

TARGETING CANCER STEM CELLS

AN INDUSTRY REPORT

A new paradigm is emerging in the treatment of cancer, based on an hypothesis that attributes malignancies to a special cell of embryological origin, the stem cell. This working premise states that among the various cells comprising a tumor is a small population of cells capable of replicating the malignancy genotypically and phenotypically. As such, these cells are believed to be the source of metastases and the root from which cancer recurrence grows. Key questions remain regarding the pathways that lead to their initial formation, the proportion of cancer stem cells (CSCs) in a given tumor, the best means of distinguishing these cells from others, and the optimal ways in which they may be targeted therapeutically.

The stem cell hypothesis of cancer has nonetheless offered up rational approaches to preventing, diagnosing, and treating malignant growth. Accordingly, it has shifted the pharmaceutical industry's research focus ever more sharply onto medicines that attend to the unique attributes of these CSCs – notably, a resistance to both chemotherapy and radiation therapy. This report provides a brief review of five areas in which drugs are nearing a crucial stage of development, the Phase II clinical trial that provides human proof-of-concept data. The five areas covered are: Notch signaling, PI3K/Akt pathway, immunotherapeutics, molecular chaperones, and hedgehog signaling. Moreover, we mention drugs that are exemplary of the therapies being developed in these areas and programs at both major drug corporations and emerging biopharmaceutical companies.



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TABLE OF CONTENTS

The Challenge of Cancer 3

Normal Stem Cells 4

Stem Cell Hypothesis of Cancer 4

 Implications of the Stem Cell Hypothesis 4

 Cancer Stem Cells 5

Targeting Cancer Stem Cells 6

 Notch pathway 7

 PI3K/Akt Pathway 8

 Immunotherapies 10

 Molecular Chaperones 11

 Hedgehog Pathway 12

Companies Mentioned in this Report 13

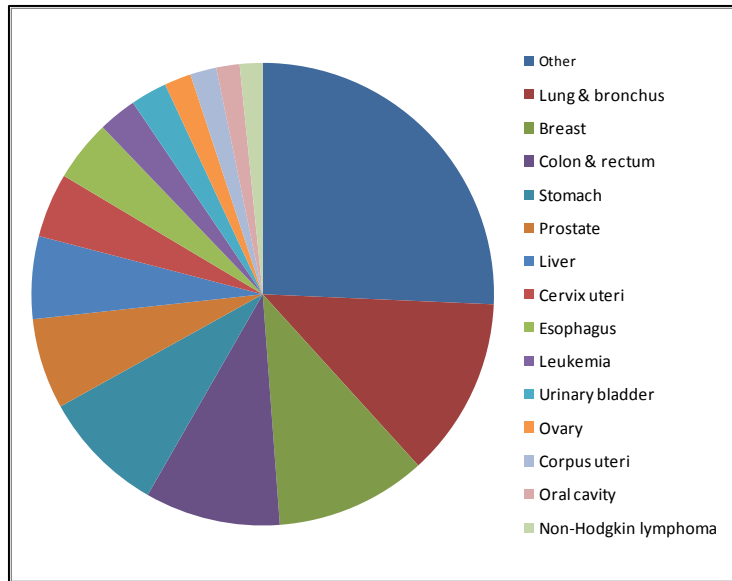
Disclosures 14

THE CHALLENGE OF CANCER

Malignancies are the second most common cause of death worldwide, just behind heart disease, and that statistic is not likely to improve quickly. Indeed, the American Cancer Society estimates that deaths due to cancer will increase from 7.6 million in 2007 to 17.5 million in 2050, simply because the world's population is aging.¹

From even a quick glimpse of Figure 1, it is apparent that cancer is a disease that arises in virtually every part of the body. Multiple factors contribute to oncogenesis, and that explains in part why the disease has proven so difficult to treat.

Figure 1. A Breakdown of Estimated New Cancer Cases Worldwide by Organ/Tissue¹



With the advent of new technologies, the pharmaceutical industry has begun to develop targeted inhibitors of pathways that appear to contribute to tumorigenesis and metastases. These new medicines offer the promise of treating many types of cancer with greater efficacy and fewer unwanted side effects than heretofore possible. An important premise guiding this work is the cancer stem cell hypothesis.

¹ Global Cancer Facts & Figures 2007, published by the American Cancer Society.

NORMAL STEM CELLS

Normal stem cells give rise to all parts of the body during development. As such, they comprise a large proportion of the early embryo, but diminish in number as the organs are formed via cell differentiation into the specialized components of each type of tissue. Much of this occurs during embryological development. However, there are three organs that undergo significant development postnatally, the brain, breast, and prostate. Normal stem cells also have an important role in tissue maintenance, as cell turnover occurs at distinct rates depending on the tissue. In the skin, gut, and blood, cells turn over rapidly, while in other areas, notably the nervous system, they turn over more slowly. A third function of stem cells is to aid in tissue repair after injury, and again, this capability differs significantly between organs. For instance, the liver has considerable regenerative capacity, while nerves can suffer permanent damage. In all three roles – development, tissue maintenance, and tissue repair – it is the self-renewal property of stem cells that enables them to fulfill their task.

