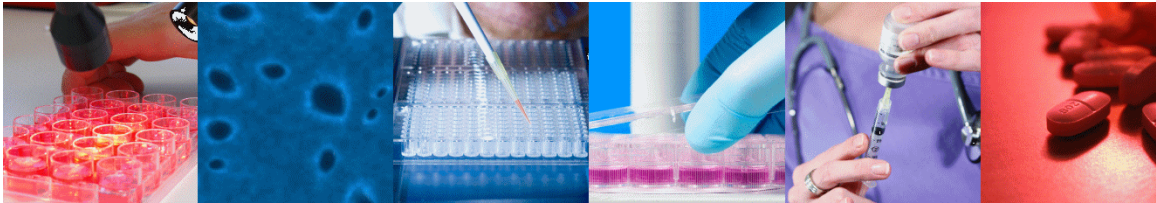


Cancer Stem Cells



Emerging Therapeutic, Diagnostic
and Market Opportunities (2008)

By John Bates PhD

About the Author

John Bates is an experienced consultant and writer in the cancer field. After a first degree in biochemistry and chemistry (1978) and a PhD in cancer research in 1981, John joined Upjohn Ltd, managing a team of research scientists, later taking a similar role with Glaxo Group Research. In 1989 he co-founded the contract pharmaceutical research company Melbourn Scientific Ltd, establishing the company's technical and commercial activities. In 2000 John joined ANGLE Technology Ltd, providing due diligence on new pharmaceutical technologies, later joining Acumen Bioscience (drug discovery instrumentation) as Technical Director. He has provided board level management to companies including Cizzle Biotechnology (lung cancer), Pro-Cure Therapeutics (cancer stem cells), Membrasense (sensor technology) and consultancy services to pharmaceutical companies, life science investors and university enterprise departments.

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Overview

A substantial body of evidence indicates that tumours contain a unique subset of cancer cells that drive the development of cancer. These cells, Cancer Stem Cells (CSCs), have been identified in leukaemia, myeloma, breast, prostate, pancreas, colon, brain and lung cancers and are believed to be responsible for the development and metastatic spread of this disease. If these findings are now confirmed in the clinic, the selective targeting of CSCs will offer a new paradigm in cancer therapeutics and diagnostics. Currently there are more than 30 CSC R&D programmes in progress, around 50% of which are at Phase I or beyond. Patient data from the first clinical trials of drugs believed to target CSCs, are now being reported. Most CSC R&D programmes are being taken forward by SME's and > 90% of the 70 patents in this area (which more than doubled in 2007) have been filed by Universities. Substantial opportunity for collaboration in this field has recently led to agreements between SMEs and major pharmaceutical companies. This includes a recent announcement of a collaboration between GSK and Oncomed Pharmaceuticals, Inc.

Drug Discovery and Pipeline

More than ten different strategies are being actively researched to enable the selective therapeutic targeting of CSCs, which are described in this report. In December 2007, clinical data were reported on GSK's Tykerb (which is already approved in the US for breast cancer), that targets the CD44+/CD24+ population of breast cancer cells i.e. breast CSCs. ChemGenix has also recently published clinical data from a Phase II/III CML clinical trial of omacetaxine mepesuccinate, which is believed to target CSCs. Stemline Therapeutics have reported that their investigational CSC-targeting molecule SL-401, has demonstrated single agent anti-tumour activity in acute myeloid leukaemia in a multi-centre Phase I/II dose escalation study. Currently around 30 companies or commercially based research organisations are progressing R&D activities in the CSC field, 65% of which are SME's. This report identifies five "Top-25" pharmaceutical companies that are

progressing developments relating to CSCs. Of the current 30 R&D programmes, around 50% are at Phase I or beyond. The availability of isolated CSCs to drug discovery companies offers opportunities to identify new druggable targets and to re-examine existing drug libraries that have not been screened against this unique subset of cells, or xenograft models developed from them.

