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Concise Review: Emerging Drugs Targeting Epithelial Cancer Stem-Like Cells

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ABSTRACT

Increasing evidence suggests that cancer cell populations contain a small proportion of cells that display stem-like cell properties and which may be responsible for overall tumor maintenance. These cancer stem-like cells (CSCs) appear to have unique tumor-initiating ability and innate survival mechanisms that allow them to resist cancer therapies, consequently promoting relapses. Selective targeting of CSCs may provide therapeutic benefit and several recent reports have indicated this may be possible. In this article, we review drugs targeting CSCs, in selected epithelial cell-derived cancers. STEM CELLS *2017;35:839–850*

SIGNIFICANCE STATEMENT

Drugs selectively targeting cancer stem-like cells (CSC) continues to evolve, and hold significant promise for the next phase of cancer therapeutics. This review article highlights recent achievements in our understanding of CSC signaling and its mediators and summarizes advances in the discovery and development of targeted therapy and novel therapeutics/drugs making their way to the clinic. We focused on the unique challenges of working with epithelial cell-derived CSC, including the characterization of cell populations, the identification of druggable targets and pathways, their validation in preclinical models, and the translation to the clinic.

INTRODUCTION

Cancer stem-like cells (CSCs) are a subpopulation of tumor cells that have the extraordinary characteristic of self-renewal and the ability to generate cellular heterogeneity within a tumor [1–3]. These cells possess a number of distinctive features that allow them to become resistant to anticancer therapies and tumor-targeted drugs, which in turn, helps them to survive treatment and initiate tumor recurrence [3]. CSCs are immortal tumor-initiating cells with multipotent capacity. They are a major driving factor for tumor development, progression, metastasis, resistance to chemotherapy, and relapses after cancer treatment [1, 3, 4].

Over the last three decades, treatment options including chemotherapy, surgery and radiotherapy have only made incremental improvements in terms of patients survival [5]. Chemotherapy and radiotherapy primarily target differentiated and proliferating cancer cells while being less effective in targeting the relatively undifferentiated and quiescent CSCs [6, 7]. Many novel anticancer drugs, including tyrosine kinase inhibitors and monoclonal antibodies (mAbs) reduce the tumor size but fail to eliminate CSCs, which has been associated with cancer recurrence [8, 9].

Emerging evidence also indicates that some conventional anticancer drugs not only fail to eliminate CSCs [10, 11], but selectively enrich CSCs [7, 12, 13] possibly by inducing dedifferentiation or trans-differentiation [6, 7]. For example, in breast cancer patients receiving systemic chemotherapy, breast tumors show strong selection for CSC survival and expansion [14, 15]. This was demonstrated by increased $CD44^{hi}/CD24^{lo}$ marker profile and increased sphere-forming ability in breast cancer after conventional chemotherapy treatment with paclitaxel [15, 16]. Such observations on existing cancer treatments underline the need for a strategy based on selectively targeting of CSCs [17] (Fig. 1). In this article, we review the recent developments on new drugs targeting for CSCs, with a focus on major epithelial-derived cancers.

SELECTIVE TARGETING OF CSCS

Selective targeting of CSC is a huge challenge from the therapeutic point of view as strategies that are not sufficiently selective for CSCs

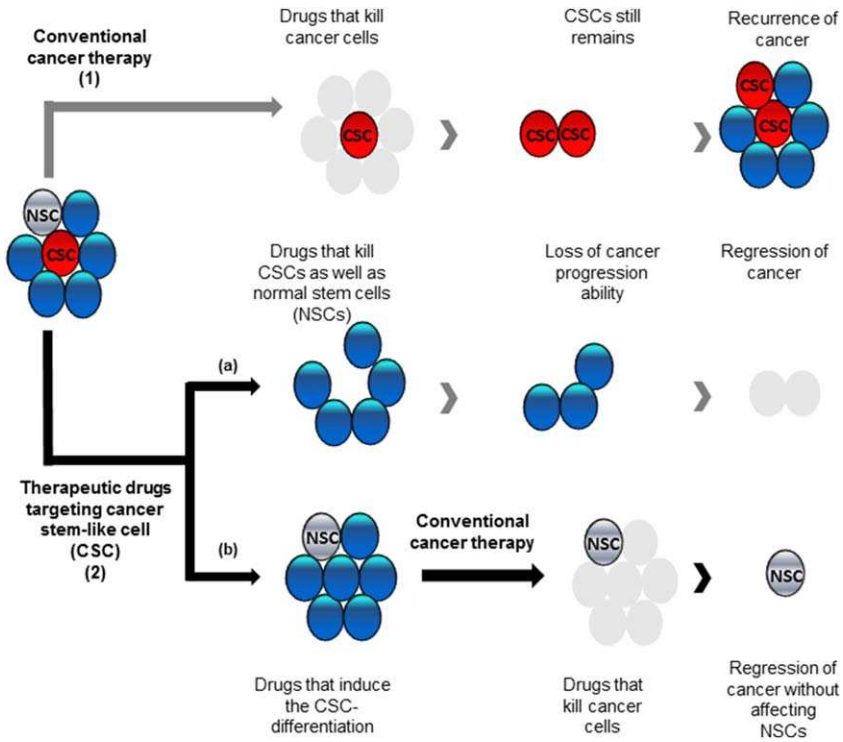


Figure 1. Different approaches in cancer treatment. **(1)** Recurrence of cancer by the remaining CSCs after conventional cancer treatment. **(2)** CSC-targeted therapies will **(A)** eliminate both CSCs and NSCs and cause loss of cancer progression, and **(B)** induce differentiation to CSCs and make them susceptible to conventional cancer therapy. This approach, when combined with conventional cancer treatment will result in regression of cancer without affecting the NSCs. Abbreviations: CSCs, cancer stem-like cells; NSC, normal stem-like cells.