STEM CELL HYPOTHESIS OF CANCER

The stem cell hypothesis of cancer attributes the origin of tumors to a loss of regulation governing self-renewal by stem and/or progenitor cells. This population of cells is believed to give rise to differentiated tumor cells, while a portion retains stem-cell properties and persists as the tumor grows. It is this relatively small number of cells, that are normally in a quiescent state and are resistant to common therapeutic interventions, that play a key role in metastatic disease and disease recurrence.

IMPLICATIONS OF THE STEM CELL HYPOTHESIS

The cancer stem cell hypothesis has important implications for disease prevention, detection, and treatment, as shown in Figure 2.² (Note that curcumin and genistein, which have antitumor activity, are examples of natural products that may be incorporated into normal diets.^{3,4})

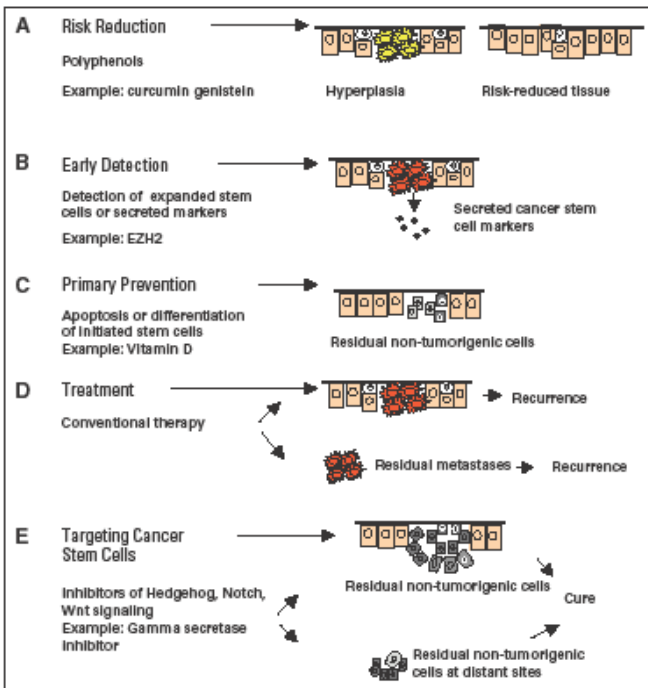


Figure 2. Implications of the stem cell hypothesis of cancer. (A) Risk reduction via a change of diet and/or life style. (B) Early detection of cancer via circulating CSCs or biomarkers. (C) Primary prevention via prophylactic intervention. (D) Treatment with a conventional therapy, possibly to debulk. (E) CSC-targeted therapeutic intervention.
 Source: Kakarala, M, and Wicha, MS.²

² Karakala, M, and Wicha, MS. Implications of the cancer stem cell hypothesis for breast cancer prevention and therapy. J Clin Oncol 2008; 28(17): 2813.

³ Verma, SP, et al. Curcumin and genistein, plant natural products, show synergistic inhibitory effects on the growth of human breast cancer MCF-7 cells induced by estrogenic pesticides. Biochem Biophys Res Commun 1997; 233(3), 692.

⁴ Singletary, K and Milner, J. Diet, autophagy, and cancer: a review. Cancer Epidemiol Biomarkers Prev 2008; 17(7): 1596.

CANCER STEM CELLS

The idea that a small population of cells is responsible for tumors can be traced to the late 19th century,⁵ though much of the progress in identifying and targeting cancer stem cells (CSCs) has taken place only in the past decade. The first report substantiating the existence of CSCs showed the presence of primitive leukemic cells similar to hematopoietic stem cells that could give rise to acute myeloid leukemia in immunodeficient mice.⁶ Since that discovery in 1994, these cells have been found in most solid tumors, including breast,⁷ brain,⁸ colon,⁹ prostate,¹⁰ pancreas,¹¹ ovary,¹² and melanoma.¹³ Skeptics of the CSC hypothesis have argued that cancer cells may be capable of de-differentiating (i.e., returning to a less differentiated state akin to a stem cell) and that methods used to isolate CSCs are inadequate to select for all cells with the capacity for self-renewal. Regardless, the hypothesis provides an explanation for differences between cells in a tumor and offers a new rationale for drug design.

Proving that CSCs exist has not been straightforward, as special techniques have been required to select for these cells out of the vast number in a solid tumor and then to demonstrate their multilineage differentiation capacity in serial xenografts. The task was made all the more difficult because CSCs often comprise about 1% of a tumor, though higher proportions have been reported.⁴ Selection has hinged on the identification of cell surface markers, some of which are specific for their respective tissue types, while others are common to primary tumors found in a variety of organs. This combination of different markers for cell selection and the variability that can be introduced during culture and/or the use of different xenograft techniques has caused some controversy over the existence of a CSC population in all tumors. A list of stem cell markers, shown in Table 1, provides an illustration of how some markers have been identified on multiple tumors, while others appear to be specific for only certain malignancies.

