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*Review Article*

# Emerging drug discovery approaches for selective targeting of "precursor" metastatic breast cancer cells: highlights and perspectives

Moulay A Alaoui-Jamali, Krikor Bijian, Gerald Batist

*Lady Davis Institute for Medical Research and Segal Cancer Center, Jewish General Hospital, Departments of Medicine, Oncology, and Pharmacology and Therapeutics, McGill University, Montreal, Qc H3T 1E2, Canada.*

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**Abstract**: Breast cancer is a prevalent disease and a major cause of morbidity and cancer-related deaths among women worldwide. A significant number of patients at the time of primary diagnosis present metastatic disease, at least to locoregional lymph nodes, which results in somewhat unpredictable prognosis that often prompts adjuvant systemic therapies of various kinds. The time course of distant recurrence is also unpredictable with some patients sustaining a recurrence within months after diagnosis, even during adjuvant treatments, while others can experience recurrence years or decades after initial diagnosis. To date, clinically approved therapeutics yielded marginal benefits for patients with systemic metastatic breast disease, since despite high clinical responses to various therapies, the patients virtually always become resistant and tumor relapses. Molecular profiling studies established that breast cancer is highly heterogeneous and encompasses diverse histological and molecular subtypes with distinct biological and clinical implications in particular in relation to the incidence of progression to metastasis. The latter has been recognized to result from late genetic events during the multistep progression proposed by the dominant theory of carcinogenesis. However, there is evidence that the dissemination of primary cancer can also be initiated at a very early stage of cancer development, originating from rare cell variants, possibly cancer stem-like cells (CSC), with invasive potential. These precursor metastatic cancer cells with stem-like properties are defined by their ability to self-renew and to regenerate cell variants, which have high plasticity and intrinsic invasive properties required for dissemination and tropism toward specific organs. Equally relevant to the CSC hypothesis for metastasis formation is the epithelial-mesenchymal transition (EMT) process, which is critical for the acquisition of cancer cell invasive behavior and for selection/gain of CSC properties. These exciting concepts have led to the formulation of various approaches for targeting precursor metastatic cells, and these have taken on greater priority in therapeutic drug discovery research by both academia and pharmaceuticals. In this review, we focus on current efforts in medicinal chemistry to develop small molecules able to target precursor metastatic cells via interference with the CSC/EMT differentiation program, self-renewal, and survival. It is not meant to be comprehensive and the reader is referred to selected reviews that provide coverage of related basic aspects. Rather, emphasis is given to promising molecules with CSC/EMT signaling at the preclinical stage and in clinical trials that are paving the way to new generations of anti-metastasis drugs.

**Keywords:** Breast cancer, metastasis, cancer stem cells, EMT, experimental therapy

## Introduction

Most advanced cancers, including breast cancer (BCa) are increasingly highly treatable by a growing number of therapeutic agents. While clinical responses may be greater than 50%, metastatic carcinomas inevitably develop therapeutic resistance, and metastases are the ultimate cause of death for the patients. Molecular profiling studies of primary BCa reveal it to be a highly heterogeneous entity, which includes diverse histological and molecular subtypes with distinct biological behaviors and clinical implications, including prognosis, choice and predicted response to specific therapeutics, incidence of spread and recurrence [1, 2]. These include basal cell type, so-called 'triple negative', Her-2 driven, and also luminal cell-derived tumors that can be further divided into prognostic subgroups and predicted efficacy to

specific therapies (e.g. anti-hormonal, trastuzumab, etc.). Much remains to be done with respect to classifying and understanding the biological behavior, including response to particular therapies, for these patients.

Although currently used BCa biomarkers demonstrated great clinical values, none can fully predict the risk nor the clinical outcome of invasive disease. For instance, some breast cancer patients free of axillary lymph-node metastasis still present disseminated disease. In patients with metastatic disease that have amplified Her -2, the clinical response to regimens containing the anti-Her-2 antibody trastuzumab can be as high in 40% of patients, and results in increased survival, but the patients eventually relapse. The triple negative BCa subtype, which does not express significant levels of estrogen receptor, progesterone receptor and Her-2 receptor, is usually associated with poor outcome and high incidence of metastasis compared to other tumor subtypes, and lack benefit from currently approved targeted therapies. Therefore, novel paradigms and approaches are emerging toward further understanding BCa heterogeneity and progression. In parallel, discovery of alternative therapeutics such as targeting "precursor" metastatic cells or "cancer-stem like cells", proposed to be present as a rare sub -population of cells with distinct intrinsic invasive capacity and believed to respond poorly to conventional chemotherapy and available targeted agents like anti-hormones and trastuzumab, are currently under extensive investigations by several groups.