may cause toxicity to healthy tissues and increase risk of recurrence among the patients [18]. Many approaches exploit the differences in cell surface markers to identify compounds that selectively target CSCs. These targets include the ATP binding cassette (ABC) transporter superfamily, anti-apoptotic factors, detoxifying and DNA repair enzymes and distinct oncogenic cascades such as Wnt/β-catenin, TGF-β, Hedgehog (Hh), EGFR, PTEN, BMI-1, NF-κB, Bcl-2, JAK/STAT, Notch, and PI3K/Akt/mTOR pathways [1, 19–22]. Compounds that preferentially induce the terminal differentiation of CSCs may also provide a valid therapeutic rationale for eliminating CSCs. For example, in humans, both normal breast and breast cancer stem cells express the *OCT*4 gene but neither express the connexin 43 gene. These two genes serve two diametrically opposed functions. OCT4 maintains the stemness and undifferentiated state of both normal and breast CSCs, while expression of connexin 43 gene is required for differentiation [23]. Selective expression of *OCT4* and *connexin 43* genes may be exploited to induce the CSCs to terminally differentiate.

Recently, several compounds have been identified to target CSCs, a combination of which with conventional chemotherapy drugs has been shown to significantly suppress self-renewal, induce differentiation, inhibit tumor growth and metastasis, and eventually eliminate CSCs [19, 24] (Fig. 1). Table 1 shows the clinical status of some drugs and compounds identified for targeting of CSCs in different cancers.

Breast Cancer

Signaling pathways such as Notch, Wnt, and Hh have received much interest as key targets for CSC-based therapies in breast cancer [49]. The Wnt/β-catenin pathway is essential for CSC survival, self-renewal and resistance, and downregulating this pathway has been shown to eliminate CSCs in breast cancer cells [50, 51]. Oxymatrine and curcumin downregulated Wnt1, β-catenin, c-Myc, and Cyclin D1 in MCF-7 and MDA-MB-231 human breast cancer cells, causing inhibition of self-renewal and "tumorsphere" formation [3, 42]. The antitumor effect of curcumin was confirmed in vivo in a nude mouse model bearing MDA-MB-231 xenograft tumors [52]. Given their safety profile, curcumin and oxymatrine, may constitute promising candidates for breast cancer therapy, although additional studies are needed to validate this therapeutic approach.

Breast CSCs exhibit increased Notch expression, and at present, anti-Notch mAbs and γ-secretase inhibitors (GSI) are under clinical evaluation for advanced breast cancer [53]. Targeting the Notch pathway has been reported to reduce stem-like cell activity in vitro using breast-cancer-derived secondary mammospheres, and patient-derived tumor formation in vivo [54]. Upon combination with docetaxel, both mAbs and GSIs enhance the efficacy of docetaxel and reduce self-renewal and tumor growth. This effect was particularly seen in patient-derived xenografts obtained from the Sum149 triple-negative breast cancer (TNBC) cell line and TNBC primary cancer cells [21, 24, 54]. Patients with TNBC have an exceptionally poor prognosis. While current treatments with doxorubicin and cyclophosphamide fail to eradicate CSCs and lead to recurrence within 4-6 weeks [3, 43], inhibition of self-renewal by mAbs or GSI may provide an effective strategy against this aggressive form of cancer.

A number of other studies suggest that metformin may selectively target breast CSCs. For example, metformin

Table 1. Drugs and novel compounds identified for selective cancer stem-like cells targeting