Table 1. Markers Used to Identify and Isolate Stem Cells from Malignancies¹⁴

CANCER	CD44	CD24	CD133	ALDH1	ESA	B1	α6	CD138	CD34	CD166	CD20
Breast	+	-	+	+	+	+	+				
Colon	+		+	+	+					+	
Prostate	+		+	+		+	+				
Head and neck	+			+							
Pancreatic	+	+	+	+	+						
Lung			+								
Brain			+								
Liver			+								
Melanoma	+		+				+				+
Multiple myeloma				+				-	+		+
Leukemia	+			+						+	

Definitions for the human CD (cluster destination) molecules are: CD44, hyaluronate receptor (p-glycoprotein1); CD24, heat stable antigen; CD133, prominin1; ALDH1, aldehyde dehydrogenase 1A1; ESA, epidermal surface antigen (Flotillin/20); B1, integrin B1 chain; α6, integrin α6 chain (CD49F); CD138, heparin sulfate proteoglycan fibroblast growth factor receptor (syndecan proteoglycan 1); CD34, hematopoietic progenitor cell antigen (GP105- 120); CD166, activated leukocyte cell adhesion molecule; CD20, B-lymphocyte cell- surface antigen B1, membrane- spanning 4- domains, subfamily A, member 1.

⁵ Julius Cohnheim (1839-1884) experimental pathologist. JAMA 1968; 206: 1561.

⁶ Lapidot, T, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994; 367: 645.

⁷ Al-Hajj, M, et al. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci 2003; 100: 3983.

⁸ Singh, SK et al. Identification of a cancer stem cell in human brain tumors. Cancer Res 2003; 63: 5821.

⁹ O'Brien, CA, et al. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 2007; 445: 106.

¹⁰ Wang, S, et al. Pten deletion leads to the expansion of a prostatic stem/progenitor cell subpopulation and tumor initiation. Proc Natl Acad Sci 2006; 103(5): 1480.

¹¹ Li, C, et al. Identification of pancreatic cancer stem cells. Cancer Res 2007; 67: 1030.

¹² Bapat, SA, et al. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Res 2005; 65: 3025.

¹³ Fang, D, et al. A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res 2005; 65: 9328.

¹⁴ Epenetos, A. Trojantec: Targeting Cancer Stem Cells, presented at the JP Morgan Conference January, 2009.

TARGETING CANCER STEM CELLS

CSCs are noted for their resistance to both chemotherapy and radiation. Indeed, this trait has even been used to isolate CSC populations from some tumors. The mechanisms behind the resistance differ. Radiation resistance is attributable to increased DNA repair, while resistance to chemotherapy is frequently related to accelerated drug transport and to drug metabolism. Another factor involved in sensitivity to some chemotherapeutic agents is that they are cell cycle dependent, which means that quiescent CSCs are unlikely to be affected by this type of intervention.

The development of drugs that target CSCs must avoid damaging normal stem cells to be clinically useful, given the normal cells' importance to tissue maintenance and repair. This isn't a new challenge for the pharmaceutical industry, though, as most oncology drugs are toxic to normal cells. Fortunately, a number of opportunities are available for targeting CSCs pharmacologically, as presented in Table 2.

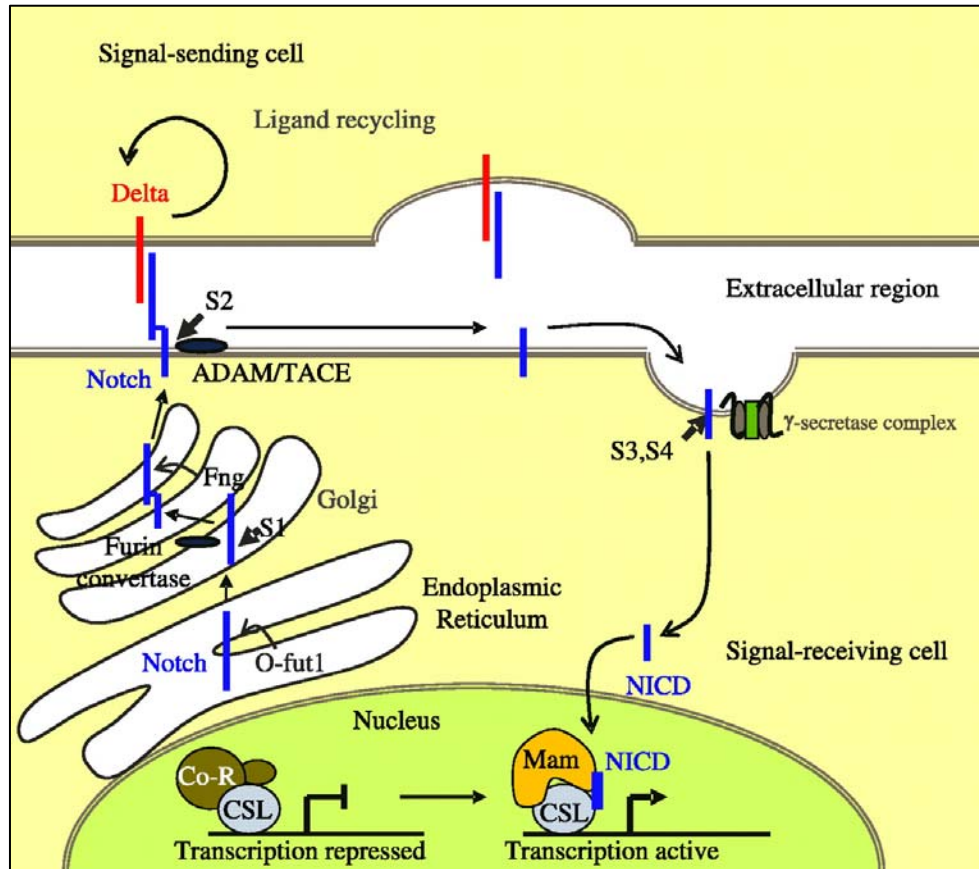
Table 2. New Drugs Targeting Cancer Stem Cells

