcome disparities across the population, the discovery of molecular sub-types is advancing our understanding of differences in breast cancer incidence and prognosis.

Major research priorities in breast cancer epidemiology include:

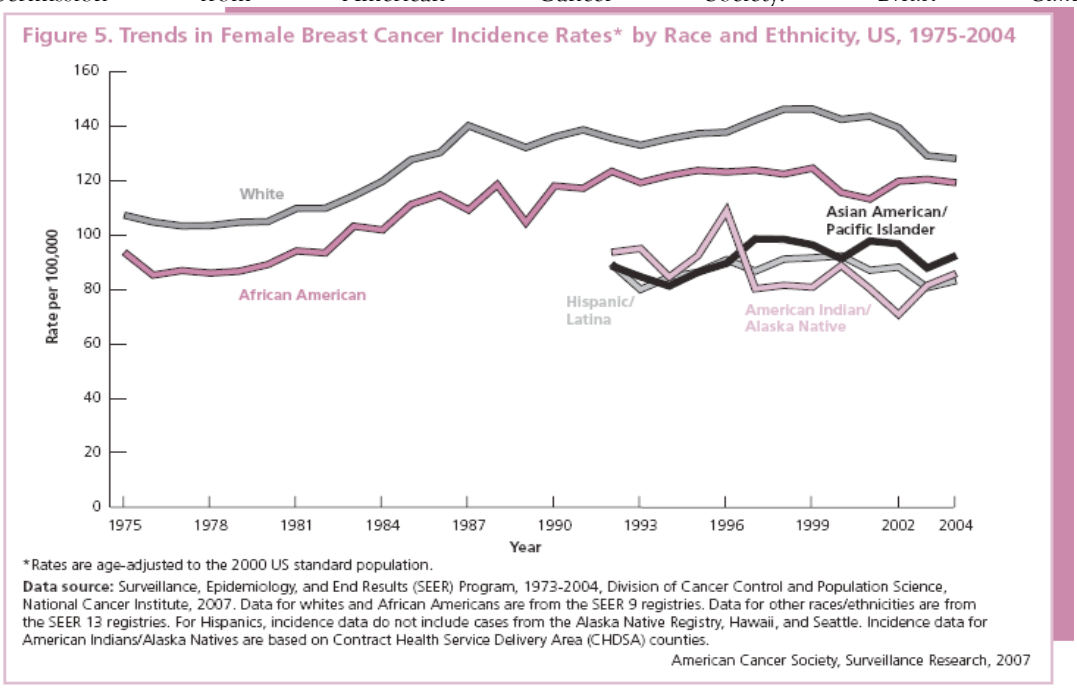
1) An improved understanding of the role of modifiable (lifestyle, hormonal, environmental) risk factors in breast carcinogenesis;

2) Identification of common genetic variants responsible for clinically relevant increases in breast cancer risk; and

3) Discovery of tumor and patient-specific factors which predict benefit from specific therapies.

An over-riding goal of epidemiologic breast cancer research is to improve targeting of early detection, prevention and treatment interventions, in order to optimize the risk-benefit ratio for individual patients.

Figure 1. Breast cancer incidence and mortality in the United States (US). Reproduced with permission from American Cancer Society. *Breast Cancer Facts and Figures 2007-2008.* Atlanta: American Cancer Society, Inc.



