

## Chemoresistance in Cancer Stem Cells and Strategies to Overcome Resistance

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Received date: Feb 11, 2014, Accepted date: Mar 26, 2014, Published date: Mar 31, 2014

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### Abstract

In cancers, there exists a subpopulation of cells which are referred to as cancer stem cells (CSCs) or tumor initiating cells that have enhanced tumor-initiating capacity and metastatic potential, and drive tumor progression. Since the initial identification of acute myeloid leukemia CSCs in 1997, CSCs have been found in many types of cancer and have intrinsic resistance to the current chemotherapeutic strategies. With increased levels of detoxifying enzymes, enhanced DNA repair abilities, impressive efflux capacity, and a slower cell-cycle; CSCs present a formidable obstacle against effective chemotherapy. Several methods of specifically targeting CSCs have been developed in recent years, and these compounds have potential as adjuvant therapies. The following is a review of the mechanisms responsible for chemoresistance in CSCs, with an emphasis on potential strategies to overcome this resistance.

**Keywords:** Cancer stem cells; Chemoresistance; Targeted therapies

### Introduction to Cancer Stem Cells

For many years, tumors had been thought of as monoclonal populations of rapidly dividing cells, and that all cells had equivalent cancer-initiating abilities. Over time, it has become evident that tumors are heterogeneous in nature and that certain cells have increased tumor-initiating abilities. These tumor-initiating cells are also referred to as cancer stem cells (CSCs) and are hypothesized to self-renew (maintaining a population of CSCs) and to differentiate into less tumorigenic Non-CSCs [1]. First identified by Bonnet & Dick as the tumor-initiating cells of acute myeloid leukemia, CSCs were later isolated from solid tumors by Al-hajj et al. in breast cancer, as well as in brain tumours by Singh et al. [2-4]. Since these seminal publications, CSCs have been isolated from many cancers, including colon, pancreatic, liver, and prostate, lung, head and neck, ovarian, and stomach cancers [5-12].

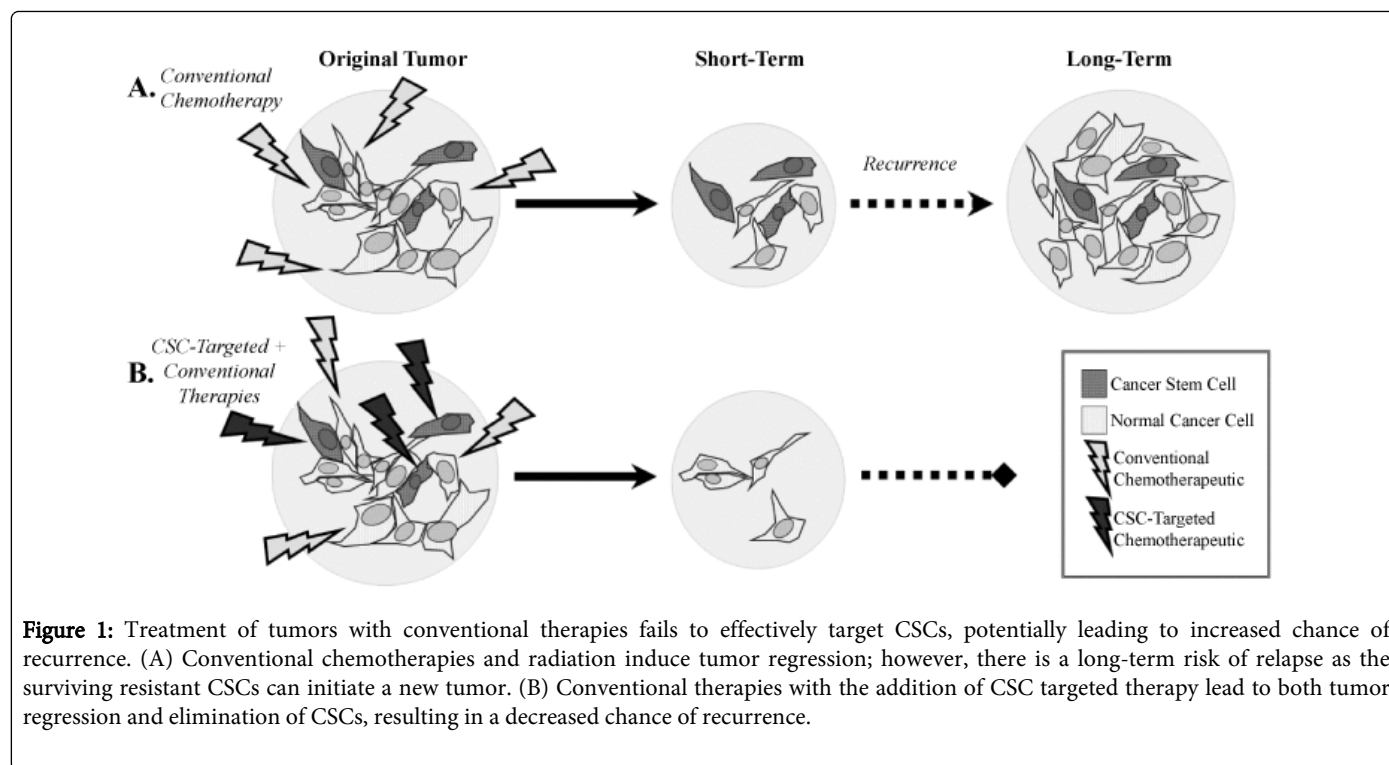
CSCs are functionally defined by their ability to initiate new tumors in severely immunocompromised mice [1]. A number of biomarkers are associated with more tumorigenic cells and can be used in combination to identify or isolate CSCs. These biomarkers are cancer-type specific and are often cell surface markers or based on increased aldehyde dehydrogenase (ALDH) activity as measured by the Aldefluor assay. For example, in breast cancer, cells sorted based on CD44+CD24- are enriched for CSCs, and high ALDH activity is also found in this CSC-enriched population [3,13]. Throughout this paper, CSC biomarkers will be referred to these biomarkers are either well-established identifiers for the cancer type discussed, or were verified by the authors to be prevalent in the population of cells that initiated tumors in immunocompromised mice.