Molecule	Company	Stage of Development	Indications
Notch (γ-secretase inhibitors)			
MK-0752	Merck	Phase I/II	Glioblastoma, medulloblastoma, breast, solid tumors
R4733	Roche	Phase I/II	Solid tumors
Notch (inhibitor of activation)			
TR-4	Trojantec	Preclinical	Breast, colon, pancreatic, cervical, prostate, SCLC, neuroblastoma
PI3K (kinase inhibitors)			
CAL-101	Calistoga Pharmaceuticals	Phase I/II	Leukemia, lymphoma, multiple myeloma
XL-147	Exelixis	Phase I/II	Solid tumors, NSCLC, endometrial carcinoma, ovarian
GDC-0941	Genentech	Phase I	Solid tumors, metastatic breast, non-Hodgkin's lymphoma
BGT-226	Novartis	Phase I/II	Breast, solid tumors
BEZ-235	Novartis	Phase I/II	Breast, CNS, colorectal, gastrointestinal, lung, lymphoma, melanoma
PX-866	Oncothyreon	Phase I	Solid tumors
AKT (kinase inhibitors)			
Perifosine	Keryx Biopharmaceuticals	Phase I-III	Various solid tumors and hematopoietic malignancies
Archexin	Rexahn Pharmaceuticals	Phase II/III	Renal, renal cell carcinoma, glioblastoma, ovarian, stomach, pancreatic
Immunotherapy			
IMUC-121	ImmunoCellular Therapeutics	Preclinical	Glioblastoma
IMUC-109/037	ImmunoCellular Therapeutics	Preclinical	Pancreatic, breast, colon
IMUC-69	ImmunoCellular Therapeutics	Preclinical	Ovarian cancer, multiple myeloma
Diagnostic/therapeutic	MabCure	Preclinical	Ovarian, prostate
OMP-21M18	OncoMed Pharmaceuticals	Phase I	Solid tumors
Molecular Chaperones			
CUDC-305	Curis/Debiopharm Group	Preclinical	Solid and hematological cancers
XL888	Exelixis	Phase I/II	Solid tumors
MPC-3100	Myriad Pharmaceuticals	Phase I/II	Refractory cancer
SNX-5422	Pfizer	Phase I/II	Solid tumors, lymphomas
STA-9090	Synta Pharmaceuticals	Phase I/II	Leukemia
TR-1	Trojantec	Preclinical	Ovarian, colon
Hedgehog (pathway inhibitors)			
BMS-863923	BMS/Exelixis	Phase I	Skin, solid tumors
GDC-0449	Genentech/Curis	Phase I/II	Skin, colorectal, ovarian, medulloblastoma, solid tumors
IPI-926	Infinity	Phase I	Solid tumors

NOTCH PATHWAY

Notch signaling is an evolutionarily conserved local signaling method that regulates cell fate during development and in the adult, and plays an important role in oncogenesis. There are four Notch receptors that bind with ligands (dubbed Delta and Jagged ligands) on an adjacent cell's surface. As shown in Figure 3, this interaction results in proteolytic cleavages, one of which is mediated by the enzyme γ -secretase. An intracellular portion, the Notch intracellular domain (NICD), is thus released, whereupon it translocates to the nucleus to effect DNA transcription. As such, Notch is both a transmembrane receptor and a transcription factor.

Figure 3. The Basic Notch Signaling Pathway



Source: Fiuza, UM and Arias, AM.¹⁵

Given the “up-close and personal” nature of Notch signaling, it isn’t surprising that this pathway is involved in the proper spacing of cells during embryogenesis.¹⁶ Other cell processes in which Notch signaling plays a role are differentiation, proliferation, adhesion, migration, and angiogenesis. For instance, it determines the fate of stem cells during formation of the nervous system and is essential in maintaining melanocyte stem cells in adults.^{15,17}

Notch & Cancer: Notch involvement in cancer depends upon its role in normal cells of that same tissue.¹⁵ For instance, if Notch acts as a gatekeeper of stem cells or regulator of precursor cell fate under normal conditions, it acts as an oncogene in promoting malignant growth. On the other hand, in tissues in

¹⁵ Fiuza, UM and Arias, AM. Cell and molecular biology of notch. J Endocrinol 2007; 194(3): 459.

¹⁶ Bolos, V, et al. Notch signaling in development and cancer. Endocrine Rev 2007; 28: 339.