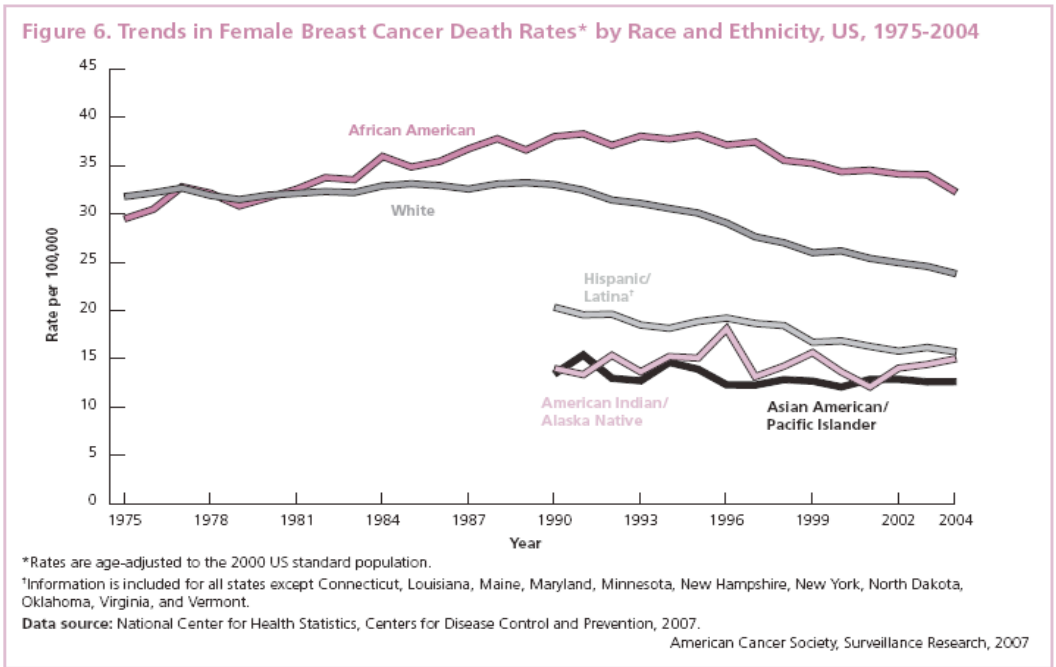


Table 1. Established breast cancer risk factors

Risk Factor	Range of Associated Relative Risk
Oral contraceptive use	<1 - 1.5
Menarche < 12 years of age or menopause > 55 years of age	1 - 1.5
Post-menopausal obesity	1 - 1.5
Menopausal hormone use in past 5 years (estrogen plus progestin)	1.5 - 5
Alcohol, > 1 drink per day	1.5 - 5
Nulliparous or first child born after 30 years of age	1.5 - 5
Dense breast tissue found on mammogram	1.5 - 5
Proliferative breast tissue or fibrocystic change on biopsy	1.5 - 5
Atypical hyperplasia or carcinoma in situ on biopsy	2 - 5
Family history of breast cancer in first-degree relative	2 - ≥5
History of chest radiation therapy before 30 years of age	≥5
Mutation in cancer susceptibility gene (such as *BRCA1/2, p53*)	≥5
Age greater than 50 years	≥5
Female sex	≥20

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The Case for Investments in Innovation and Discovery During a Time of Resource Constraint: Cancer as a Model

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Forty years have passed since the passage of the National Cancer Act of 1971. While some have questioned the benefit of the investments in cancer research through the National Cancer Institute, it is undeniable that significant progress has been made in understanding cancer biology, human genetics, stem cell biology, immunology, imaging as well as targeted approaches for a number of malignancies. More importantly, the opportunities for future discoveries and innovations that can change the face of cancer seem unparalleled and opportune for continued investment. At the same time, our nation's investment in science, discovery and innovation are at risk from the conflation of separate but interrelated forces. The unifying factor is the downturn of the US economy and rising debt burden that could compromise continued investments in research. This is made worse by the looming deficits faced by individual states across the US – among which California stands as an unwelcome example. Furthering the challenge is the rising cost of healthcare that now consumes 17% of the US GDP and which is increasing at an unsustainable rate. Among the drivers for healthcare costs are the fee-for-service model of reimbursement in the US that has led to a proliferation of technology driven procedures and therapies. A disease-based model of medical care, almost regardless of prognosis, has driven the healthcare focus of hospitals and doctors as well as the broad array of medical and related industries. In many ways cancer and its care stands a model of excellence as well as example of what has gone wrong in US medicine and science.

On the positive side of the ledger is the quality and excellence of cancer care that has emerged from the multidisciplinary team approach to diagnosis and management – which is particularly notable in Cancer Centers. Evidence-based protocol management has stood as example of clinical effectiveness research in clinical oncology. While this has reached a pinnacle of excellence in pediatric oncology, where over 70% of children are treated on a defined protocol, childhood cancer is rare and constitutes less than 2% of malignancies in the US. In contrast less than 5% of adults are treated on defined protocols. This has led to a highly variegated approach to the management of cancer and the proliferation of "cancer centers" at community hospitals and in private practice settings. A major reason for this is that the reimbursement for cancer care has been lucrative for hospitals (given the use of imaging and related diagnostic technologies, especially in ambulatory settings) and for doctors (who have benefited from establishing ambulatory infusion centers). The desperation and fear associated with the diagnosis of cancer has also prompted the use of expensive technologies, even when data is not supportive (e.g., stem cell transplantation for breast cancer even when outcomes showed not significant benefit) and expensive modalities that may contribute to only limited survival (e.g., Provenge for metastatic prostate cancer). The boundaries of expectation are complicated further by the surprisingly limited willingness of clinical oncologists to engage in end-of-life planning with patients, even when the course of disease is defined. Unabated this will worsen in the future given the projected increases in longevity and dramatic increases in the elderly during the next two decades – at the very time that Medicare funding is increasingly fragile and state support for Medicaid highly vulnerable.