As research on CSCs gained notoriety [14-16], it became clear that these seemingly ubiquitous tumor-initiating cells were resistant to radiation and chemotherapy. The presence of these cells in tumors contributes to a patient's likelihood of recurrence post-treatment, and may be the cause of resistance in tumors that do not respond to anti-cancer therapies. Herein, we will review the literature with regards to chemotherapeutics that CSCs are resistant to, mechanisms of CSC chemotherapeutic resistance and finally we discuss novel targeted therapies that are being developed which show efficacy towards killing CSCs.

### Drug Resistance in Cancer Stem Cells

The success of most chemotherapeutics is judged on the drug's ability to decrease tumor size or induce short-term remission. While this measure of success is intuitive and many drugs evaluated by these criteria are used in effective chemotherapeutic regimens, it is becoming increasingly evident that in some cases, eliminating the bulk of cancer cells may effectively select for resistant cells. As we discuss below, CSCs have a higher intrinsic resistance to chemotherapy than do normal cancer cells, and may be the source of post-therapy relapse [17] (Figure 1).

Cancer cells may acquire resistance to chemotherapy, or may have a high basal level of resistance through a variety of mechanisms (Table 1). These mechanisms have been well studied in cancer cells, and the same concept is being applied to CSCs. As discussed in detail later, there is evidence of increased drug inactivation through increased expression of detoxifying ALDH enzymes, enhanced DNA repair activity which thwarts platinum and alkylating agents, reduced drug activation via quiescence, and increased drug efflux by upregulation of ABC transporters.



**Figure 1:** Treatment of tumors with conventional therapies fails to effectively target CSCs, potentially leading to increased chance of recurrence. (A) Conventional chemotherapies and radiation induce tumor regression; however, there is a long-term risk of relapse as the surviving resistant CSCs can initiate a new tumor. (B) Conventional therapies with the addition of CSC targeted therapy lead to both tumor regression and elimination of CSCs, resulting in a decreased chance of recurrence.