## Molecular modeling of breast cancer progression to metastasis

Metastasis formation has been recognized as a late event resulting from progressive accumulation of genetic events in a subset of tumor cells according to the multistep theory of carcinogenesis. Alternative models owing to recent progress in molecular profiling and clinical observations support the hypothesis that dissemination of primary BCa may be initiated at a very early stage of cancer development from rare and genetically programmed cell variants with intrinsic invasive capacity [2-11]. Among these cell variants the propensity of metastasis can be greatly influenced by host tissue microenvironment factors. For instance, (i) disseminated circulating BCa cells can be detected in over 10% of patients at primary diagnosis and without clinical or pathological signs of metastases [5, 6]; (ii) the occurrence of metastases in patients with early stage and lymph node negative BCa and in patients with unseen primary cancer [7, 8] (iii) the concept of cancer dormancy where metastatic cells may colonize distant organs early on and stay viable and clinically dormant for a prolonged period of time until undefined factors trigger their growth; (iv) the striking clinical reports of rapid "explosion" of undetectable metastases observed in some patients after removal of pathologically classified early primary cancers [9], and (v) the identification of deregulated gene clusters in early disease that are predictive of metastasis occurrence [4]. Hence, rare cancer cell variants, possibly cancer stem cell-like cells, with intrinsic high invasive capacity exist at early stages of cancer development. Indeed, exciting fundamental studies in the field have identified two key aspects to these observations; the presence of rare invasive cancer cells which express cell surface markers that distinguish stem cells, "cancer stem-like" cells, and their connection to the process of epithelial-mesenchymal transition (EMT), which plays a critical role in cancer cell invasion.

## Precursor "metastatic" breast cancer cells: the CSC concept and its connection to the EMT process

The cancer stem cell hypothesis that attempts to explain the origin of precursor invasive cancer cells has added exciting impetus to ongoing efforts to deepen our basic understanding of the complex biology of the metastatic process. In the case of BCa, molecular profiling studies on BCa tissues and cell lines revealed a high biological and histological heterogeneity. In this context, the CSC concept recapitulates several features of BCa cell heterogeneity since it supports the presence of cellular hierarchy within a tumor where cells with self-renewal and invasive capacity coexist with more differentiated and weakly or non-invasive cells (**Figure 1**). In support of this hypothesis, tissues from patients with invasive BCa, including triple negative breast cancer (TNBC) were found to contain a significantly higher proportion of $CD44^{+}/CD24^{-/low}$ CSC compared to tissues from less invasive subtypes [12]. This is of particular interest in view of the finding that disseminated cancer cells detected in bone marrow of BCa patients