Cancer Diagnostics

CSCs are believed to be causally linked to the development and metastatic spread of cancer. If this is confirmed in man, this will likely place CSCs at the heart of cancer diagnostics. Researchers have identified a number of surface proteins such as CD44, CD133 and many others, which may have important application as biomarkers and diagnostics. Some of these surface proteins are found on a number of different CSCs, whereas others appear to be unique to certain CSCs. A number of intracellular markers found in CSCs, may also have diagnostic utility. These developments are described in this report.

CD133 mRNA levels in peripheral blood, measured using RT-PCR, have been found to predict colon cancer recurrence. There is a need for new methodologies that isolate and characterise circulating tumour cells (CTCs) in the blood, that can be applied to CSCs. CTC technologies using the EpCam marker to isolate these cells are able to predict colon cancer recurrence. The adaption of these techniques based on specific CSC phenotypes may provide sensitive new methods for identifying CSCs in the body. Innovation in advanced microfluidic, chip-based and genetic/phenotypic screening technologies are anticipated in the future. OncoMed Pharmaceuticals have announced the discovery of a gene expression profile of CSCs (breast and other cancers), that correlate with clinical outcome.

This Report

This report gives a comprehensive up-to-date review of global R&D on CSCs and strategies to target them. This includes around 30 companies or commercially based

research organisations (including 20 SMEs and five major pharmaceutical companies) that are progressing drug discovery activities, including drug pipeline (pre-clinical to Phase III), discovery strategy, candidate molecules, drug targets, current clinical trials and related areas. Also covered are current developments on the detection of CSCs and new diagnostic approaches. Commercial opportunities in drug discovery and diagnostics relating to CSCs are also presented. More than 50 academic research teams from 13 different countries are reviewed, together with leading discoveries in this field. Current patents referring to CSCs (70+ by Dec 2007) are also presented.

From the Report

Table 3.1 Development pipeline of CSC-targeting candidate drug molecules

Company	Molecule	Cancer	Research	Phase I	Phase II	Phase III/IV
		Breast				→
				→		
		Myeloma		→		
			→			
		AML		→		
		AML		→		
		Breast	→			
			→			
			→			
				→		
			→			
		Prostate, Breast	→			
		AML	→			
			→			
				→		
				→		
		Brain		→		
				→		
		CSC Research	→			
			→			
			→			
		Prostate	→			
		Leukaemia and Breast Cancer	→			
			→			
		Leukaemia		→		
		CML			→	
				→		
		Glioblastoma		→		
		metastasis		→		

3.1 Thinking Differently

The proliferative nature of cancer has dominated scientists' thinking on this disease. Most conventional cancer drugs target the replicative machinery of cancer cells and success is measured by how effectively these processes are blocked. Clinicians commonly monitor therapeutic response by measuring tumour size before, during and after chemotherapy and tumour shrinkage is used as a measure of success.

This model does not apply in the case of CSCs, as they represent a small proportion of the cells within a tumour. Destroying this population of cells may not produce significant changes in tumour size or volume. New methods therefore need to be developed to monitor the destruction of CSCs, alongside the bulk of normal (non-tumorigenic) cancer cells.

For example, in studies of the CSC-targeting GSK drug Tykerb[®] (a combined EGFR/HER2 tyrosine kinase inhibitor) in a breast cancer clinical trial^{3,1}, levels of tumorigenic CD44⁺/CD24^{-/low} breast cancer cells were monitored to measure pathologic response. This revealed that CSC levels fell from 10.6% to 4.7% (a drop of 56%) in this study and other tests showed reduced self-renewal capacity. This neoadjuvant trial (before surgery in this case) demonstrated an improved pathologic complete response (i.e. disappearance of all clinical evidence of the disease) using Tykerb[®], which may indicate how effectively the breast CSCs were targeted and destroyed.