¹⁷ Moriyama, M, et al. Notch signaling via *Hes1* transcription factor maintains survival of melanoblasts and melanocyte stem cells. J Cell Biol 2006; 173(3): 333.

which Notch initiates terminal differentiation, it has tumor suppressor activity. Among the tissues in which Notch acts as an oncogene are: T cell malignancies (e.g., acute lymphoblastic leukemia/lymphoma) and some B cell malignancies, breast cancer (dependent on Notch 1 and Jagged-1 interaction); gastrointestinal cancer; lung cancer; melanocyte-derived carcinoma, in which it enables primary melanoma cells to gain metastatic capability; cervical cancer (Notch 1 signaling in early-stage disease), and prostate cancer, in which Notch signaling facilitates the epithelial-mesenchymal cell transition, leading to cells with greater migratory capabilities.

Notch-Targeting Drugs: A number of compounds have been tested for their ability to inhibit γ -secretase, but currently only two are in clinical trials.¹⁸ Merck is testing MK-0752 in several early human studies against various solid tumors, including two brain cancers, glioblastoma and medulloblastoma. Roche's R4733 is in a single Phase I/II trial involving patients with solid tumors. Among the cancers that have shown sensitivity to γ -secretase inhibitors are lung cancer,¹⁹ Kaposi's sarcoma,²⁰ multiple myeloma,²¹ and T cell acute lymphoblastic leukemia.²²

An alternative approach that is under development at Trojantec inhibits the Notch pathway within the nucleus at the point of transcription. Specifically, the drug combines a proprietary cell-penetrating peptide, an antennapedia, with a truncated version of Mastermind that behaves in a dominant negative fashion to inhibit Notch signaling. (This target is depicted in Figure 3 where NICD is interacting with Mam (Mastermind) and CSL transcription factor in activating DNA transcription.) The cell-penetrating peptide has been shown to carry the inhibitor to cells throughout the tumor.¹⁴ The drug may prove useful against tumors that over-express Notch signaling components: T-cell acute lymphoblastic leukemia, basal cell carcinoma, and neuroblastoma, as well as pancreatic, small cell lung, breast, cervical, and prostate cancers.

PI3K/AKT PATHWAY

Several drugs targeting this signaling pathway are under various stages of development in the pharmaceutical industry. The attention is well deserved, because of the central role that one of the three classes of phosphoinositol-3 kinases (PI3K) plays in proliferation, survival, growth, and glucose homeostasis.²³ The key components of the PI3K/Akt pathway, which are presented in Figure 4, include the class I PI3K, an enzyme that is recruited by activated membrane receptors to convert an extracellular message into an intracellular message (phosphoinositol triphosphate, or PIP₃). This activity is counterbalanced by a phosphatase (PTEN) that attenuates the signal to turn off the signal. The next participant is Akt, which is protein kinase that is activated by PIP₃. This enzyme acts on various substrates, including the transcription factor FOXO and a protein designated as TSC2. Phosphorylation of FOXO prevents transcription of genes that regulate the cell cycle and apoptosis, while phosphorylation of TSC2 disrupts cell cycle regulation. Akt also inhibits apoptosis directly by acting on another protein, BAD; down-regulates expression of the tumor suppressor p53 via MDM2; and disrupts another regulatory pathway controlling apoptosis, one mediated by nuclear factor κ B (NF- κ B).

¹⁸ Based on searches performed on www.clinicaltrials.gov in early September 2009.

¹⁹ Konishi, J, et al. γ -Secretase inhibitor prevents notch3 activation and reduces proliferation in human lung cancers. *Cancer Res* 2007; 67(17): 8051.

²⁰ Curry, CL, et al. γ -Secretase inhibitor blocks Notch activation and induces apoptosis in Kaposi's sarcoma tumor cells. *Oncogene* 2005; 24: 6333.

²¹ Nefedova, Y, et al. Inhibition of Notch signaling induces apoptosis of myeloma cells and enhances sensitivity to chemotherapy. *Blood* 2008; 111(4): 2220.

²² Lewis, HD, et al. Apoptosis in T cell acute lymphoblastic leukemia cells after cell cycle arrest induced by pharmacological inhibition of notch signaling. *Chem Biol* 2007; 14: 209.

²³ Chalhoub, N and Baker, SJ. PTEN and the PI3-kinase pathway in cancer. *Ann Rev Pathol* 2009; 4: 127.

IMMUNOTHERAPIES

CSCs display molecules on their surfaces that support cellular communication, adhesion, nutrient uptake, and other biological processes. These may differ in quantity and quality from those found on normal cells of the same original tissue, and as such, they provide an opportunity to target CSCs immunologically. The surface antigens that are being targeted by the five drugs listed in Table 2 typify the research being conducted:

- **ImmunoCellular Therapeutics** is utilizing two approaches to recruiting the immune system in the fight against cancer. Its drug ICT-121 is an example of active immunization, which involves administering an off-the-shelf antigen to stimulate the immune system to recognize the antigen as foreign. In this case, the antigen is a nine amino acid epitope of CD133, which is found on many different types of CSCs, as shown in Table 1. ImmunoCellular has a patent on this molecule and on its use as a vaccine. Clinical development of ICT-121 is scheduled to commence early next year, initially targeting glioblastoma. A second clinical path will follow, probably with pancreatic cancer as the next indication. In both instances, ICT-121 is likely to be used in combination with other anticancer agents.