Mechanisms of Chemoresistance	
<b>Increases drug export</b>	<b>Reduced drug uptake</b>
<b>Increased drug inactivation</b>	<b>Reduced drug activation</b>
Changes in Drug Target interaction	
Enhanced EGFR and MAPK/ERK signalling	
Enhanced DNA repair	
Inhibition of apoptosis	

**Table 1:** General mechanisms of chemoresistance in cancer cells  
Footnote: Mechanisms of chemoresistance that are more prevalent in CSCs are in bold.

### Chemoresistance of Cancer Stem Cells Due to Aldehyde Dehydrogenase Activity

The Aldefluor assay (Stem Cell Technologies, Inc.) was originally designed to isolate viable hematopoietic stem cells in human umbilical cord blood by identifying cells with high ALDH enzymatic activity [18]. The population of Aldefluor+ cells is often referred to as ALDH+ or ALDH<sup>bright</sup>. Highly tumorigenic cells isolated based on high Aldefluor activity were first identified in breast carcinomas and leukemia [19,20] and since then, Aldefluor+ cells have efficiently initiated xenograft tumors of liver, head and neck, stomach, lung, pancreatic, cervical, thyroid, prostate, colon, bladder, and ovarian cancers [7,21-29].

ALDHs are a super-family of enzymes involved in oxidizing aldehydes to carboxylic acids, and increased activity of some isoforms is associated with detoxification capabilities [30]. Due to the general function of ALDH enzymes in detoxification, it has been hypothesized

that Aldefluor activity associated with CSCs would confer resistance to chemotherapeutics as well. Indeed, in a breast cancer study, tumor samples with high ALDH protein levels were associated with patient resistance to paclitaxel and epirubicin [31]. Additionally, Aldefluor+ cells from lung cancer cells lines demonstrated a high resistance to multiple chemotherapeutic agents (cisplatin, gemcitabine, vinorelbine, docetaxel, doxorubicin and daunorubicin) when compared to Aldefluor- cells [22]. Though none of the aforementioned drugs seem to be metabolized directly by ALDH; other chemotherapeutics, such as the alkylating agent cyclophosphamide, are detoxified by the enzyme.

Abundant in the environment, and unavoidable in living cells, alkylating agents are a family of compounds defined by their ability to add alkyl groups to a variety of molecules [32]. This process damages DNA by generating covalent adducts that lead to mutations in the sequence. These mutations may result in apoptosis or replication failure. The DNA damaging effects of alkylating agents are utilized in chemotherapy for a variety of cancers; examples of alkylating agents include cyclophosphamide, melphalan, ifosfamide, carmustine, procarbazine, and temozolomide. While these agents are usually effective against most non-CSCs, it seems that CSCs are resistant to these drugs via ALDH detoxification and through increased DNA repair.

Cyclophosphamide is used to treat breast, lung and ovarian cancers, as well as acute myeloid leukemia, chronic myeloid leukemia, neuroblastoma, and lymphoma [33-38]. Cyclophosphamide is an inactive prodrug that is converted to 4-hydroxycyclophosphamide and aldophosphamide once inside of the cell, and eventually results in phosphoramide mustard which forms the DNA crosslinks [39]. Despite widespread effectiveness in many cancer types [40], cyclophosphamide is less effective in the presence of ALDH which interferes with the drug's decomposition to aldophosphamide[39]. Metabolism of cyclophosphamide by ALDH has been suspected for decades [41], and the enzyme's role in chemotherapy resistance was

first elucidated for leukemia. Studies using the murine leukemia cell line L1210 found that 4-hydroxycyclophosphamide was detoxified by ALDH and that cell line resistance could be attributed to ALDH activity [42-44].