Compound	Mode of actions/targets	Cancer types	Clinical status	References
3-O-methylfunicone (OMF)	Inhibits CD24, CD29, CD44, CD133, CD338; reduces Survivin, hTERT and Nanog	Breast CSC	Preclinical	[3, 25]
BBI503	Stemness kinase inhibitor	Advanced colorectal CSC	Phase 1/2	[26]
BBI608	STAT3 inhibitor	Colorectal CSC	Phase 3	[27]
Berberine	Quaternary ammonium salt	Breast CSC, colorectal CSC	Phase 2/3	[28]
Catumaxomab (anti-EpCAM/ anti-CD3)	Bispecific antibody	CSCs in malignant ascites induced by human ovarian, gastric and pancreatic cancer	Phase 1/3	[3]
Cyclopamine,	Smo antagonist, Hedgehog pathway inhibitor	Glioblastoma CSC, multiple myeloma CSC, chronic myeloid leukemia SC, gastric CSC, breast CSC, prostate CSC, pancreatic CSC	Phase 1	[3, 29]
Curcumin, analog GO-Y030, diflurinated curcumin (CDF)	Wnt inhibitor; affects many CSC regulators (Hedgehog, Notch, PI3K/Akt/mTOR pathway.	Glioblastoma SC, colon CSC, pancreatic CSC, breast CSC	Phase 2	[3, 30]
Epigallocatechin gallate (EGCG) and its synthetic analogs	Downregulates mTOR pathway; activates AMPK and upregulates p21	Prostate CSC, pancreatic CSC, breast CSC, advanced solid tumors, SCLC	Phase 1/2	[3, 31]
G007-LK	Wnt inhibitor	Colorectal CSC	Preclinical	[32]
G244-LM	Wnt inhibitor	Colorectal CSC	Preclinical	[32]
Genistein	Wnt inhibitor	Colon CSC	Phase 2	[33]
IWR-1	Wnt inhibitor	NSCLC, colorectal CSC	Preclinical	[32]
JW55	Wnt inhibitor	Colorectal CSC	Preclinical	[32]
LDE-225[a]	Hedgehog inhibitor	SCLC CSC, pancreatic CSC, breast CSC, basal cell carcinoma	Phase 1	[34, 35]
LGK974	Porcupine inhibitor	Colorectal CSC	Phase 1	[36]
Metformin	Reduces EMT related ZEB1, TWIST1 and Slug	Breast CSC, pancreatic CSC, thyroid CSC, prostate CSC, solid tumors	Phase 1/3	[23, 37, 38]
Mithramycin	Telomerase inhibitor	Lung CSC,gastrointestinal CSC, Breast CSC	Phase 2	[39]
MT110	(Anti-EpCAM/anti-CD3) bispecific antibody	Colon CSC, pancreatic CSC, advanced solid tumors	Phase 1	[40]
OMP-21M18	Anti-DLL4 monoclonal antibody	NSCLC-SC, colon CSC, breast CSC	Phase 1	[41]
OMP-18R5	Frizzled −1, −2, −5, −7, −8 receptors	Intestinal solid tumor	Phase 1	[36]
Oxymatrine	Downregulates Wnt1, β-catenin, c-Myc, Cyclin D1, LEF1.	Breast CSC	Preclinical	[3, 42]
P245	Anti-CD44 monoclonal antibody	Breast CSC	Preclinical	[43]
Parthenolide, dimethylaminoparthenolide LC1	NF-B inhibitor, targets proto-oncogene tyrosine protein kinase *Src*	AML-SC, lymphoid leukemia SC, breast CSC, prostate CSC, myeloma-SC	Phase 1	[3]
PRI-724	CBP/Catenin antagonist	Advanced intestinal solid tumor SC	Phase 1	[36]
PTC-596	BMI-1 inhibitor	Advanced solid tumor SC	Phase 1	[44]
Resminostat	HDAC inhibitor	Advanced colorectal CSC	Phase1/2	[45]
Repertaxin	CXR1 and CXR2 inhibitor	Breast CSC	Preclinical	[3]
Resveratrol	Wnt inhibitor; upregulates miR622 and miR633	Medulloblastoma SC, breast CSC, pancreatic CSC, glioblastoma SC	Phase 1/2	[46]
Salinomycin	Inhibits ALDH, SOX2, CXCR4; reduces CD133, vimentin; induces E-cadherin	Breast CSC, AML SCs, GIST SC, gastric CSC, lung CSC, osteosarcoma SC, colorectal CSC, squamous cell carcinoma SC, prostate CSC, pancreatic CSC	Phase 1/2	[22]
Sulforaphane	Wnt inhibitor	Pancreatic CSC, breast CSC, prostate CSC, CML SC	Preclinical	[3, 47]
TG4010	Recombinant vaccine	NSCLC SC	Phase 2/3	[32]
Tranilast	Agonist for AHR; decreases Oct4, CD133	Breast CSC	Preclinical	[3]
Vismodegib (GDC 0449)	Smo antagonist, Hedgehog inhibitor	Pancreatic CSC, lung CSC, medulloblastoma, basal cell carcinoma, glioblastoma, chondrosarcoma, gastric carcinoma colon, ovarian, breast CSC	Phase 1/2	[3, 48]
WIKI4	Wnt inhibitor	Colon CSC	Preclinical	[32]
XAV939	Tankyrase inhibitor	Colon CSC	Phase 1	[33]

[a]FDA approved for basal cell carcinoma.

Abbreviations: EMT, epithelial-mesenchymal transition; miRs, microRNAs; HDAC, histone deacetylase; NSClC, non-small cell lung cancer; SCLC, small cell lung cancer.

resensitized MCF-7 breast CSCs to radiation [55]. Metformin also caused significant inhibition in both self-renewal and CSCs proliferation in MCF-7-derived mammospheres that are enriched for $CD44^+/CD24^{-/low}$ CSC populations [23]. Furthermore, metformin reduced expression of epithelial-mesenchymal transition (EMT)-related gene products like ZEB1, TWIST1, and SLUG in $CD44^+/CD24^{-/low}$ CSCs from four other breast cancer cell lines [37]. The selective effect of metformin on CSCs contrasted with a commonly used anticancer drug, doxorubicin. Doxorubicin significantly killed cancer cells but spared the CSCs [56]. When combined with metformin, doxorubicin caused a reduction in both cancer cells and CSCs from the heterogeneous tumor population, indicating the effectiveness of combination therapy [56]. In another study, metformin was shown to suppress the self-renewal and proliferation of trastuzumab-resistant human breast CSCs, and to act synergistically with trastuzumab in vitro [37]. Moreover, metformin also showed significant anti-TNBC effects both in vitro and in vivo. For example, MDA-MB-231 cell derived xenografts showed significant reduction in tumor outgrowth upon pretreatment with metformin [57].