The company is also pursuing a passive immunization approach to combating cancer. This involves administering a monoclonal antibody preparation to identify the CSCs for subsequent destruction by the immune system. Two programs in this area are ongoing, one of which, ICT-69 was recently outlicensed to Roche in a deal that could generate \$32 million in milestone payments. This antibody has shown high affinity and specificity for multiple myeloma and ovarian cancer cells in preclinical tests and may be used for both diagnostic and therapeutic purposes. The other program targets two cell adhesion molecules, CEACAM5 and 6, that are considered tumorigenic, based on their expression by precancerous cells of the colon and breast. Indeed, CEACAM5 has a central role in the formation of liver metastases by colon cancer, and CEACAM6 is associated with invasive forms of breast and pancreatic cancer. ImmunoCellular's two antibodies in this program are ICT-109 and ICT-37.

- **OncoMed Pharmaceuticals'** lead drug candidate is OMP-21M18, an antibody that blocks DLL4 signaling. (DLL4 is a Delta ligand for the Notch receptor, shown in Figure 3.) A preclinical study has demonstrated that the antibody reduces cancer stem cell frequency in colon and breast tumors when administered as a monotherapy or in combination with other chemotherapeutic agents.²⁸ It also significantly delays tumor recurrence and disrupts tumor angiogenesis. OMP-21M18, which is currently in a Phase I clinical trial involving patients with advanced solid tumors, is part of a \$1.4 billion Notch collaboration between OncoMed and GlaxoSmithKline that was signed in December 2007.
- **MabCure** is developing antibodies against specific malignancies, but it is operating on the belief that the optimal antigens for therapeutic purposes are not those that are easily recognized by the immune system, but rather are the least antigenic. This is based on the observation that the immune system typically destroys aberrant cells that it can detect, and so, for an early CSC to survive, it must escape detection. Later, when the tumor has expanded sufficiently, its growth is too rapid for the immune system to target effectively. Based on this hypothesis, the company has developed proprietary techniques for stabilizing the surfaces of cells to preserve subtle features of even weak antigens, and it has optimized its technique for generating monoclonal antibodies. Thus far, the company has prepared antibodies against ovarian and prostate cancers, as well as melanoma. MabCure's business plan is to commercialize diagnostic tests for ovarian and prostate cancer first, initially in Europe and then in the United States, while it prepares antibodies against other malignancies such as breast, colon, and lung cancers. The results of small trials of the antibodies have been promising, as ovarian cancer was detected correctly in 13/13 patients' blood and in 6 patients who were considered

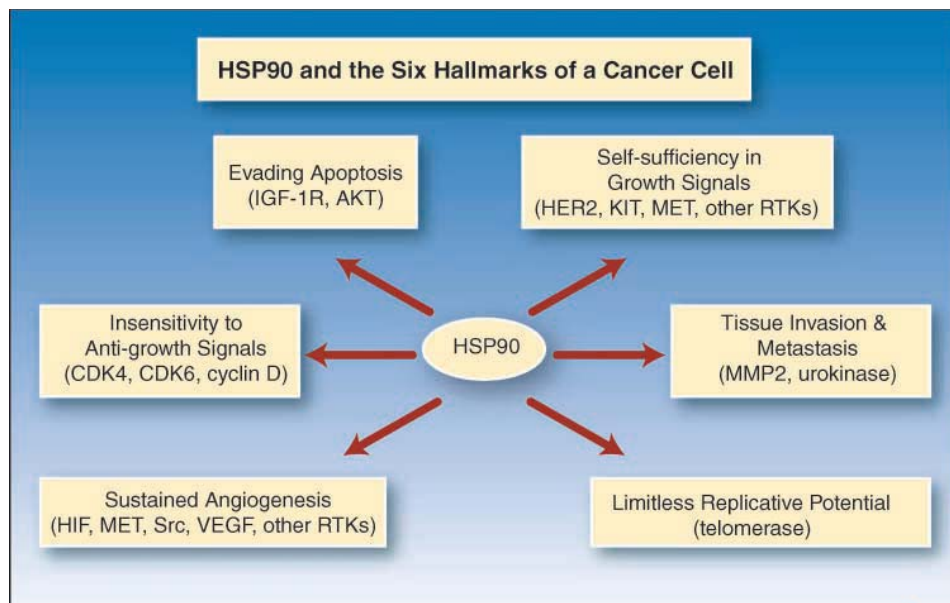
²⁸ Hoey, T, et al. DLL4 blockade inhibits tumor growth and reduces tumor-initiating cell frequency. *Cell Stem Cell* 2009; 5(2): 168.

in clinical remission, but later suffered a relapse.²⁹ Similar results were obtained with immunohistochemical analyses of metastatic melanoma (30/30 correct). In another study, the anti-melanoma antibody identified primary (10/10) and metastatic (10/10) ocular melanomas, indicating that a single antibody can recognize both early and advanced stages of the disease.