There are 19 isoforms of ALDH present in the human genome [30], and the specific isoforms responsible for cyclophosphamide detoxification in cancer cells is not fully known, however some studies indicate the involvement of at least two isoforms. Induced expression of ALDH1A1 in L1210 cells led to increased resistance to cyclophosphamide [45]. Moreb et al. determined that siRNA knockdown of isoforms ALDH1A1 and ALDH3A1 resulted in an 84% increase in cyclophosphamide toxicity in lung adenocarcinoma cell line A549 [46]. In breast cancer patient tumors, immunohistological staining determined that increased expression of ALDH1A1 and ALDH3A1 was found in tumors that did not respond to cyclophosphamide therapy, and in tumors that had undergone cyclophosphamide therapy [47]. These results implicate ALDH1A1 and ALDH3A1 in resistance to cyclophosphamide. Notably, expression of the ALDH1A1 isoform is associated with the Aldefluor activity of the CSCs of many cancers [48-51]. Therefore, there is a direct link with ALDH1A1 expression, CSCs and cyclophosphamide-resistance in cancer.

### **Chemoresistance of Cancer Stem Cells by Enhanced DNA Repair Mechanisms**

The platinum group of chemotherapeutic agents (including the common analogues cisplatin, carboplatin, and oxaliplatin) induce tumor regression by causing DNA damage. Cancer cells often have defective DNA repair pathways, and due to rapid proliferation, these cells are often in S-phase which is a vulnerable phase for DNA damage [52]. Thus, these DNA-damaging chemotherapeutics are selectively deleterious to cancer cells in S-phase, which due to impaired repair mechanisms, are not able to recover from the damage. When the DNA repair cascades are unable to adequately fix the damage, cell-cycle checkpoint components are activated which can recruit additional DNA repair components or activate apoptosis. Data from many studies imply that CSCs have elevated levels of DNA repair [53-61], these provide one explanation for the resistance of some tumor types to platinum agents.

*In vitro* evidence suggests that CSCs in lung, ovarian and breast cancer cell lines are resistant to DNA-damaging agent cisplatin, since these cells were enriched post-treatment [62]. Similar CSC enrichment was observed in mice bearing breast tumor xenografts post-cisplatin treatment [63]. Perhaps more importantly, primary clinical samples support the hypothesis that CSCs are more resistant to treatment with platinum analogues. Patients with advanced ovarian cancer showed an elevated percentage of CSCs in sampled ascites when they had been treated with cisplatin compared to chemotherapy-naïve patients [64]. In glioblastoma, resistance of CD133<sup>+</sup> CSCs to chemotherapeutics was attributed to increased expression of DNA repair and anti-apoptosis proteins [65]. Furthermore, the authors also showed that patients with recurrent glioblastoma had higher expression of CD133 in their tumors post-treatment with chemotherapeutics.

### **Chemoresistance of Cancer Stem Cells Due to Quiescence**

Anti-mitotic drugs target the reorganization of microtubules essential for proper cell division and proliferation (Gascoigne &

Taylor, 2011 for review of mechanism)[66]. The two classes of antimetabolites currently approved for cancer therapy are vinca alkaloids (vincristine, vinblastine, vindesine and vinorelbine) which prevent the polymerization of microtubules, and taxanes (paclitaxel, docetaxel) that stabilize existing microtubules. Both classes effectively inhibit the formation of the mitotic spindle, inhibiting the mitotic phase of the cell cycle. Many other antimetabolites are in development; however, they have not yet been approved for clinical use [67]. The relation of CSCs to progression through the cell cycle is inconclusive; however, there is evidence to suggest that CSCs may be more quiescent or slower-cycling than their associated non-CSCs. Quiescence and a slower progression through the cell cycle in CSCs would likely render these cells less susceptible to cell-cycle targeted therapies such as the antimetabolite class of chemotherapeutics [68].

In glioblastoma, CD133<sup>+</sup>- identified CSCs were resistant to a variety of chemotherapeutic agents, including paclitaxel [65]. A later study, using CD133<sup>+</sup> cells derived from patients with treatment-refractory recurrent gliomas, demonstrated that these CSCs had gene expression profiles consistent with quiescent cells [69]. Similarly, Pece et al. determined that the gene expression profiles of high-grade breast tumors matched the profiles generated from quiescent mammary stem cells [70]. Additionally, mammospheres formed from higher-grade cells retained high levels of the quiescence marker, PKH26. Mammospheres are an *in vitro* measure of CSC tumorigenicity; thus, this data suggests that the CSCs are quiescent when compared to non-CSCs. The authors suggest that tumor progression can be associated with an increase in the number of quiescent cells, which maintain their stem-like tumorigenicity. Inducing cell-cycle entry in these cells may be an interesting option for CSC-targeted therapy, and data from a leukemia model [71], demonstrates that stimulating quiescent CSCs to divide improves the efficacy of cell-cycle dependent chemotherapy.