Selective targeting of breast CSCs was also observed with salinomycin. Salinomycin inhibited mammary tumor growth in vivo and induced epithelial differentiation of tumor cells [15]. Compared to paclitaxel, salinomycin was 10-fold more potent in decreasing the number of tumorspheres and 100-fold more potent in reducing the CSC population in breast cancer cell lines [15]. More importantly, it enhanced the cytotoxicity of conventional cancer drugs like doxorubicin, gemcitabine, etoposide, paclitaxel, docetaxel, vinblastine, and trastuzumab, suggesting that it is worthwhile to explore and evaluate the usefulness of salinomycin-based combination therapies for breast cancer CSCs treatment [22, 58]. The combination of salinomycin with a histone deacetylase (HDAC) inhibitor LBH589 showed synergistic inhibitory effect on TNBC stem-like cells in vivo. In xenograft mouse models, this combination inhibited the tumor growth of ALDH1-positive cells by inducing apoptosis and cell cycle arrest [59].

Agonists of the retinoic acid receptor and peroxisome proliferator-activated receptor -γ selectively inhibited tumorspheres obtained from the MCF7 cell line by suppressing the activity of the NFκB/IL6 axis which is highly active in breast cancer derived tumorspheres. By contrast, normal mammary gland derived tumorspheres or nontumorigenic MCF10 cell lines were not inhibited [60]. Iinhibition of focal adhesion kinase (FAK) and related signaling pathways by genetic manipulation caused suppression of tumorigenesis and reduction in breast CSC progression in vivo [61]. Since these cells possess intrinsic chemoresistance [62], FAK-inhibitor based therapy may help to overcome drug resistance, eliminating breast CSCs and preventing breast cancer recurrence [21]. In this context, an anti-alcoholism drug disulfiram has cytotoxic effects in breast CSCs by interfering with self-renewal, apoptosis and by re-sensitizing breast CSCs to cytotoxic drugs [63]. Furthermore, recent studies reported that treatment with azithromycin and O-methylfunicone (OMF, a metabolite produced by *Penicillium pinophilum*) resulted in depletion of tumorsphere formation and CSCs population in breast cancer cell lines [25, 64]. It was demonstrated that, in contrast to cisplatin, OMF treatment caused a marked reduction in both the number and the size of tumorspheres as judged by the complete disappearance of CD24, CD29 CD44, CD133, and CD338 in breast CSCs. Moreover, OMF treatment resulted in induction of apoptosis and downregulation of Survivin, hTERT and NANOG expression demonstrating the effectiveness of OMF in selective targeting in breast CSC [25]. Additionally, recent studies have also suggested that the repertaxin may selectively target human breast CSC. Repertaxin treatment caused a marked decrease in breast CSCs population and their sphere-forming ability [65], and selectively inhibited IL-8 mediated EMT, angiogenesis, metastasis, and chemotherapy resistance in breast cancer [66]. Altogether, these findings provide evidence for the development of strategies to target the breast CSCs phenotype.

Colorectal Cancer

At present, conventional chemotherapy of colorectal cancer (CRC) is primarily based on 5-fluorouracil (5-FU), oxaliplatin, and irinotecan. However, the response rates of CRC to these chemotherapies are about 40%–50% and in many cases CSCs can survive the treatment [22]. CRC cells are highly dependent on constitutively active Wnt signaling for cell survival, growth, and differentiation [36]. CRC-SCs typically have high levels of β-catenin activity [15]. At present, several therapies are being evaluated for their ability to inhibit Wnt signaling [67], destabilize β-catenin or disrupt the β-catenin/TCF [53]. For example, resveratrol inhibits the Wnt pathway in colonic mucosa and thereby prevents cancer development [46]. It also downregulates several oncogenic microRNAs (miRs) and upregulates tumor suppressive miRs like miR-622 and miR-633 [68]. Recent advances also include the development of highly promising mAbs against the Wnt cascade, Fz receptors, or secreted Fz-related proteins for clinical use [69, 70].

Besides atypical activation of cellular signaling, CRC-SCs have been associated with resistance to therapy. Several studies revealed that the drug resistance of CRC-SCs may be overcome by pretreatment with HDAC inhibitors such as vorinostat or romidepsin [45, 71]. Vorinostat is a broad-spectrum HDAC inhibitor targeting class I, II, and IV HDACs, whereas romidepsin is a class I HDAC inhibitor. These inhibitors change the level of pro- and anti-apoptotic molecules, induce cell cycle arrest at G1/S or G2/M transition thereby enhancing differentiation and apoptosis [45]. Vorinostat and romidepsin treatment of CRC-SCs resulted in a reduction of Wnt expression and induced differentiation [71]. Therefore, these inhibitors may provide a novel way to make the CRC-SCs more susceptible to conventional chemotherapy [21].

Similar to its effect on breast CSCs, salinomycin also inhibited a number of CRC-SC characteristics including colonosphere formation, migration and invasion. It also selectively reduced the $CD133^+$ cell population by inducing E-cadherin downregulation and upregulation of vimentin in HT29 CRC cells [22]. Moreover, salinomycin induced the cytotoxicity and cell death of $CD44^+$ $EpCAM^+$ population in HCT116 CRC cells and in a dose dependant manner, inhibited growth of HCT116 xenografts in mouse models in vivo [72]. As a result, salinomycin drug is now in clinical trial phases 1/2 and initial evidence suggests it to be a potential drug for CRC. In recent years, cellular prion protein (PrPC) has also been considered as a promising target molecule for cancer therapies and mAbs targeting $CD44^+$ CRC-SCs expressing $PrPC^+$ inhibited metastatic capacity [73].