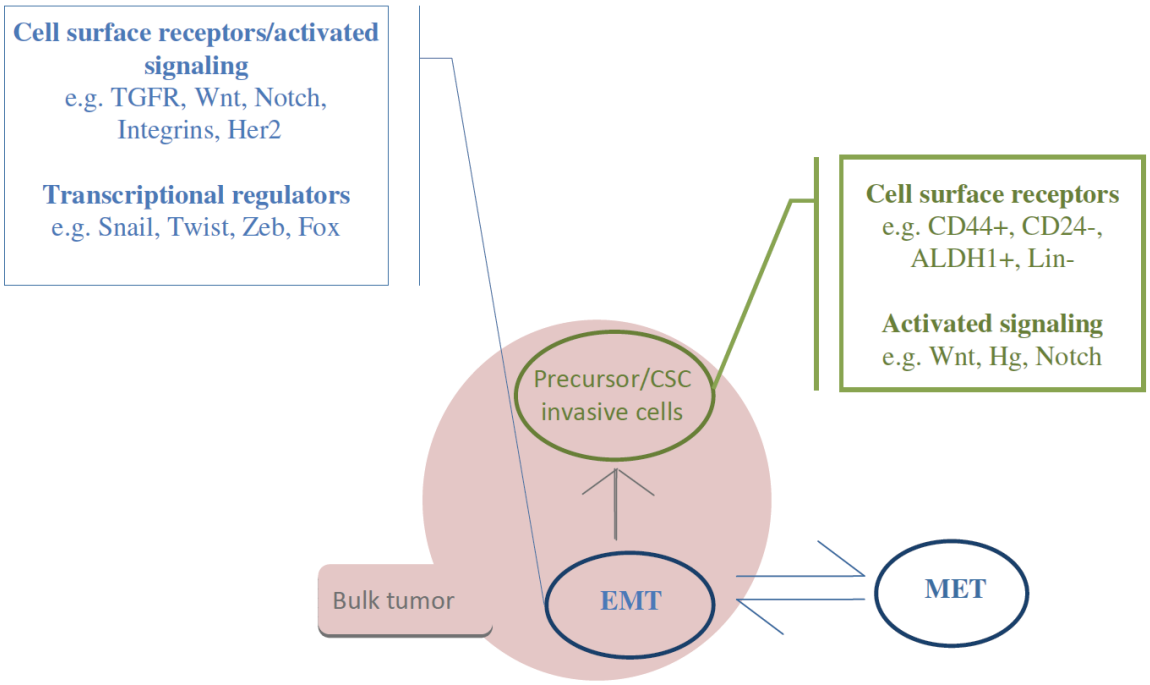



**Figure 1.** A simplified schematic representation of potential origins of precursor invasive cancer cells. In primary tumor mass, precursor invasive cells might be present as a tiny cell population that coexists at early stage of tumor development with more abundant mature/differentiated cancer cell populations. These invasive precursor cells might be represented by cancer stem cells or cell variants with cancer stem cell-like properties, and express stem cell surface markers and signalling molecules found to be activated in CSC (shown in the insert). In addition, the cells' ability to switch back and forth from epithelial to mesenchymatic states, a phenomenon referred to as Epithelial-Mesenchymal Transition (EMT) or mesenchymal-epithelial transition (MET) process, has been recognized as a key contributor to cancer cell heterogeneity and invasiveness, as well as selection of cancer cell variants with stem cell-like properties. EMT is characterized by phenotypic and molecular changes associated with the mesenchymal phenotype, e.g. activation of transcriptional repressors of E-cadherin. For MET, it is postulated that once the migratory cancer cells generated by EMT invade distant tissue beds, they can form secondary tumors exhibiting an epithelial phenotype. In all cases, tissue microenvironment "niche" remodelling may drive selection, self-renewal and survival of EMT/CSC cells. Therapeutic approaches to tackle precursor invasive cells aim to target cell surface markers, activated signalling pathways, or mechanisms associated with the EMT process.

also express stem cell markers ($CD44^+/CD24^{-/low}$), and are found at this point in a higher proportion of tumor cells [13].

Moreover, a link between the gain of cancer stem-cell properties and EMT phenotype exists. For instance (i) the EMT process is sufficient to induce a population with stem cell characteristics from well-differentiated and oncogene transformed mammary cells; (ii) human mammary cells undergoing EMT in response to TGFβ, or ectopic expression of Snail or Twist exhibit enhanced stem-like features; (iii) SAGE analysis of $CD44^+/CD24^{-/low}$ compared to $CD44^+$ cells from clinical samples revealed that predominant TGFβ-related signalling is predictive of patients' prognosis [14-16]; (iv) transplantation of EMT-like BCa tumors from conditional mutant BRCA1 mice gave rise to enriched $CD44^+/CD24^{-/low}$ cell population [17]; (v) gene expression patterns indicative of EMT was observed in basal B subgroup of BCa cells, in the luminal subgroup, and in isolated BCa stem cells [18]; (vi) EMT signatures were observed in stem-like cancer cells with increased clonogenic ability [19]; and a high proportion of $CD44^+/CD24^{-/low}$ cancer stem-like cells concomitant with activation of EMT-regulated genes have been observed in the basal-like subtype of human BCa, which is associated with a particularly aggressive behavior and poor prognosis [20-23]. Moreover, the aggressive and triple negative claudin-low BCa tumors are found to have low to nil expression of luminal differentiation markers,

while they overexpress markers of both EMT and cancer stem-like cells [24]. In a similar manner, signaling associated with Her-2 overexpression has been shown to contribute to the induction of EMT as well as the maintenance of cancer stem-like cells, both of which have been associated with poor prognosis of Her-2+ BCa [25, 26].