### **Chemoresistance of Cancer Stem Cells by Enhanced Drug Efflux Mechanisms**

CSCs are enriched in the side population (SP) of tumor cells which have high efflux of Hoechst dye [72-77]. The efflux capacity of the SP is attributed to increased expression of ATP-binding cassette (ABC) transport proteins ABCB1, ABCC1, and ABCG2 [76,78-81]. These ABC transporters are able to efflux a wide array of chemotherapeutic drugs (e.g. colchicine, doxorubicin, etoposide, vinblastine, and paclitaxel) and their expression is a major cause of multi drug resistance in cancers [82]. Upregulation of these three ABC transporters is often seen in CSCs, and contributes to chemoresistance. For example, increased expression of ABCB1 was shown in the CD44<sup>+</sup>CD24<sup>-</sup> identified breast CSCs, which were also comparatively resistant to doxorubicin [83].

### **Targeting Cancer Stem Cells to Overcome Chemoresistance**

CSCs exhibit dysfunctional signalling via three key embryonic pathways: the Wnt/ $\beta$ -catenin, Notch, and Hedgehog (Hh) pathways. The reliance of CSCs on these dysregulated signalling paradigms have generated potential targets for anti-CSC-directed therapies. Here we review several strategies for CSC focused therapy: targeting the Wnt, Notch, and Hh signaling pathways; inhibition of ALDH; and inhibition of ABC transport proteins.

## Targeting Wnt Signalling

Wnt signaling is essential for controlled cell proliferation, cell fate decisions during development, and adult stem cell maintenance. Briefly, the binding of extracellular Wnt ligands to membrane-bound frizzled receptors results in the recruitment of disheveled proteins that block glycogen synthase kinase 3 from interacting with its substrates, which include  $\beta$ -catenin [84].

Enhanced Wnt signaling has been observed in the CSCs of many different cancer types. In chronic myeloid leukemia, deletion of  $\beta$ -catenin in combination with imatinib depleted leukemic CSCs; however, deletion of  $\beta$ -catenin alone did not prolong survival in mice [85]. In a breast cancer model with spontaneous lung metastasis, Malanchi et al. determined that periostin, a key regulator of metastatic colonization, recruits Wnt ligands and likely promotes the maintenance of CSCs in their niche [86]. Additional evidence supports aberrant Wnt signaling in lung, colon, and gastric CSCs [87-89]. Strikingly, Teng et al. observed increased  $\beta$ -catenin and OCT-4 (a marker of stemness, see Pesce, 2001) [90] expression in cisplatin-selected A549 lung adenocarcinoma cells.

Resveratrol, a natural polyphenol, has anti-oxidant properties, may have a role in somatic cell reprogramming and can be used in reprogramming mouse embryonic fibroblasts into induced pluripotent stem cells [91]. Furthermore, is hypothesized to exhibit anti-CSC properties via inhibition of Wnt signaling. Resveratrol inhibits fatty acid synthase (FASN) in breast cancer cell-line-derived CSCs, thus suppressing their proliferation [92]. Overexpression of FASN has been associated with increased stability of  $\beta$ -catenin [93] thus, inhibition of FASN likely results in decreased Wnt signaling. More recent findings suggest that resveratrol may be able to inhibit CSC migration and invasion in pancreatic cancer [94]. However, a phase 1 trial of resveratrol treatment in colon cancer inhibited the Wnt pathway in normal colonic mucosa, but not in cancerous colon tissue [95], suggesting toxicity may be a concern with using resveratrol as anti-cancer therapeutic, despite its potential anti-CSC activity.

An isoflavone, genistein, primarily acts as a specific tyrosine-kinase inhibitor [96] and has been demonstrated to inhibit the tumorigenicity of CSCs in prostate, gastric and breast cancers [97-100]. However, it is unclear whether this is due to attenuation of Wnt signaling [101,102] or of the Hedgehog pathway [98,100]. A phase 2 study of genistein on localized prostate cancer patients revealed a decrease in serum PSA in patients treated with genistein [103], as more focal cancer was observed among genistein-treated patients, although effects on CSCs is unclear at this time.