Porcupine and interferon (IFN) inhibitors may be used to target CRC-SCs. Porcupine inhibitors such as LGK974 inhibit palmitoylation of Wnt and since this is a required step in Wnt secretion and Wnt action. For example, such effect was mediated by suppression of tumor growth in murine xenografts established by mouse mammary tumor virus-driven Wnt1 expression [36]. IFN is a cytokine typically used in the treatment of viral diseases and it also has antitumor activity. In HT29 CRC cells, IFNα has been shown to suppress CRC-SCs self-renewal [74], by inducing apoptosis and differentiation.

CRC-SCs express the polycomb gene BMI-1, which is associated with poor patient outcome and resistance [21]. Treatment of primary CRC-derived xenografts with BMI-1 inhibitors like PTC-209, resulted in CRC-SCs loss with long-term and irreversible impairment of tumor growth [75]. Furthermore, the AP20187 treatment has increased 5-FU-induced cell death of CRC-SCs. AP20187 is a synthetic, cell-permeable drug that can induce dimerization of fusion proteins containing a growth factor receptor signaling domain. For example, along with increased apoptosis, a significant decrease in tumor size and $CD133^+$ CRC-SCs loss were observed in mouse models upon treatment with AP20187 in vivo [76].

Curcumin in combination with the widely used FOLFOX (Folinic acid, 5-FU, and oxaliplatin) regimen appeared to interfere with CRC-SCs [77]. It affects multiple CSC regulators including Wnt/β-catenin, Sonic Hh, Notch and PI3K/Akt/mTOR signaling, while sensitizing CRC-SCs [78, 79]. Increasing data suggest that the curcumin in combination therapy is highly effective in eliminating the CSCs in chemo-resistant colon cancer cells. The combination of curcumin and thetyrosine kinase inhibitor dasatinib, eliminated FOLFOX-resistant CRC-SCs more efficiently than the single agents [80]. Synthetic analogs including GO-Y030, GO-YY078 and difluorinated curcumin (CDF) have been developed to improve the bioavailability of curcumin, and these showed enhanced tumor suppression in vitro and in vivo in $Apc^{(580D/+)}$ mice [78]. These findings suggest that a nontoxic agent such as curcumin or its analog(s) by itself or together with the conventional chemotherapeutic could be an effective treatment strategy for preventing the emergence of chemoresistant colon cancer cells by reducing/eliminating CSCs.

Lung Cancer

Lung cancer is the most lethal form of cancer worldwide. About 80%-85% of lung cancers are non-small cell lung cancer (NSCLC). NSCLC grows and spreads more slowly than small cell lung cancer (SCLC) which makes up 15%-20% of the lung cancer cases. With the existing treatment options for lung cancer, 5 years overall survival rate is still very poor (<15%) [32]. Currently used treatment options for lung cancer include chemotherapy drugs such as cisplatin, etoposide, irinotecan, gemcitabine, and docetaxel. Emerging evidence indicates that in lung cancer, a number of current anticancer therapies may enrich stem-like cell subpopulations. For example, in NSCLC, even a low dose cisplatin treatment significantly enriches $CD133^+$ cells in H460 and H661 human NSCLC cell lines [32].

The presence of lung cancer SCs is usually evaluated by the expression of variety of CSC markers [32]. A number of studies have shown that the neuroendocrine cells in lung are the origin of lung CSCs and as such lung CSCs are highly responsive to neuropeptides. For example, Sarvi et al., study suggests that neuropeptide antagonists inhibit the tumor growth and selectively target the chemoresistant $CD133^+$ cells and inhibit tumor growth [81]. Likewise, trifluoperazine is a well-known antipsychotic drug that downregulates CD44 and CD133, and inhibits tumor growth and spheroid formation in lung cancer both in vitro and in vivo [82]. Combining trifluoperazine with conventional gefitinib or cisplatin could make the enriched $CD133^+$/$CD44^+$ lung stem-like cells responsive to therapy and downregulate gene signatures of drug resistance. Indeed, in mouse models of gefitinib-resistant CL97-L2G tumors, the combination of gefitinib with trifluoperazine suppressed tumorigenesis and exhibited the lowest tumor burden [82].

As with the other types of epithelial derived tumor, an elevated level of Notch is also associated with poor outcomes in NSCLC. Upregulation of Notch genes like Notch1, Notch2 and Hes-1 has been observed in $CD133^+$ NSCLC cells [32]. The cisplatin-induced enrichment of $CD133^+$ cells as mentioned before is also mediated through Notch signaling. A number of Notch inhibitors have been developed to decrease the ALDH+ components in lung cancer cells. The major Notch inhibitors in clinical analysis are the γ-secretase inhibitors. These inhibitors prevent release of intracellular domain into the cytoplasm and subsequent translocation to nucleus, thus inhibiting the Notch activation [83]. γ-secretase inhibitors like DAPT, MRK-003, and RO4929097 significantly decreased cisplatin-induced expression of transporter proteins ABCG2 and ABCB1 in lung cancer. This may be why these inhibitors increase sensitivity to cytotoxic drugs, particularly doxorubicin and paclitaxel [34]. Furthermore, Arasada et al., has also reported that γ-secretase inhibitors can reverse erlotinib enrichment of $ALDH^+$ cells in EGFR mutated lung cancer cell lines [83].