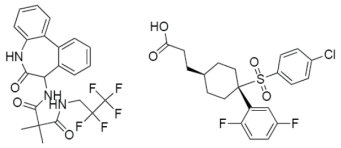
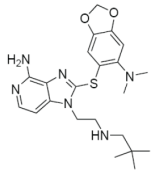
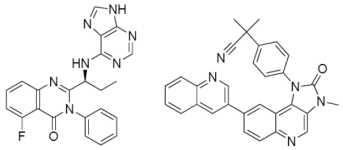
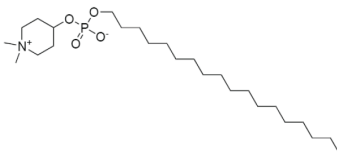
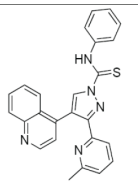
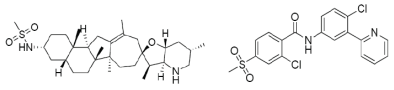
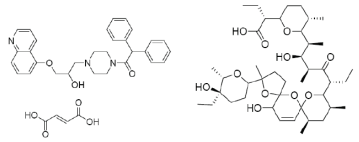
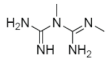
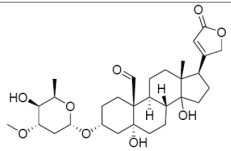
Given these and other data, the question is whether targeting CSC/EMT cells is sufficient to eradicate precursor invasive cells and metastatic disease. Any evidence that alter the trajectory of the disease, in mice or humans, would be supportive of the relevance and applicability of the observations summarized above. Indeed, encouraging results have emerged from studies in Chronic myeloid leukemia (CML), where the CSC concept has been well investigated. For example, restricted expression of the Bcr-Abl oncoprotein in the mouse hematopoietic stem cell compartment was found to be sufficient to induce CML with features similar to human CML, and elimination of these CSCs by genetic means eradicate the tumor in contrast to the Bcr-Abl inhibitor imatinib, which failed to do so [27]. Moreover, in colorectal cancer the CSC marker CD133 is found to be expressed in a subpopulation with tumor-initiating capacity when transplanted in immunodeficient mice [28], and in BCa, expression of the transcriptional repressor Snail was sufficient to promote the recurrence of BCa [29], suggesting that CSC/EMT surface markers and their coupled signaling pathways might represent rate-limiting targets.

## Progress in experimental approaches for targeting precursor invasive cancer cells

The CSC paradigm and its connection to the EMT process as key players for the generation of precursor metastatic tumors presents novel therapeutic targets for metastatic BCa, either at the microscopic or macroscopic state of disease. At present, the most encouraging results are seen with therapeutic antibodies targeting CSC-specific cell surface antigens "receptors" identified in a number of tumor types, including BCa. For example in the in acute myeloid leukemia (AML), a neutralizing antibody against CD123 (CD123 9IL3 receptor) was found to discriminate between leukemic stem cells and normal hematopoietic cells [30] and to selectively inhibit cell survival of $CD34^+/CD38^-$ AML cells [31]. In a similar manner, antibodies against CD44, a positive marker of tumor-initiating cells in particular in BCa ($CD44^+/CD24^{low/-}/lin^-$) inhibited tumor growth and prevented cancer recurrence [32]. On the other hand, activating anti-CD44 antibody was found to induce terminal differentiation of human AML stem cells [33]. Antibodies targeting CD33, an antigenic glycoprotein expressed on the surface of AML and leukemic blasts, conjugated to a chemotherapy drug called calicheamicin is reported to selectively target CSC [34]. Moreover, antibodies targeting EpCAM, a pan-epithelial differentiation adhesion molecule expressed in CSC of a variety of tissues and associated to cancer cell invasion and poor prognosis, has been shown to induce antibody- and complement-mediated cytotoxicity against EpCAM positive human colorectal cancer cells [35]. Presently, many studies are underway to establish the potential of therapeutic antibodies targeting CSC cell surface markers to improve the outcome and induce long-term cure. These will provide proof of principle that targeting cell surface receptors can not only discriminate between CSC and normal stem cells, but can also overcome CSC heterogeneity that may occur during cancer progression [36, 37].