OncoMed Pharmaceuticals, Inc. (OncoMed) and Bayer have initiated a Phase 1b dose-escalating clinical trial of a decoy receptor for Wnt ligand, Fzd8-Fc. This trial, using Fzd8-Fc (OMP-54F28) in combination with paclitaxel and gemcitabine for patients with first-line Stage 4 pancreatic cancer, follows a Phase 1 trial in patients with solid tumors (NCT01608867). In addition, OncoMed has developed an anti-frizzled monoclonal antibody, vantictumab. Results from a Phase 1a study were presented at the European Cancer Congress (2013), suggesting that vantictumab is well tolerated. As well, prolonged stable disease was observed in 3 patients with neuroendocrine tumors. Phase 1b trials of vantictumab in combination with standard chemotherapeutic regimens are ongoing in untreated stage 4 pancreatic cancer (NCT02005315), previously-treated NSCLC (NCT01957007), and locally recurrent or metastatic breast cancer (NCT01973309).

Prism Pharma Co., Ltd. and iNventiv Health Clinical are investigating PRI-724 in clinical trials. PRI-724 blocks the recruitment of  $\beta$ -catenin to Wnt-responsive elements in the genome, thus preventing activated transcription. Ongoing trials include those in patients with advanced solid tumors, pancreatic cancer, or myeloid malignancies (NCT01606579, NCT01764477, NCT01302405). Therefore, Wnt targeted treatment has potential as an adjuvant therapy in a wide range of cancer types, and presents a very promising avenue for future anti-CSC targeted therapy research.

## Targeting Notch Signalling

The Notch signalling pathway is essential for cell-fate determination and pattern formation throughout vertebrate development; its absence results in lethal hyperplasia of the nervous system [104,105]. Notch signalling is initiated by ligand binding of transmembrane receptors Notch1, Notch2, Notch3, and Notch4, which induce proteolytic cleavage of the receptors' intracellular domains by the presenillin- $\gamma$ -secretase complex [106,107]. The intracellular domains enter the nucleus, and regulate transcription of target genes. Aberrant Notch pathway signalling has been demonstrated in the CSCs of a number of cancers. Notch pathway inhibition depleted CD133+ glioblastoma cells and inhibited tumor growth neurosphere formation [108]. Activating Notch1 mutations are seen in ~50% of T cell acute lymphoblastic leukemia (T-ALL) cases [109] and Notch signalling appears to be of greater importance in CD43<sup>+</sup> CD7<sup>+</sup> T-ALL leukemia stem cells [110]. Other work has specifically identified Notch4 as contributing to Notch activity in breast CSCs [111].

A phase 1 study of  $\gamma$ -secretase inhibitor (GSI) RO4929097 (Hoffman-La Roche) in refractory metastatic disease or patients with locally advanced solid tumors had mostly favourable results and spawned a number of subsequent clinical studies (e.g. NCT01238133, NCT01154452, NCT01196416) [112]. Another GSI in clinical trials is MK-0752 (Merck), which was shown to decrease CD44+CD24- and Aldefluor+ CSC populations in breast tumor xenografts [113]. In addition, a phase 1 clinical trial of MK0752 in combination with docetaxel decreased breast CSCs over the course of treatment. Unfortunately, due to the involvement of  $\gamma$ -secretase throughout the gastrointestinal tract, dose-limiting gastrointestinal side-effects are of concern in using this particular mode of therapy. Additional toxicity may be observed due to goblet cell metaplasia [114,115]. A third GSI, GSI-18 been shown to deplete the Aldefluor+ CSCs and decrease colony formation and xenograft engraftment in pancreatic cancer models [116]. Similar results were seen in DAOY medulloblastoma cells [117]. We have yet to see if GSI-18 has clinical effects.