CSCs are thought to drive the metastatic spread of cancer and several investigational drugs are in development which, it is hoped, will selectively target these cells. More research needs to be carried out to understand how CSCs emerge and their involvement in the development and progression of cancer. Methods are required to allow these cells to be detected and measured, and to help scientists establish the mechanisms that drive the migration of these cells in the body. It is hoped that these methods will enable early detection of cancer, provide guidance on therapeutic decision-making, allow the monitoring of therapeutic response and give information on patient prognosis.

In blood cancers such as leukaemia, ease of sample collection may allow a relatively simple measurement of CSC levels. This may not be the case with solid tumours, where the need for a conventional biopsy would be anticipated. Findings by Balic and co-workers^{3.2} suggest that CSCs enter the circulation and move to the other sites in the body early in the development of the disease. In these studies breast CSCs were identified in the bone marrow, potentially offering an alternative site to investigate the migration of CSCs in the body.

If CSCs do migrate from tumours early in their development, this will make their detection all the more important. However, given the low numbers of these cells in tumours, their detection amongst many millions of other cells in the general circulation or in other tissues, presents a significant technical challenge. The further development of technologies that are currently used to detect and isolate CTCs (circulating tumour cells), adapted to measure circulating CSCs, is anticipated in the future.

3.2 Resistance to Therapy

There is evidence that CSCs are resistant to a number of conventional cancer therapies, including radiation treatment. This is believed to be due to several factors including increased activity of the ATP-binding cassette (ABC) drug transporters in these cells, their relative quiescence, increased levels of DNA repair and a lowered tendency to enter apoptosis due to higher expression levels of anti-apoptotic proteins^{3.3}.

Active drug efflux transporters are a family of proteins that have a direct effect on the behaviour of drugs and affect parameters such as bioavailability and excretion. These proteins also affect the penetration of drugs into tissues. The quiescence of CSCs may protect them from drugs that target the mechanisms of cell division (e.g. DNA replication). Scientists also report the discovery of a "shuttling protein" which may explain the resistance of CSC to radiation treatment^{3.4}.

Ma and co-workers^{3,5} recently reported the identification of CSCs in hepatocellular carcinoma (HCC), characterised by their CD133 phenotype. The sensitivity of these cells to the two chemotherapeutic agents doxorubicin (DNA-interacting drugs) and fluorouracil (inhibition of thymidylate synthase) was examined and it was found that CD133(+) HCC cells contribute to chemoresistance through preferential activation of Akt/PKB and Bcl-2 cell survival response. These researchers suggested that the targeting of this survival-signalling pathway might provide a novel therapeutic model for the disease.

In other studies, Kang and Kang^{3,6} studied drug resistance in Glioblastoma Multiforme (GBM), the most common brain cancer. These studies were based on the use of a "dissociated cell system" of human GBM cells, A172 and established GBM2 cells, that show resistance to 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). After exposure to a lethal dose of BCNU, a small number of surviving GBM cancer cells were reported to contain subpopulations of stem-like cells that expressed CD133, CD117, CD90, CD71, and CD45 cell-surface antigens. These cells were also found to show multipotency and to initiate new tumours when transplanted into the brain of SCID mice. These surface antigens may also provide drug-targeting opportunities.

Studies by Jeremy Rich^{3,7} at the Duke University Medical Centre have shown that CSCs derived from human glioblastoma surgical specimens and xenografts are radiation resistant. This was thought to be due to increased activation of the DNA damage checkpoint. These same sub-populations were found to promote tumour angiogenesis through increased expression of vascular endothelial growth factor. Rich suggested that hypoxia and stem cell maintenance pathways may provide therapeutic targets to sensitise cancer stem cells to cytotoxic therapies.

3.5 Telomerase

The enzyme Telomerase is a reverse transcriptase, which directs telomere synthesis by adding specific sequences of DNA to the end of chromosomes. This enzyme is believed to be key to the immortality of stem cells. Telomerase activity is found in embryonic stem cells in sufficient levels to maintain telomere length, but shows

little or no activity in normal stem cells^{3.11}. Telomerase activity is not found in normal somatic cells, but it is found in cancer cells, where it is able to maintain replicative immortality. Telomerase is now regarded as a universal marker in cancer cells^{3.12}.