In parallel to biologics, exciting efforts have been made in the refinement of medicinal chemistry approaches toward selective targeting of precursor invasive cancer cells/CSC, and in particular the use of ligand-target chemistry approaches that link bioactivity to phenotype. An increasing number of available chemical libraries, privileged scaffolds, protein secondary structure mimetics, DOS and BIOS libraries rich in chiral and functional groups are being exploited to identify selective small molecules against CSC/EMT. In contrast to biologics, such molecules offer practical advantages in that they are amenable for structure–activity relationship studies to generate versatile derivatives from lead molecules able to induce subtle changes in protein dynamics or be adapted to affect one or multiple targets suitable for complex multifactorial signaling such as those involved in the regulation of CSC self-renewal and survival. Several hypothetical levels of intervention using small molecules can be envisioned to target precursor invasive cancer cells. These include (i) the use of selective differentiation/de-differentiation agents for reprogramming precursor invasive cells/CSC to induce cell death or

**Table1.** Examples of the structural diversity of agents undergoing clinical trials for targeting cancer-stem like cells

| Inhibitors | Target |
|---|---|
|  RO4929097 MK-0752 | Inhibitors of Notch activation |
|  CUDC-305 | HSP90 inhibitor |
|  CAL-101 BEZ-235 | Inhibitors of phosphatidylinositol 3-kinase-δ (PI3K-δ) |
|  Perifosine | Inhibits Akt activation |
|  A-83-01 | Inhibitor of TGF-β type I receptor ALK5 |
|  IPI-926 Gdc-0449 | Hedgehog pathway inhibitors |
|  MS-209 Salinomycin | Drug transporter inhibitors |
|  Metformin | Inhibits cellular transformation and selectively kills CSCs |
|  Cymarin | Induces SOX17 expression in ESCs |

to exit from quiescence to a more differentiated state, which can render these cells more susceptible to cytotoxic chemotherapy drugs or, alternatively, to promote entry into a dormant state; (ii) use of inhibitors that interfere with key signaling pathways hyperactivated in CSC/EMT and required for cell renewal and survival, and (iii) targeting the tissue microenvironment "CSC niche" believed to be required for CSC survival, self-renewal, and migration. While many of these approaches have been explored in physiological contexts and in the field of regenerative medicine, their utility has only recently started to spark great interest in the cancer field (**Table 1**).

Particular classes of molecules involved in the cell reprogramming aspect that modulate cell differentiation and de-differentiation have emerged. Several agents have been identified to alter cancer cell phenotype via promotion of cell differentiation and cell death, including leukemias and more recently CSC, e.g. SAHA, retinoic acid, Aza-cytidine, valproic acid [38, 39]. Advanced chemistry applied to regenerative medicine has indeed revealed that selectivity and potency can be achieved via sequential activity of two or more functionally distinct molecules. A relevant example is represented by the HDAC inhibitors IDE1 and IDE2. These small molecules act as TGFβ/activin signaling mimicking agents, including induction of Smad 2 phosphorylation and are found to promote differentiation of embryonic stem cells into definitive endoderm

[40]. This exciting study also highlights the potential of sequential small molecules to manipulate specific stages of cell differentiation, e.g. chemically derived endoderm following IDE1 or IDE2 treatment can further be differentiated into pancreatic progenitors with (-)-indolactam V [40]. Taking the TGFβ/activin signaling as an example, the potential of these inhibitors to deorbit pluripotent cells to undergo a differentiation state via modulation of activin signaling may have relevance to CSC as well, in particular because activin A modulates an important signaling pathway for the initiation step of stem cell differentiation. This pathway is also involved in BCa metastasis [41] and inhibitors of activin signaling have been shown to block EMT and prevent cancer progression to metastasis [42]. The requirement of sequential molecules to achieve optimal response such as seen with IDE1 and IDE2 has also been reported for 5-aza -2'-deoxycytidine followed by SAHA (suberoylanilide hydroxamic acid) in inducing optimal apoptosis in $CD34^+$ cells from primary myelofibrosis, which originates from pluripotent hematopoietic stem cell [43], as well as when targeting the Wnt signaling pathway [44].