The Hh pathway has also been implicated in chemotherapy resistance in lung cancer and maintenance of lung CSCs [84]. The monoclonal antibody 5E1 directed against Shh-N inhibited the Hh signaling and decreased tumorigenicity in H249 and H1618 SCLC cell lines both in vitro and in tumor xenotransplants in nude mice [32]. Wnt inhibitors such as XAV-939, IWR-1, and Wnt-2 monoclonal antibody have been shown to downregulate canonical Wnt signaling and showed antitumor activity and induced apoptosis in NSCLC cells [35, 85]. Likewise, EMT was reversed by inhibitors such as PHA-665752 and PF-2341066 that blocked Met receptor phosphorylation in chemoresistant SCLC. The combination of etoposide and PF-2341066 showed a significantly decreased in tumor growth in previously resistant lung cancer cells [86].

In a study comparing salinomycin to paclitaxel, salinomycin showed inhibition of both tumorsphere formation and expression of ALDH, SOX2, CXCR4, and SDF-1 in lung adenocarcinoma A549 stem-like cells [22]. Even though paclitaxel initially decreases tumor volume, progressive treatment causes an increase in SC markers levels such as ALDH, CXCR4, and SDF-1 and promotes metastatic spread in in vivo models. However, this drug still requires further studies to address its efficacy in clinical settings [87].

It has been reported that stem cell factor (SCF) and its receptor c-kit have major role in lung CSCs and blocking SCF-c-kit leads to the inhibition of CSC proliferation and survival induced by chemotherapy [88]. Upon combination, however with cisplatin, this can eliminate both CSCs and bulk tumor growth in the heterogeneous tumor population [89].

Table 2. Drugs that are currently under evaluation to target (a) breast cancer, (b) colorectal cancer, (c) lung cancer, and (d) pancreatic cancer stem-like cells

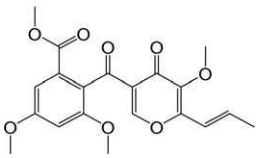
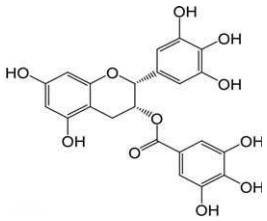
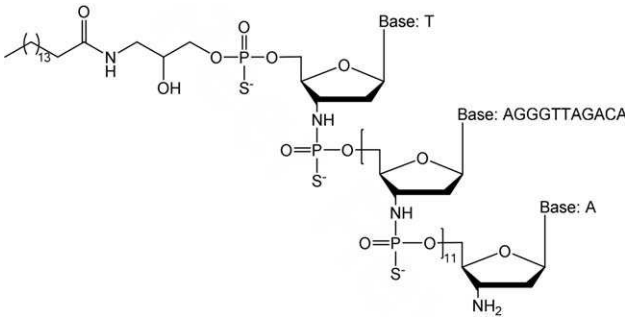
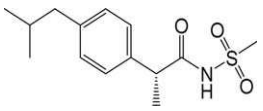
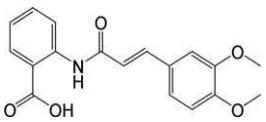
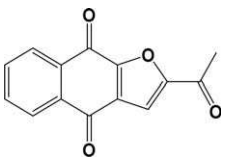
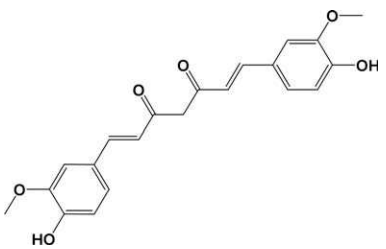
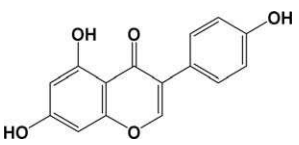
Compound	Molecular structure	References
(a) Breast cancer		
3-OMF (Pubchem CID: 10548301)		[25]
EGCG and its synthetic analogs (Pubchem CID: 65064)		[3, 31]
Imetelstat		[95]
Repertaxin (PubChem CID: 9838712)		[24, 65, 66]
Tranilast (PubChem CID: 5282230)		[3, 97]
(b) Colorectal cancer		
BBI-608, Napabucasin PubChem CID: 10331844		[36]
Curcumin PubChem CID: 969516		[30, 42, 78]
Genistein PubChem CID: 5280961		[33]

Table 2. *Continued*

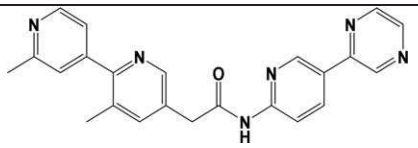
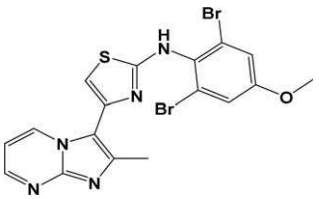
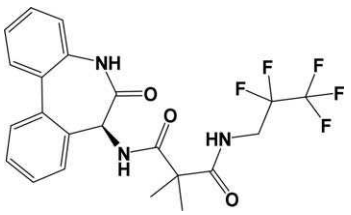
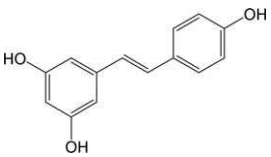
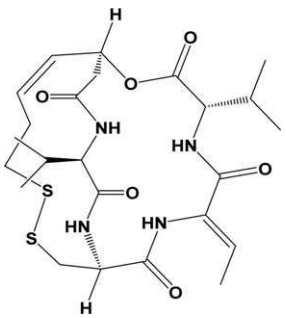
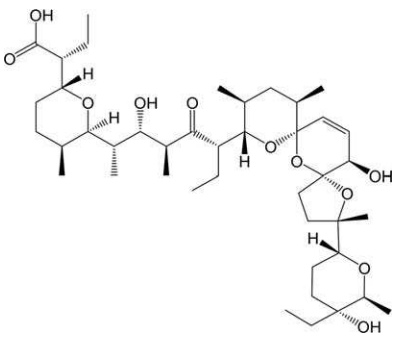
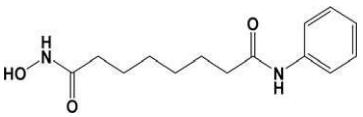
Compound	Molecular structure	References
LGK974 PubChem CID: 46926973		[36]
PTC-209 PubChem CID: 1117196		[44, 75]
RO4929097 PubChem CID: 49867930		[34]
Resveratrol PubChem CID: 445154		[46, 98]
Romidepsin PubChem CID: 57515973		[71]
Salinomycin PubChem CID: 6473797		[22, 53]
Vorinostat PubChem CID: 5311		[71]