MOLECULAR CHAPERONES

Molecular chaperones, also known as heat shock proteins (Hsps), are integral to the production of new proteins, protection of existing proteins from environmental stress and degradation, and the identification of proteins that are beyond repair. Among the chaperones is Hsp90, which normally functions to allow for some variation in protein structures, thereby permitting genetic diversity (which may be important from an evolutionary viewpoint). Hsp90 is also directly involved in cell motility during development, as it is found on the surface of neurons where it serves as an essential component supporting cell migration. But it has also been implicated in oncogenesis, as it is overexpressed in many tumors, thereby protecting them from chemotherapy and radiation.³⁰ Its importance to malignant cells is apparent from its client proteins, some of which are provided in Figure 5. Many of these proteins, which normally rely on Hsp90 for stabilization, support the six characteristics of cancer cells. As a result, Hsp90 is a potentially intriguing target for therapeutic intervention.

Figure 5. HSP 90 Sustains the Cancer Cell



Source: Xu, W and Neckers, L.³⁰

Drugs targeting Hsp90: Potential therapeutic applications of drugs that inhibit Hsp90 function are varied as the six hallmarks of a cancer cell and the molecular contributors to those traits. For instance, KIT has been implicated in gastrointestinal stromal tumors; IGF1R (insulin-like growth factor 1 receptor), in non-small cell lung cancer; cyclin D, in esophageal tumors; and telomerase, in cervical cancer.

Five drugs listed in Table 2 are small molecules that inhibit Hsp90 binding with its client proteins, largely by interfering with the binding of ATP. If they prove successful in clinical trials, they may have use as monotherapies. However, they will almost certainly be used in combination with other chemotherapeutic agents and with radiotherapy, since blocking Hsp90 should render the malignant cells more sensitive to these types of anticancer treatments. Recent preclinical research on Curis's CUDC-305 attests to the

²⁹ Personal communication from MabCure.

³⁰ Xu, W and Neckers, L. Targeting the molecular chaperone heat shock protein 90 provides a multifaceted effect on diverse cell signaling pathways of cancer cells. Clin Cancer Res 2007; 13(6): 1625.

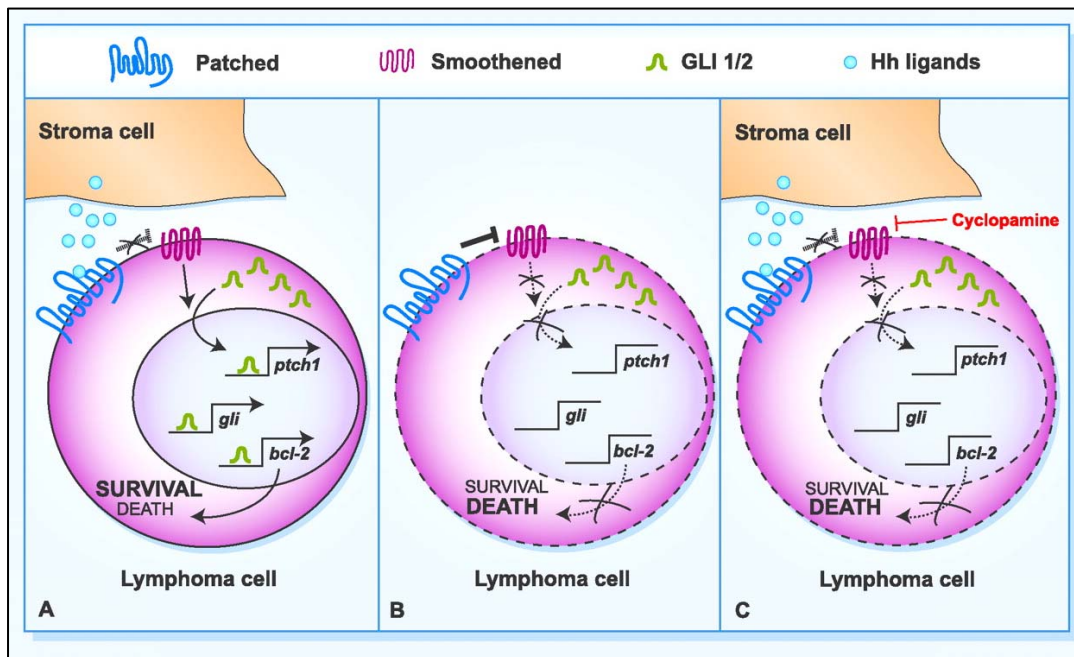
broad impact of inhibiting Hsp90 by lowering expression of oncoproteins and members of the PI3K/Akt and MAPK pathways, and increasing the expression of members of the apoptotic pathway.³¹ Moreover, the drug caused tumor regression in mouse models of glioblastoma, non-small cell lung, breast, and gastric cancers.

Molecular chaperone therapy: Rather than inhibiting a pro-oncogenic Hsp, Trojantec has created a medicine that tilts the balance between survival and apoptosis by increasing intracellular concentrations of the Hsp derived from the p21 gene. Like TR4, which was discussed in the Notch pathway section, this fusion protein uses a cell-penetrating peptide to deliver the active moiety. In animals implanted with colorectal and ovarian cancer xenografts, TR1 significantly reduced the tumor burden, when it was administered alone and in combination with chemotherapeutic agents.¹⁴ Moreover, the effects of this novel drug had no toxic side effects and displayed no immunogenicity.