A candid assessment of the "state of health" of the American healthcare "system" and future investments in innovation and discovery does not depict a reassuring prognosis – which has significant implications for academic medical centers, including Stanford. Academic medical centers are comprised of one or more professional schools and teaching hospitals affiliated with a comprehensive university or health sciences center. Sixty-six are National Cancer Institute Designated Cancer Centers. Academic medical centers have four missions: education, research, patient care and community service. Three of these missions are "cost-centers", and in many academic medical centers, revenues from clinical care are used to subsidize the missions in education, research and community service. Given the likely widening gap in

research funding, the continued costs for education and the probable increasing community needs that will increase with the aging population together with the withdrawal of state and federally funded benefit and entitlement programs, it seems inevitable that the stress on academic medical situations will worsen. At the same time, clinical revenues and especially those from in current cancer care (e.g., infusion centers, imaging, expensive technologies and drugs unsupported by clinical evidence) are vulnerable and will further impact cancer centers and their communities – and academic medical centers.

The current projected budget for the National Institutes of Health for the rest of FY11and for FY12 and the years ahead will have major implications for medical research and the investigators and trainees who conduct it. Coupled with this, the Affordable Care Act of 2010 that will continue to unfold over the next decade, will also impact academic medical centers and cancer centers. The shifting emphasis to maintaining health, managing populations and not simply individuals, the migration from fee-for-service care to integrated care and bundled payments, the focus in clinical evidence and review of technologies and interventions, will surely impact Medicine, as we know it today. And while it is possible to focus on the dire consequences, there is also an opportunity to use this "crisis" as an opportunity for change.

Cancer treatment and prevention has been an exemplar for protocol based-care and clinical trials. This approach to evidence-based care needs to become the rule rather than the exception. Coupled with this should be a focus on novel ways to deliver cancer care, including an expansion of clinical protocols in more dispersed settings and by different constellations of providers. A focus on regulatory science as well as population management and science will need to be better coupled with innovations in bioscience and technology. There is an opportunity for cancer centers to emerge as leaders of innovation in treatment and prevention and to help redefine the boundaries of care. And there is the opportunity for Cancer Centers to develop novel ways to construct clinical research, include the majority of patients on clinical protocols and engage the interface between basic science, translational medicine and community partnerships. Cancer Centers should take leadership in developing patient-centered care and become proactive in addressing challenging issues like "end-of-life" and palliative care in more comprehensive ways.

Healthcare reform coupled with greater competition for research funding will make Cancer Centers more important in the lives of faculty and institutions. They will need to be an anchor for education and career development, a center for discovery and innovation, a mechanism for resource management through core services and facilitated interactions, a ultimately a robust connection between science, clinical medicine, industry and the community.

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Mechanisms for Maintaining Genome Stability at the Replication Fork

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Genome instability is a hallmark of cancer cells and a driving force in cancer progression. Various forms of DNA damage can lead to genomic alterations, but double-strand breaks (DSBs) are particularly deleterious as they are a precursor of genome instability and can lead to the significant loss of genetic material or chromosomal translocations. Cells possess a complex response to DNA damage that facilitates repair of damaged DNA and prevents further genomic alterations. This response is directed in large part by activation of the DNA damage checkpoint kinases, ATM and ATR, which phosphorylate downstream molecules involved in a surprisingly diverse array of processes. Importantly, defects in DNA damage response (DDR) pathways have been linked to a growing number of human diseases, among which are a number of syndromes associated with a predisposition to cancer and congenital and developmental defects. Furthermore, the DDR itself acts as a barrier to cancer progression by promoting senescence or apoptosis in damaged cells.