Noticeably, the therapeutic potential of differentiation agents may rely on the fact that unlike embryonic stem cell, where differentiating agents have the capacity to induce pluripotency, in the partially committed adult stem cells, which include CSC, it is hypothesized that such agents can promote differentiation into limited lineages of "multipotent" cells with less susceptibility to induce tumor formation, although this remains to be established. In addition, a major drawback for the use of differentiation agents in cancer remains the unpredictable effect resulting from the impaired and/or heterogeneous differentiation markers in cancer cells. For example, inhibitors of TGF, FGFR, Wnt, and Rock-GTPase signaling have been shown to either block embryonic stem cell differentiation or promote self-renewal of pluripotent progenitor pools: the later activity may not be desirable in cancer therapeutics. Such double-edged effects have been shown for GSK3, a serine/threonine protein kinase involved in cell differentiation and self-renewal and downstream of numerous signaling activated in CSC such as the WNT and Hedgehog pathways. For instance, stem cell quiescence resulting from delay in cell cycle progression can be induced in hematopoietic stem cells by using the GSK-3β inhibitor 6-bromoindirubin 3'-oxime, which upregulates CDK inhibitor p57 and downregulate cyclin D1 [45]. In the context of cancer the potential use of GSK3 inhibitors is debated, partly due the negative regulatory function of GSK3 on β-catenin but some evidence support the potential utility of GSK inhibitors for certain leukemia and solid cancers [46]. Presently, several GSK3 inhibitors e.g. tideglusib, are at the clinical stage for other applications, such as neurodegenerative disease and regenerative medicine, and the outcome of these studies will pave the way for other applications such as in cancer.

In contrast to cell differentiation, cell de-differentiation remains poorly understood in cancer. One example is the purine analogue reversine, which was reported to dedifferentiate myoblastic cell line C2C12 into multipotent cells able to be redirected to differentiate into other types of cells, e.g. osteoblasts or adipocytes depending on differentiation culture medium [47]. Reversine also demonstrated inhibitory activity against acute myeloid leukemia cells [48]. However, reversine is also found to be a non-selective agent targeting myosin II, Aurora kinase, MEK2, and MSP2. These targets have a broad function both in differentiated and non-differentiated cells. To date, no data is available for the use of reversine in CSC.

A more widely used alternative to interfere with CSC is the use of inhibitors targeting signaling pathways activated in CSC or involved in the regulation of EMT, which can enrich for CSCs. Among these are inhibitors of the TGFb signaling pathway, which have a critical role in the regulation of both the EMT and CSC; proteins involved in cell-cell interaction and polarity such as PAR6A, Notch1, Hedgehog, Wnt, integrins, polycomb repressive complex 1 (PRC1) protein Bmi-1, claudin, numerous receptor and non-receptor tyrosine kinase inhibitors and Rho GTPases inhibitors, such as the p160-Rho-associated coiled-coil kinase (ROCK) inhibitor Y-27632, which has been reported to block cell differentiation, reduce apoptosis and hinder EMT [49], and many have revealed anticancer activity in preclinical models. However, the outcome of inhibitors currently at various stages of clinical trials, e.g. Notch inhibitors such as MK-0752; TGF inhibitors such as the orally active LY-2157299, and the Hedgehog inhibitor IPI-926, will certainly shed useful information on the utility of targeting these molecules for metas-

tatic disease. In addition to these signaling inhibitors, the use of microRNAs, which generally act as negative regulators of CSC, is emerging as an attractive approach for therapeutic application [50]. Nevertheless, although many signaling pathways associated with both microRNA and growth factor receptors are found to be active in cancer stem cells, selectivity toward CSC versus mature cancer and normal stem cells has not been established.

Small molecules that target the CSC tissue microenvironment, the CSC "niche", are also emerging as potential therapeutic approaches to interfere with precursor invasive BCa cells. CSC niches are believed to represent CSC-specific tissue sanctuaries that favor, promote and maintain CSC survival and self-renewal activity, thereby providing a selective targeting opportunity. Indeed, the rare CSCs are believed to be concentrated in vascularized sanctuaries that provide appropriate CSC-endothelial cell interactions, necessary for self renewal, differentiation, and survival [51]. The relationship between CSC and the vascular niche is believed to be bi-directional such that the niche supports both the growth and renewal of CSC. For example glioma CSCs secrete high levels of VEGF required for EC proliferation and tube formation [52]. Moreover, cancer cell plasticity requires recruitment of neovessel formation via tumor stem-like cell differentiation to endothelial cell progenitors; this seems to involve Notch and VEGF signaling [53, 54]. Not surprisingly, targeting CSC niches such as via direct (anti-VEGF) or indirect (anti-ErbB2) inhibition of angiogenesis caused a reduction in CSC and tumor growth [51]. Other important emerging targets for CSC “niche” are chemokines and cytokines. For example, targeting CXC chemokine receptor 1 (CXCR1) by reparixin has been shown to block the formation of breast cancer stem cells that drive tumor growth and metastasis [55]. CXCR1 is a receptor for IL-8 (CXCL8), a proinflammatory chemokine that has been implicated in the metastasis and progression of multiple malignancies, and IL-8 has also been shown to stimulate self-renewal of breast cancer stem cells in vitro.