HEDGEHOG PATHWAY

Hedgehog signaling provides another intercellular mechanism of regulation, one that serves essential functions in the normal proliferation and differentiation of stem cells during embryonic development. It also provides regulatory oversight in controlling stem cells in the adult. As shown in the illustration in Figure 6 of hedgehog signaling with a lymphoma cell, the pathway includes some components with exotic names, but the communication process isn't that different from other ligand-receptor interactions: In mammals, there are three types of hedgehog ligands that can interact with the Patched receptor. This results in a depression of Smoothened and the subsequent activation three transcription factors, GLI 1, 2, and 3. Mutations in this pathway figure in tumorigenesis, as exemplified by Gorlin syndrome that can lead to basal cell carcinoma, medulloblastoma, and other malignancies. Treatment with the experimental compound cyclopamine interrupts hedgehog signaling by preventing the depression of Smoothened and activation of GLI transcription factors – a condition that favors apoptosis. Other mutations that activate this CSC maintenance pathway involve loss of repressors and overexpression of factors that stimulate it.

Figure 6. Basic Components in the Hedgehog Pathway



Source: Lindemann, RK.³²

³¹ Bao, R. Antitumor activity of CUDC-305, a novel oral Hsp90 inhibitor, in solid and hematological tumor xenograft models. Presented at the annual meeting of the American Academy of Cancer Research, April 19, 2009.

³² Lindemann, RK. Stroma-initiated hedgehog signaling takes center stage in B-cell lymphoma. Cancer Res 2008; 68(4): 961.

Drugs Targeting the Hedgehog Pathway: Three drugs are listed in Table 2 that interfere with hedgehog signaling and are in clinical trials with cancer patients. Two of the compounds, IPI-926 and GDC-0449, are derivatives of cyclopamine, which has been studied extensively in preclinical cancer models. In fact, cyclopamine is a drug that Champions Biotechnology studied with its Biomerk Tumorgraft™ model, demonstrating that the drug works well in combination with gemcitabine, because it targets CSCs in pancreatic tumors, while gemcitabine kills proliferating cells.³³ This is consistent with an observation that cyclopamine renders esophageal carcinoma cells more sensitive to radiation and to chemotherapy.³⁴ Activation of this pathway is known to increase drug efflux from cells by increasing transporter proteins and to alter cell-cycle checkpoint and DNA repair systems.^{35,36}

COMPANIES MENTIONED IN THIS REPORT

Company	Ticker	Market Cap #
Bristol-Myers Squibb	BMY	\$44.2 B
Calistoga Pharmaceuticals	private	
Champions Biotechnology	CSBR.OB	\$20.2
Curis	CRIS	\$148
Exelixis	EXEL	\$685
Genentech	private	
ImmunoCellular Therapeutics	IMUC	\$14.7
Infinity Pharmaceuticals	INFI	\$181
Keryx Biopharmaceuticals	KERX	\$63.6
MabCure	MBCI.OB	\$623.4
Merck & Company	MRK	\$68.6 B
Myriad Pharmaceuticals	MYRX	\$116.0
Novartis	NVS	\$108 B
OncoMed Pharmaceuticals	private	
Oncothyreon	ONTY	\$148
Pfizer	PFE	\$110 B
Rexahn Pharmaceuticals	RNN	\$51.7
Roche Holdings	RHHBY	\$137 B
Synta Pharmaceuticals	SNTA	\$95.1
Trojantec	private	

Market capitalizations are in millions, except for those designated with "B" for billions. Market data is based on closing prices as of September 11, 2009.

³³ Jimeno, A, et al. A direct pancreatic cancer xenograft model as a platform for cancer stem cell therapeutic development. *Mol Cancer Ther* 2009; 8(2): 310.

³⁴ Chen, YJ, et al. Targeting the hedgehog pathway to mitigate treatment resistance. *Cell Cycle* 2007; 6(15): 1826.

³⁵ Sims-Mourtada, J, et al. Sonic hedgehog promotes multiple drug resistance by regulation of drug transport. *Oncogene* 2007; 26(38): 5674.

³⁶ Leonard, JM, et al. Sonic hedgehog signaling impairs ionizing radiation-induced checkpoint activation and induces genomic instability. *J Cell Biol* 2008; 183(3): 385.

DISCLOSURES

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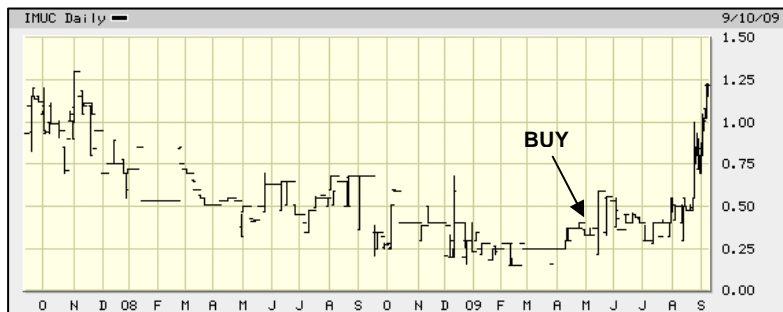
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2-YEAR PRICE CHART FOR IMMUNOCELLULAR THERAPEUTICS:

Source: BigCharts.com

4/27/2009 – Initiating Coverage: share price: \$0.37; rating: BUY; 12-month price target: \$2.50.

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