Disruption in many cellular processes can lead to activation of the DDR and/or DSB formation, most prominent of which are DNA replication, DNA repair and the DNA damage response itself. The genome is particularly vulnerable during DNA replication, when it is susceptible to errors in its duplication as well as exogenous and endogenous events that damage the DNA or block replication fork progression. Stalled replication forks pose a significant challenge for the cell, and many types of DNA damage can stall replication forks. Moreover, there are natural sites in the genome where replication can be slow and ongoing replication forks are blocked. When a replication fork stalls, it is critical that the fork remains in the proper configuration and that components of the replication machinery, or so-called replisome, remain associated with that fork. A failure to do so causes the collapse of stalled forks and the formation of double-stranded breaks.

Because of the threat presented by stalled replication forks, all organisms have evolved a response that actively stabilizes and restarts stalled and collapsed forks. An essential element of this response centers around the checkpoint pathways. In mammalian cells, the ATR kinase is the primary mediator of events induced by stalled replication forks. ATR responds to inhibitors of DNA replication, such as the polymerase inhibitor aphidicolin; inhibitors of nucleotide synthesis, such as hydroxyurea; and various forms of endogenous and environmentally-induced DNA damage that block progression of the normal replicative polymerases. Data from our lab and others suggests that the ability of ATR to respond to these different agents results, at least in part, from the formation of primed single-stranded DNA (ssDNA) at stalled replication forks and upon processing of DSBs. In contrast, the ATM kinase responds to agents that induce DSBs, phosphorylating its downstream effectors in all phases of the cell cycle.

When ATR is absent, replication forks are not properly stabilized and cells accumulate DSBs in the absence of exogenous stress. These breaks lead to the activation of ATM, and they accumulate to an even greater extent in the presence of aphidicolin. The precise effectors of ATR in mediating stabilization of the replication fork stability are not clear. There is some evidence that the checkpoint acts to stabilize the association of replisome components with stalled forks. Whether this stabilization is through direct modification of these proteins or through more indirect means is not yet clear. It is hypothesized that this stabilization maintains the fork in a competent state, protecting the newly synthesized DNA and the fork from enzymatic activities that can lead to its rearrangement and/or processing. Other checkpoint-independent pathways may also act to stabilize the fork as well.

Recent data suggest that many cancer cells have high levels of replication stress, suggesting they are dependent upon ATR and other fork stabilization pathways for survival. Indeed, inhibitors of ATR and its downstream effector kinase, Chk1, strongly sensitize cancer cells to drugs causing further replication stress, and in some cases can cause cell death on their own. This has led to an interest in targeting ATR

and Chk1 in the clinic, and a need to understand replication stress and the response to this stress in greater detail.

In this presentation we will discuss the processes a cell uses to maintain genome stability during DNA replication, and our work in this area. Recently our laboratory performed a genome-wide siRNA screen in human cells to define the processes and proteins that, when altered, cause increased DNA damage in otherwise unperturbed cells and in cells under replication stress. We used H2AX phosphorylation as a marker for DSB formation as it is one of the earliest events occurring at a DSB site. Phosphorylation of the histone variant H2AX (γH2AX) depends on the checkpoint kinases (ATM, ATR and DNA-PK). A large number of proteins and pathways were identified in our screen, and enriched among this group were many of the expected effectors, including genes involved in DNA replication, DNA repair and checkpoints. We also identified components of known pathways not previously linked to the DNA damage response, as well as a number of previously uncharacterized proteins. Among our hits may be genes that may drive cancer progression when lost, or which could sensitize cancer cells under replication stress when inhibited. They also represent potentially novel mediators of replication fork stability.

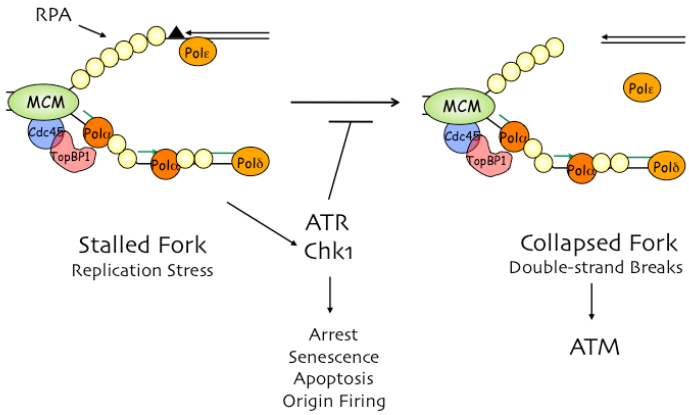

Figure. Simple cartoon of stalled fork activating ATR and fork collapse after prolonged stalled or ATR loss.