Geron Corporation is now carrying out clinical trials of two different types of telomerase inhibitor, a vaccine (GRNVAC1) and a lipidated drug (GRN163L). Geron reports that GRN 163L kills CSCs and the outcome of current clinical trials of this molecule are awaited with interest.

3.7 Hedgehog and Wnt

Hedgehog signalling is an important regulator in animal development and involves the intracellular signalling polypeptide ligand, Hedgehog (Hh). This protein is involved in adult stem cell signalling and in tissue regeneration. Activation of the Hedgehog pathway has been linked to the development of cancers^{3.15} and a number of companies are developing molecules that target the Hedgehog pathway, including Infinity Pharmaceuticals, Curis Inc and Genentech.

Peacock and co-workers^{3.16} have shown that a subset of multiple myeloma cells which show Hh (Hedgehog) pathway activity are concentrated within the tumour stem cell compartment. This group reports that the Hh ligand promotes expansion of this compartment, whereas blockade reduces it.

Research by Philip Beachy and colleagues^{3.17} has shown high levels of Hh in prostate cancer cells and these data were linked with the state of the cancer, namely whether it was metastatic, and therefore likely to spread, or benign and likely to remain in the prostate. Hh levels were found to be much lower in samples taken during radical prostatectomy, compared with samples taken from secondary tumours. Beachy's group have also reported that the drug Cyclopamine blocks the Hedgehog pathway and produces regression of grafted human tumours in animal models. These findings may offer clinicians the possibility of using Hedgehog levels in cells as a predictor of the metastatic stage of the cancer.

Cyclopamine (11-deoxojervine) is a naturally occurring alkaloid and teratogen found in the corn lily. This molecule inhibits the Hedgehog signalling pathway through its effects on the levels of active and inactive Smoothed protein. Researchers at the Laboratory of Genomic Diversity, NCI-Frederick are investigating how molecules such as Cyclopamine might be involved in the control or the self-renewal of CSCs.

Bara and coworkers^{3.18} recently reported on the study of Hedgehog signalling in brain cancer. These scientists were interested to discover whether Hedgehog blockade could target the stem-like population in glioblastoma multiforme. Hedgehog pathway blockade using Cyclopamine caused a 40–60% reduction in growth of adherent glioma with confirmed Hedgehog pathway activity but not in those lacking evidence of pathway activity. These and other studies were interpreted as showing depletion of clonogenic cancer stem cells. It was also found that GBM cells injected intracranially following Hedgehog blockade no longer gave rise to tumours in athymic mice, suggesting a loss of the CSC population.

Wnt proteins are a group of highly conserved signalling molecules involved in the regulation of cell interactions during embryogenesis and are also linked to cancer. Van de Wetering and co-workers^{3.19} have reported that by switching off the Wnt signalling pathway, which is active in colorectal cancer cells, it is possible to bring about the differentiation of colon cancer cells into non-proliferative cells, indicating that Wnt could be an important signal in the control of colon cancer.

3.13 Drug Pipeline

OncoMed Pharmaceuticals

OncoMed Pharmaceuticals, Inc. (www.oncomed.com), a discovery-based company, was established in 2004 by Michael Clarke and Max Wicha, both leading researchers in the CSC field. The company is developing antibodies that target critical CSC pathways. In December 2007, Oncomed announced a worldwide strategic alliance with GSK, to develop antibody therapeutics that target CSCs. Under this agreement,

GSK has an option to license four candidates against multiple CSC targets from OncoMed's antibody library.

OncoMed report that they will receive an undisclosed initial payment, equity investment and milestone payments up to \$1.4 billion. OncoMed have said that they will receive double-digit royalties on all collaboration product sales. This alliance also includes OncoMed's lead antibody, OMP-21M18, scheduled to enter the clinic in 2008 (source: Oncomed website press release, 2007).

Research being carried out by Oncomed also has application in cancer diagnostics and in January 2007^{3,28} the company announced the discovery of a novel gene expression signature associated with breast cancer stem cells. This gene profile showed strong correlation with patient prognosis.