OncoMed has also targeted the Notch pathway with demcizumab (OMP-21M18), which is a monoclonal antibody against Delta-like ligand 4 (DLL4) which binds the Notch receptor. Phase 1b trials of demcizumab in combination with gemcitabine and Abraxane in pancreatic cancer (NCT01189929) demonstrated a high clinical benefit rate (Market watch report). Additional clinical trials are ongoing in non-small cell lung carcinoma (NCT01189968) and ovarian cancer (NCT01952249).

Despite a multitude of evidence suggesting reliance of CSCs on aberrant Notch signalling, recent findings indicate that not all CSCs may be reliant [118]. Thus, while targeting Notch signalling may effectively eliminate a significant proportion of CSCs, there may be some CSCs which are not susceptible to this therapy. It remains to be

seen if anti-Notch therapies have long-term benefits in cancer patients over standard therapeutics alone.

## Targeting Hedgehog Signalling

The Hedgehog (Hh) signalling pathway is involved in the regulation of cell differentiation and proliferation in embryonic development and in the maintenance of adult stem cells. Hh ligands sonic hedgehog (Shh), indian hedgehog (Ihh) and desert hedgehog (Dhh) bind to the cell-surface receptor Patched (PTCH). The binding of ligands to PTCH triggers an accumulation of Smoothed (SMO) within the cell membrane and activates GLI transcriptional regulators. Activated GLI proteins (activators Gli1 and Gli2, and repressor Gli3) accumulate in the nucleus and control transcription of Hh target genes. Targets of Hh signalling include JAG2 and Wnt proteins, resulting in significant cross-talk between the Hh, Wnt, and Notch pathways [119].

There is a fair amount of evidence to suggest that CSCs have higher levels of Hh signalling than their non-CSC counterparts. Hh components are more highly expressed in CD44+CD24- breast CSCs and likely contribute to maintenance of self-renewal potential in these cells [120]. Inhibition of Hh signalling decreased spherogenicity in CD133+ glioma CSCs, decreased self-renewal in Aldefluor+ B-ALL cells, and inhibited clonogenicity of multiple myeloma CSCs [121,122]. In addition to depleting Aldefluor+ cells, Hh inhibition also decreased metastatic spread in a xenograft model of pancreatic cancer and inhibited the growth of human serous ovarian tumor xenografts [123,124].

Several inhibitors of Hh signalling are in various pre-clinical and clinical stages of testing. The inhibitors of Hh ligand-PTCH interactions are perhaps the least advanced in the drug development pipeline. 5E1 is an anti-Shh antibody, and has been shown to inhibit the growth of colon cancer xenografts [125]. Robotnikinin also blocks the Shh-PTCH interaction, but it remains to be seen if it is able to exert anti-cancer effects [126].

Cyclopamine inhibits Hh signalling by binding to SMO [127]. Treatment of mice with cyclopamine or its analogues inhibited the growth of medullablastoma xenografts [128]. In a phase I trial in patients with refractory solid tumors, the cyclopamine-derived SMO inhibitor IPI-926 (saridegib, Infinity Pharmaceuticals), contributed to a response in eight of 28 patients [129]. Additionally, there was substantial evidence for the use of IPI-926 in patients with pancreatic cancer [130] however, the phase 2 study (NCT01130142) was stopped after an interim analysis revealed that patients on the saridegib + gemcitabine arm had a median survival of less than 6 months, which is less than the historical gemcitabine-treatment mean of 6 months. There have been a number of other trials of IPI-926 in other malignancies (NCT01310816, NCT01371617), and we await the findings of these studies to properly evaluate the promise of saridegib.

Another inhibitor of SMO, GDC-0449 (vismodegib, Genentech), reduced growth of several lung cancer cell lines via inhibition of the Hoescht-excluding side populations [131]. A phase I trial of GDC-0449 resulted in a number of partial and complete responses among patients with basal-cell carcinoma [132]. Similarly, a case study of GDC-0449 treatment in one patient with refractory metastatic medulloblastoma resulted in rapid regression of the tumor; however, this response was incomplete and transient [133].