Table 2. *Continued*

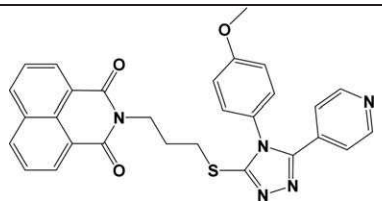
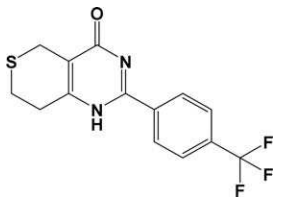
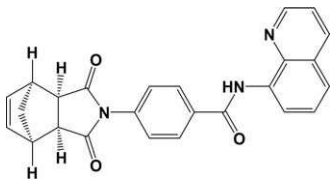
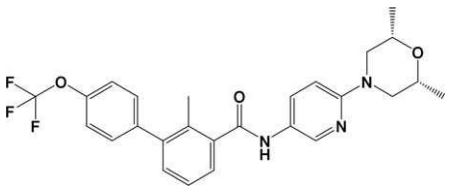
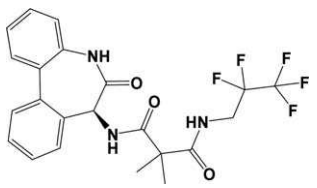
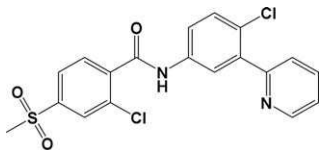
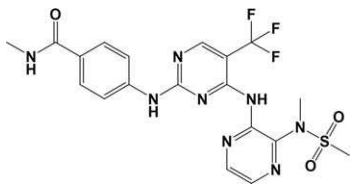
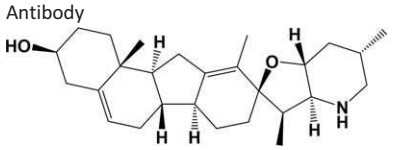
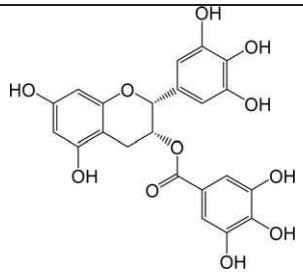
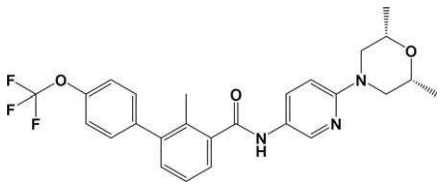
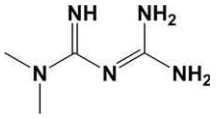
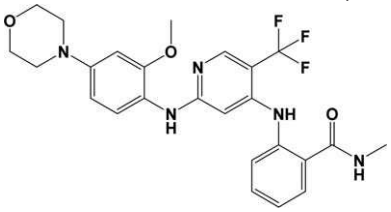
Compound	Molecular structure	References
WIKI4 PubChem CID: 2984337		[32]
XAV939 PubChem CID: 2726824		[99]
(c) Lung cancer IWR-1-endo PubChem CID: 91885421		[32]
LDE-225, Sonidegib PubChem CID: 24775005		[34, 35]
RO4929097 PubChem CID: 49867930		[34]
Vismodegib PubChem CID: 24776445		[3, 48]
VS-6063, Defactinib PubChem CID: 25117126		Clinical Trials identifier: NCT01951690
(d) Pancreatic cancer Catumaxomab	Antibody	[3]
Cyclopamine PubChem CID: 442972		[3, 29, 93]
		[31, 95]

Table 2. *Continued*

Compound	Molecular structure	References
Epigallocatechin gallate (EGCG) and its synthetic analogs (Pubchem CID: 65064)		
LDE-225* PubChem CID: 24775005		[34, 35]
Metformin PubChem CID: 4091		[37, 38]
MT110 VS-4718 PubChem CID: 25073775	Antibody 	[40] Clinical Trials identifier: NCT02651727

Recently, targeting ABC transporters with low molecular weight heparin (LMWH) has received much interest, particularly in the field of drug resistance. ABC transporters pump chemotherapeutic drugs out of the cell, conferring to resistance to chemotherapy. LMWH has been shown to reduce ABCG2 expression in six human lung cancer cell lines (REF). Such approach also induced apoptosis and eliminated CSCs when used in combination with cisplatin [90].

Telomerase has recently been demonstrated to be an essential factor for CSC immortalization [91]. Treatment with MST312, a telomerase inhibitor, significantly reduced the $ALDH^+$ CSC population and the length of the telomeres in these cells in vivo. MST312 has also been shown to induce p21, p27, and apoptosis in the whole tumor population in lung cancer [92]. As outlined above, recent progress in strategies in treatment-resistant lung cancer cells and the signaling cascades activated by CSCs are becoming increasingly important for monitoring the progress of cancer therapy and for evaluating new therapeutic approaches.