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Cancer Biomarkers and Molecular Therapeutics

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An emerging paradigm in tumorigenesis is the notion that the targeting of specific molecules may be a specific and effective therapy for neoplasia (Arvanitis 2007). Many new therapeutic agents have been identified that block specific oncogenic signaling pathways thereby eliciting tumor regression through the phenomenon that has been described as oncogene addiction (Felsher 2008; Weinstein 2008) (Figure 1). This understanding has led to a mandate to more generally identify cancer biomarkers that could be used to predict and validate the efficacy of particular drugs that will be effective in the treatment of a specific cancer.

However, there are many challenges to identifying and measuring biomarkers. First, cancers are generally highly complex, and quite heterogeneous such that it can be extremely difficult to identify biomarkers. Second, even when biomarkers have been identified, it can be quite challenging to obtain sufficient clinical tumor material to monitor biomarkers, hence, necessitating highly sensitive methods. Third, tumor tissue can be complex making it difficult to make an accurate assessment of a tumor through any single biomarker, thus, requiring approaches that are robust enough to look at more than just a few potential markers. Fourth it would appear that the best way to evaluate efficacy would be through the interrogation both before and after the initiation of drug therapy; thereby, techniques that allow for repeat measurements may be advantageous.

A multitude of approaches have been developed for identifying then assessing biomarkers in cancer specimens, each with specific advantages and disadvantages that can be broadly classified as four different approaches: genomics, proteomics and molecular imaging.

Genomics based methods have been the most widely applied and include microarrays as well as QPCR based approaches to examining gene expression changes. Gene signatures predicting outcome in lymphoma, breast and most other types of cancer have been defined. A major advantage of this approach is that a wide survey of thousands of genes can be obtained. However, a common pitfall of this approach is that it is remarkable that different investigators only rarely identify the same predictive gene signature. This most likely reflects that the specific gene signature identified is highly dependent upon the microarray platform, the methods of analysis and/or the likelihood that it is not gene expression but protein expression that is directly responsible for biologic activity.

Many proteomic methods have been developed for measuring biomarkers including nanoscale proteomic methods such as the nanoimmunoassay (NIA) and magnetonanosensors. Both approaches utilize antibody detection with an ability to measure biomarkers in picograms of clinical material in picoliter volume (Fan 2009). This approach also has the advantage of allowing one to assess many biomarkers at the same time. Flow cytometry can be used to examine biomarkers in intact tumor cells that allows for simultaneous measurements; but also requires much more clinical material that must be in single cell suspensions. Mass spectrometry based methods generally require much more clinical material but have the major advantage that they do not require antibody based detected or even the pre-existing knowledge of the specific biomarker that is to be identified.

Finally, molecular imaging methods provide an approach that can measure gene or protein expression in situ without requiring clinical material. To date, PET based imaging methods have been developed that can be used to measure general biological features of tumors including proliferation, glucose metabolism, apoptosis and angiogenesis. However, at least in experimental models, it is possible to use imaging methods that can be used to measure the expression of specific proteins.

In conclusion, many new approaches have been developed to identify and measure cancer biomarkers that will be useful in the development of molecular therapeutics and to predict, monitor and evaluate drug efficacy.