Geron Corporation

The enzyme Telomerase is expressed in a number of malignant tumours, is essential for malignant cell growth and is absent or transiently expressed at low levels in most normal adult tissues. Geron's developmental drug GRN163L is a short chain oligonucleotide and an inhibitor of this enzyme. This drug candidate possesses a 5' lipid chain, designed to increase its lipophilicity and tissue penetrating power. GRN163L has demonstrated anti-tumour effects in a wide range of haematological and solid tumour models and is reported to target CSCs.

In November 2007 Geron Corporation (www.geron.com) announced that it enrolled its first patient in a Phase I/II clinical trial of GRN163L in multiple myeloma, published by the company under the heading "Human trial with GRN163L follows preclinical data showing single-agent activity against multiple myeloma cancer stem cells and cancerous plasma cells".

The main objective of this study is to determine the safety and maximum tolerated dose (MTD) of GRN163L, when given intravenously to multiple myeloma patients.

GRN163L used as a single agent and in combination with Velcade™ inhibits cutaneous and disseminated myeloma tumour growth *in vivo* (source: Geron Corporation press release, 2007).

In December 2007 Geron Corporation announced that Merck & Co, Inc. had filed an IND for a cancer vaccine candidate that targets telomerase. Merck is developing the vaccine under a 2005 licence agreement, that gives the company exclusive rights to develop and commercialise non-dendritic cell-based vaccines targeting telomerase. This milestone triggered a \$4 million milestone payment to Geron.

Geron also announced in December 2007 that it is enrolling patients with acute myelogenous leukaemia (AML) in a Phase I/II study for its own telomerase vaccine candidate, GRNVAC1, which delivers the telomerase antigen using autologous dendritic cells. The company is also developing a second-generation telomerase vaccine based on dendritic cells made from human embryonic stem cells (source: Geron Corporation press release).

GlaxoSmithKline

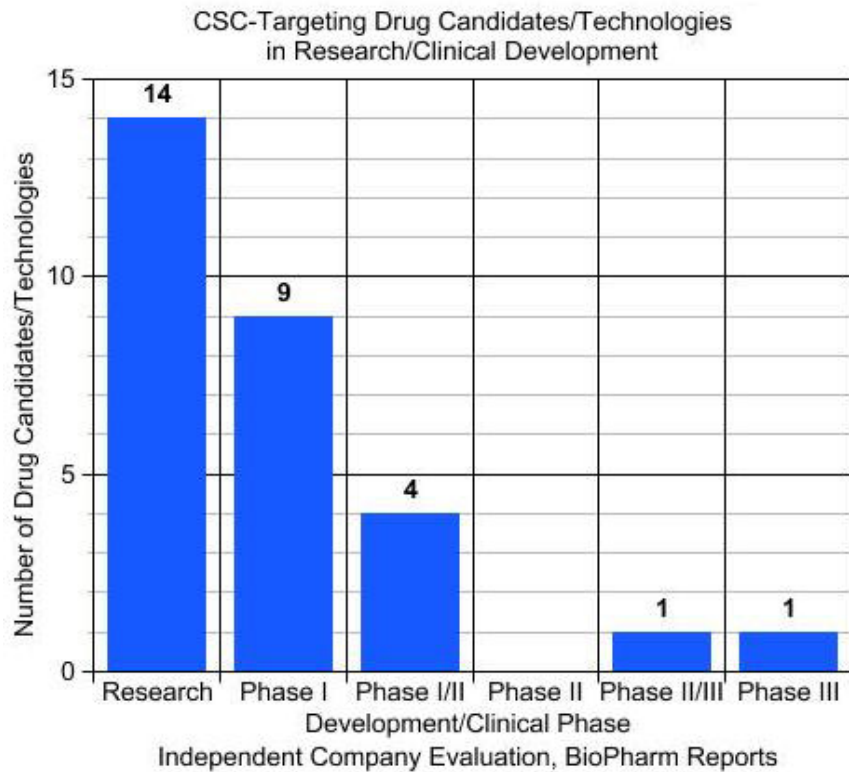
In December 2007 GlaxoSmithKline (www.gsk.com) and OncoMed Pharmaceuticals, Inc. announced that they had formed a strategic alliance to develop antibodies that target CSCs and this is summarised earlier in this chapter.