With targets further down the Hh pathway, GANT58 and GANT61 inhibit Gli-mediated transcription and blocked xenograft growth of

prostate cancer cells [134]. In particular, GANT58 decreased viability of T-ALL cells and functioned synergistically with the AKT inhibitor GSK690693 to induce cell death [135]. Targeting Gli-mediated transcription may be able to reduce cell migration [136] and may also attenuate drug resistance in some cancer types [137]; however, clinical data is required to definitively answer these questions.

## Aldehyde Dehydrogenase Inhibitors

As discussed earlier, increased ALDH activity is a common biomarker of CSCs and is involved in detoxifying certain chemotherapeutics, making ALDH inhibitors a promising avenue for anti-CSC targeted therapy development. Known inhibitors of ALDHs include chloral hydrate, cyanamide, DEAB, gossypol, molinate, pargyline, and disulfiram [138]. Disulfiram has been used for decades to treat alcohol abuse, and recently its potential in cancer treatment has been investigated [139]. The anti-cancer mechanisms of disulfiram are not limited to ALDH inhibition; principally, disulfiram is used to inhibit the proteasome and E3 ligases, and may also be a DNA-demethylating compound [140-143]. Disulfiram inhibits both ALDH1A1 and ALDH2 isoforms [138,144], and has shown anti-CSC effects in breast cancer and GBM [145,146]. Though the anti-CSC effects in the breast cancer studies have been mostly attributed to disulfiram's proteasome inhibition [146-148] in glioblastoma, ALDH inhibition by disulfiram re-sensitized Aldefluor+ cells to gemcitabine cytotoxicity [149]. After showing potential as a chemotherapy-enhancing drug, disulfiram was approved for a phase II clinical trial as a treatment for GBM (NCT0177919).

## ABC Transporter Inhibitors

The upregulation of ABC transporters in CSCs and the increased efflux capacity that accompanies that upregulation has prompted investigation of ABC inhibitors as adjuvant therapy. ABC inhibitors have already been tested in various clinical trials; however, they were originally positioned as a broad strategy to increase the efficacy of chemotherapeutics on all cells within a tumor. ABC transporter inhibition should instead be conceptualized as a way to re-sensitize the small population of CSCs with pre-existing intrinsic resistance [150]. ABCB1 inhibitors such as verapamil and cyclosporine A were among the first to be investigated and were effective in treating acute myeloid leukemia, non-small cell lung carcinoma, and breast cancer [151-153]. More recently, ABCG2 inhibition by axitinib has been investigated, and in cell lines from many cancer types, it effectively re-sensitized the side population of CSCs to topotecan and mitoxantrone [154]. The current approach is to inhibit a single ABC transporter; however there are three efflux proteins that are important to chemoresistance in CSCs (ABCB1, ABCC1, and ABCG2). Several compounds have been found to inhibit the three key ABC transporters: cyclosporine A, biricodar, PK11195, and curcumin [155]. While not all of these drugs have undergone clinical trials, inhibiting the key ABC transporters in CSCs is an avenue for future research.

## Conclusions

The bulk of a tumor is composed of non-CSCs; however, the presence of CSCs represents an important hurdle to effective cancer therapy. These tumor-initiating cells are more resistant to conventional chemotherapeutics than the majority of cancer cells, and survival of CSCs likely contributes to tumor recurrence. Mechanisms by which CSCs are resistant to chemotherapy include enhanced DNA

repair, increased detoxification capacity, and quiescence. Though CSCs are a great challenge to chemotherapy, they may be surmounted by the use of ABC transporter inhibitors, ALDH inhibitors, and through targeting CSC-specific Wnt, Hh, and Notch pathways. Theoretically, eliminating CSCs through targeted therapies would increase the efficacy of our existing treatments and lead to more favourable long-term prognoses for many cancer types.

## Financial Support

MLT is supported by a studentship from the Canadian Breast Cancer Foundation Atlantic-Chapter, administered by the Beatrice Hunter Cancer Research Institute. KMC is supported by a trainee award from the Beatrice Hunter Cancer Research Institute with funds provided by the Canadian Imperial Bank of Commerce as part of the Terry Fox Strategic Health Research Training Program in Cancer Research at the Canadian Institutes of Health Research (CIHR). Publication of this manuscript is supported by a grant to PM from the CIHR (MOP-130304).

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