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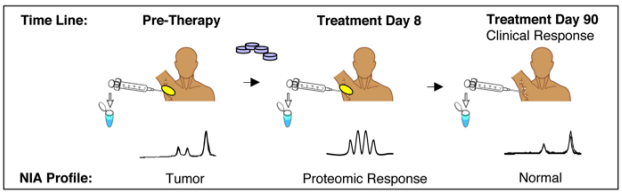
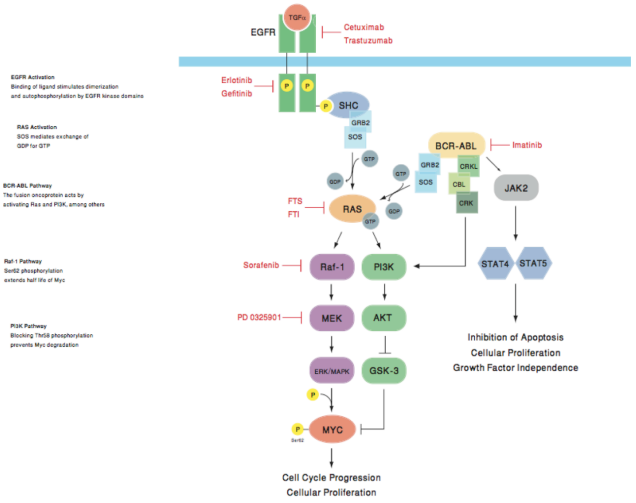
Figure 1: The measurement of cancer biomarkers are an important method for predicting and measuring the efficacy of specific signaling pathways for the treatment of cancer.





Clinical Cancer Trials Based on Target Selection

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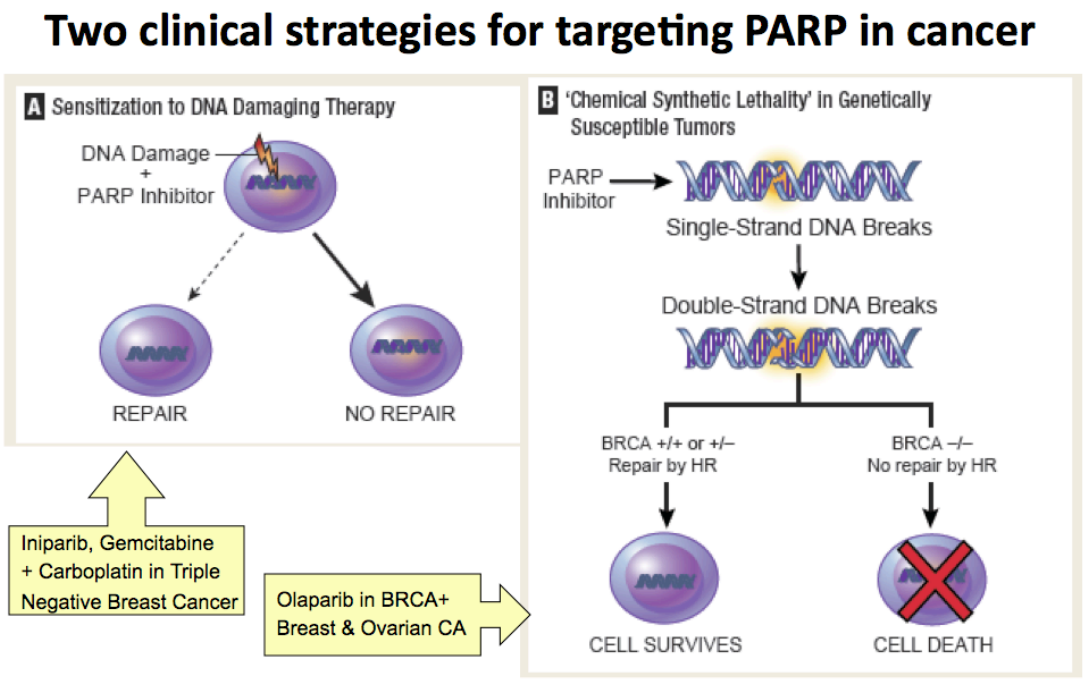
The understanding of cancer specific pathways and the development of drugs targeting these specific genetic changes has allowed for molecularly based therapeutics (1). Designing clinical trials using targeted therapeutics requires novel approaches to diagnostics and translational biomarkers. We have identified specific defects in DNA repair pathways in molecular subtypes of breast cancer, and have designed clinical trials using DNA repair inhibitors to target these defects (2-4).

Triple-negative breast cancers (TNBC), defined pathologically by the lack of expression of estrogen and progesterone receptors and the lack of overexpression or amplification of the HER2/neu oncogene, account for approximately 15% of breast cancers overall. TNBCs have an aggressive clinical course, and poorer disease-specific survival than with hormone receptor-positive subtypes. Ethnic differences in the epidemiology of triple-negative breast cancer also exist, with young, African-American and Hispanic women being disproportionately affected. Women that inherit mutations in one allele of the BRCA1 breast cancer susceptibility gene are at significantly elevated risk for developing TNBC. Unlike for patients with hormone receptor-positive or HER2-positive breast cancer, specific therapeutic targeting of the triple-negative subtype has been limited due to the absence of an identifiable target.