The GSK drug Tykerb® (lapatinib) is an inhibitor of the intracellular tyrosine kinase domains of both Epidermal Growth Factor Receptor (EGFR) and Epidermal Receptor Type 2 (HER-2) receptors. This drug is approved in the US in combination with Xeloda® (capecitabine) for the treatment of patients with advanced or metastatic breast cancer whose tumours overexpress HER2 and who have received prior therapy including an anthracycline, a taxane and Herceptin® (trastuzumab).

At the San Antonio Breast Cancer Symposium in Dec 2007^{3,32}, Li and colleagues reported on a clinical trial of Tykerb (lapatinib). In this study 30 patients with

advanced HER-2 over-expressing breast cancers received Tykerb[®] as an initial single agent for the first 6 weeks, followed by a combination of weekly trastuzumab and 3-weekly docetaxel for 12 weeks before primary surgery. Unlike results seen with chemotherapy, Tykerb[®] treatment decreased tumorigenic CD44⁺/CD24^{-/low} breast cancer cells from 10.6% to 4.7% (a fall of 56%) and also reduced self-renewal capacity. The pathologic complete response (i.e. disappearance of all clinical evidence of disease) rate after Tykerb[®] and trastuzumab/docetaxel was much higher than expected, at 63% (16/25). It is believed that the use of signalling inhibitors such as Tykerb[®] involved in CSC self-renewal offers a new strategy for the long-term eradication of cancer.

Figure 3.1 Development pipeline of candidate molecules that target Cancer Stem Cells



4.3 Circulating Tumour Cells

There is need for new methodologies that can isolate and characterise circulating tumour cells (CTCs) in the blood, as current methods are generally based on complex analytical approaches with poor yields and purity^{4,2}.

Circulating tumours cells (CTCs) have been shown to correlate with the spread of breast cancer from primary to metastatic disease and efforts are now focused on developing methods with adequate sensitivity and specificity to isolate and identify these rare cells. These developments are highly relevant to current efforts to identify CSCs and to extend the capability of these technologies in respect of CSC phenotypes, as a basis for understanding the mechanisms of metastasis, for use in drug discovery and to measure therapeutic response.

To date, methods used to isolate and characterise cells include flow cytometry, immunohistochemistry, immunofluorescent microscopy and others. These techniques, whilst important, also have limitations and in the future more sensitive and selective methods based on the protein expression or mRNA will be used. PCR and RT-PCR techniques have important application in these developing areas.

Other technology platforms are also being developed. For example, studies recently reported by Nagrath and co-workers describe methods for the isolation of rare circulating cancer cells using a microchip technology^{4,2}. This was used to isolate viable circulating tumour cells or CTCs in the blood of cancer patients. This is based on a microfluidic platform reported to be capable of separating viable circulating cancer cells from peripheral whole blood samples, based on their interaction with antibody (EpCAM)-coated microposts under controlled flow conditions, without the need for pre-labelling or processing of the samples.

This chip technology successfully identified rare viable cancer cells in the peripheral blood of patients with metastatic lung, prostate, pancreatic, breast and colon cancer in 115 of 116 (99%) samples, with a range of 5 to 1,281 cancer cells per ml, yielding a purity of approximately 50%. These cells were also reported to have been isolated in 7/7 patients with early-stage prostate cancer. Preliminary success is also

reported in the use of this technology in monitoring patient response to anti-cancer therapy. Leading companies in the CTS field include Immunicon Corporation and Biocept Inc.

Figure 6.1 Patents relating to Cancer Stem Cells, 1999–2007, by year (Source: Delphion)