## Conclusion and perspectives

Metastatic cancer remains an intractable and lethal clinical challenge, despite massive efforts at new therapy development. The cancer stem cell concept and its connection to EMT provide a new paradigm to identify precursor invasive BCa cells with potentially novel challenges in the discovery of alternative therapeutic approaches. Because precursor invasive cells are believed to be a tiny sub-population of cancer cells, at least in early stage disease, targeting these cells will certainly be beneficial to prevent cancer progression to the metastatic stage, or to treat metastatic disease once it occurs. As noted above, several biologics targeting cell surface receptors and small molecule agents targeting signalling activated in CSC are already at the preclinical stage or undergoing clinical trials (examples are shown in **Table 1**). Moreover, discovery of new agents with these targets is gaining prominence in the drug discovery field. This exciting progress is not without uncertainties in predicting the move of such knowledge toward immediate clinical applications, owing in part to the as yet incompletely understood mechanims of CSC differentiation, heterogeneity, and redundant mechanisms activated both in CSC and non-CSC. In an elegant review, Dr. Clevers [56] highlighted these caveats, and in particular current concepts used to address CSC traits, clonal evolution, dormancy, and resistance to chemotherapy. Clearly, more biology in a relevant context is needed before meaningful therapeutic outcomes could be achieved.

Moreover, there are additional limitations related to experimental models and with critical importance for drug development in the context of CSC hypothesis. For instance, molecular identification of CSCs has been based on the expression profile of a particular set of cell-surface markers (e.g. CD44, CD24, CSC functional assays such as increased aldehyde dehydrogenase activity, efflux of Hoechst 33342, and retention of labeling dyes such as CFSE). Sorting cells on the basis of these characteristics enriches in CSCs. A drawback of these functional markers remains the still debated correlation between lineage marker expression and tumor phenotype. Indeed, increasing evidence support that human cancer subtypes involve distinct cellular origins with distinct molecular features; e.g. prostate cancer previously believed to arise from luminal cells is now reported to implicate distinct cancer stem cells in both the luminal and the basal cancer subtype. This may be expected as CSC bear genetic alterations that can promote genetic instability and phenotypic heterogeneity, which can create a great challenge for targeting the overall CSC population. Therefore, before a move toward translational applications the impact of CSC

differentiation/dedifferentiation agents for example requires detailed analysis of the differentiation pathways and using cell markers of intermediate differentiation stages. Another critical issue is the monitoring of CSC surrogate biomarkers, particularly when conducting clinical trials with CSC-targeted small molecules or biologics. Progress in high resolution imaging technologies, e.g. multiphoton laser scanning microscopy, and high-resolution MRI, have clearly provided powerful tools for the detection of rare cell subpopulation in tissues and for discrimination between poorly differentiated and mature cancer and stromal cells. Also, the challenge of finding small molecules that selectively modulate signaling pathways activated in precursor invasive cells/CSC is a major hurdle, given the large size of the human genome and proteomes. Indeed, many drugs discovered in other contexts are also found to affect to some extent CSC self-renewal and/or survival in experimental models, e.g. dasatinib and imatinib. The limitations and unexpected benefits of these multitargeting agents, as some of the agents intended to target other pathways may in a similar fashion derive some of their clinical benefits from their unintended effects on the CSC/EMT-related targets. With regard to CSC niche, a number of physiological probes have been developed to image tumor microenvironment events, such as angiogenesis, protease activity, hypoxia, and apoptosis. Tracking rare cells, such as CSCs, in conjunction with microenvironment-specific probes are useful in providing additional information as to the behavior of the cells of interest under specific environmental conditions and treatment. In summary, current development in biology and chemistry are becoming instrumental in resolving the many questions as to the selective modulation of the differentiation programs in precursor invasive cancer cells and therapeutic significance of these approaches to human disease, in particular their potential to overcome the limitation of existing therapies for metastatic breast cancer.

**Address correspondence to:** Dr. Mouay Alaoui-Jamali, Lady Davis Institute for Medical Research and Segal Cancer Center, Room E534, Jewish General Hospital, Departments of Medicine and Oncology, McGill University, Montreal, Canada Tel: 514-340-8260/8222; E-mail: moulay.alaoui-jamali@mcgill.ca

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