Many effective cytotoxic chemotherapies exert their anti-tumor effect through production of DNA damage and efficient cellular DNA repair can mitigate this injury resulting in treatment resistance. Breast tumors arising in patients with a germline BRCA1 mutation are associated with reduced DNA repair capacity due to the loss of BRCA1 function and are hypothesized to rely more heavily on alternate compensatory DNA repair processes for survival. BRCA1 and BRCA2-deficient cells have been found to be markedly sensitive to inhibition of poly(ADP-ribose) polymerase (PARP), in contrast to those cells wild-type or heterozygous for BRCA1 or BRCA2 (5-6). PARP is required for base-excision repair and if inhibited, repair-associated breaks result in replication fork-mediated double-strand break formation, which require BRCA1- and BRCA2-associated recombination to resolve. Inhibition of PARP is thought to enhance the effect of DNA damage; consequently, it is hypothesized that small-molecule inhibitors of PARP will have a role in the treatment of cancer, both alone and in combination with DNA damaging agents, particularly in tumors with defects in DNA repair pathways leading to a chemical "synthetic lethality".

Sporadic triple-negative breast cancers share many pathologic and molecular features with breast cancers due to hereditary BRCA1 gene mutations, including basal-like gene expression, high histologic grade, high rates of p53 mutation, and cytogenetic abnormalities. We found that sporadic TNBCs possess similar DNA repair defects and demonstrate similar chemosensitivity as BRCA1-associated breast tumors (7-8). These observations provided the rationale to investigate DNA damaging chemotherapies and PARP inhibitors in TNBC patients.

Early reports demonstrating efficacy of PARP inhibitors in women with advanced BRCA-associated and TNBC have been reported. At present, no PARP inhibitors are FDA approved for the treatment of cancer and data is lacking in an adjuvant patient population. A number of pharmaceutical companies are developing PARP inhibitors for the treatment of cancer. Responses seen in both triple-negative and non-triple-negative patients with BRCA mutations suggest that the selection of patients based on shared DNA repair defects trumps phenotypic subtype and has important implications for future investigations. Studies investigating the role of PARP inhibitors in women with early stage TNBC are ongoing and a number of studies are in development. A single arm phase II neoadjuvant study of gemcitabine, carboplatin and iniparib for women with Stage I-III triple-negative breast cancer is ongoing at Stanford University and will soon be expanded to multiple institutions through the Eastern Cooperative Oncology Group (ECOG). These studies represent a new direction in the treatment of early stage triple-negative breast cancer patients (9). Robust correlative science aimed at understanding the determinants of response to these agents will be critical to accelerate the development of this exciting targeted treatment in a curable patient population.



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What participants had to say about the 2010 CCRTP . . .

"This week was great! The course was really well-organized. The speakers were really stimulating. Great talks! Great topics!"

"This conference is perfect ever."

"Although the majority of the lectures were not strictly relevant to my goals, I was positively impressed learning different aspects of cancer research, treatment, and related issues. It was enriching."

"This week has been a worthwhile experience for my academic career as a graduate student. I am exposed to various exciting and current research areas in cancer. The lectures are all very interesting and enlightening. I am glad that I attended this course. I think I am interested in coming next year."

"In general, great course! Very broad spectrum which should be maintained in the future. Please also stay at this wonderful congress place."

"Overall, this training program has been fantastic with presentation of many diverse relevant topics in the field of cancer biology/treatment taught by qualified, experienced knowledgeable experts of their respective fields. I learned a lot. What struck me as most impressive was that 1) all the speakers presented not only the known/successes in cancer biology, but also pointed out current challenges/issues/unknowns, and 2) the speakers presented data relevant to the course topic, not just stuff from their own labs. Finally, the conference facility was fantastic."

"I was exposed to topics and areas that I would not normally read about. It got my mind working and was very educational. Thanks. For the next year, perhaps it would be useful to have a representative(s) of the current industry members for that perspective."

"This conference has increased my understanding of cancer or its effects. Thank you for organizing this conference. Also, this conference has instilled within me a desire to not just care but find a way to prevent cancer."