Pancreatic Cancer

In the development of pancreatic cancer, the Hh pathway plays an important rate-limiting role, and small molecule antagonists targeting the Hh pathway demonstrated a significant inhibition of metastasis in xenografts derived from human pancreatic cancer cell lines and pancreatic CSCs [53]. For example, vismodegib inhibits expression of Patched1, Patched2, and Smo, key components of the Hh pathway. Even though vismodegib only marginally affects tumor size initially, it significantly reduces the $ALDH^+$ cell population with stem cell properties eventually [93]. Another Hh pathway inhibitor, cyclopamine acts synergistically with gemcitabine to reduce the $ALDH^+$ population in pancreatic CSCs. The combination of cyclopamine and gemcitabine or combinations of cyclopamine and rapamycin with chemotherapy have been shown to decrease the proportion of CSCs in pancreatic cancer xenograft models [93]. Green tea epigallocatechin-3-gallate (EGCG) also has inhibitory effects on Hh pathway receptors Smo, Patched, Gli1 and Gli2. In pancreatic CSCs, EGCG inhibits a number of pluripotency-maintaining transcription factors such as Nanog, c-Myc, and Oct4, therefore efficiently targeting CSCs [48]. A plant-derived flavonoid, quercetin, is also effective in inhibiting proliferation, self-renewal and EMT as well as in inducing apoptosis of pancreatic CSCs without causing any distinct toxicity to normal cells [94].

The telomerase inhibitor imetelstat causes telomere shortening and inhibition of telomerase in PANC1 pancreatic cancer cells and prolonged treatment with imetelstat resulted in reduced CSCs from bulk tumor cells in tumor engraft xenograft studies [95]. Likewise, salinomycin in combination with gemcitabine was reported to be more effective in eliminating pancreatic CSCs in xenografted mice more than either

salinomycin or gemcitabine alone [22]. Salinomycin inhibited the growth of CD133-expressing CSCs in tumorspheres while gemcitabine inhibited the growth of CD133-negative non-CSCs. Hence, the combination shows more potential in treating human pancreatic cancer than single agents [22].

Like in many other types of cancer, metformin targets self-renewal in pancreatic CSCs and interferes with some key transcription factors for CSC maintenance, such as Notch1, Nanog, Oct4, and EZH2 [38]. It decreases the mRNA levels of these factors and causes re-expression of the miRs of let-7 and miR-200 family, which are lost in pancreatic CSCs. All these effects eventually inhibit CSC proliferation, self-renewal, migration and invasion in pancreatic CSCs [38]. Moreover, in pancreatic CSCs derived from human primary tumors, resveratrol-induced apoptosis and resensitization, inhibited EMT, and suppressed self-renewal capacity accompanied by downregulation of Bcl-2, XIAP, Zeb-1, Slug, Snail, ABCG2, Nanog, Sox-2, c-Myc, and Oct4 [96]. Consistent with these effects, resveratrol also exhibited a reduction in pancreatic tumor growth in KrasG12D mice [96].

Sulforaphane also induces apoptosis and prevents tumorsphere formation in pancreatic cancer cells. It specifically binds to the transcriptionally active NF-κB complexes and inhibits NF-κB-mediated anti-apoptotic signaling in $CD24^{+}$ $CD44^{-}$ pancreatic CSCs [47].

Table 2 shows the summery of these key compounds or drugs currently being evaluated for the abovementioned CSCs.

Conclusion

Many advances have been made in the field of targeted therapy for CSCs. So far, approaches identified have included targeting specific markers or signaling pathways to eliminate the CSCs, altering their microenvironment, or reprogramming CSCs for differentiation, re-sensitization to chemotherapy, apoptosis, and reversal of EMT or to reduce metastasis. However, bringing evaluating these approaches in a clinical setting remains a challenge.

For example, not all CSCs express markers. There may also be non-CSC cancer cells that express the markers [20]. Hence, CSC populations are constantly being rationalized and revised for the identification of new markers. Questions about optimum mechanisms to target in different stages of cancer or the extent to which surface markers can be justified enough to distinguish CSC populations still remain to be addressed. In this context, there is a need to further develop new methods and improve existing models for isolating, identifying and targeting CSCs. The recent advent of culturing of three-dimensional (3D) spheres and organoids in a dish that allow both normal and cancer stem cells to grow as they would do in live organisms, can address CSCs associated gene(s) functions and their use in CSC specific drug screening and drug resistance studies [100–102]. It is also important to develop well-defined microenvironments for 3D spheroid and organoid culture with intricate cell-cell and cell-matrix. Such tools can then be exploited in successfully inducing CSCs differentiation to reprogram toward a more differentiated phenotype cancer cells. On the other hand, increasing evidence suggests that combination therapies targeting both CSC and differentiated cancer cells will be more effective [17]. Successful designing of innovative targeting strategies, evaluating clinical efficacy, risk-benefit ratio, and preclinical toxicity of the newly identified drugs will also be required in this regard.

New approaches will increasingly require combinations of targeting strategies against CSCs. These may include manipulating CSC programming by differentiation-inducing agents or chemo-sensitizing agents and combining them with conventional chemotherapy drugs to eliminate cancer cells. Such combinations would exert the antitumor effect more selectively with minimum effects on normal cells. Future clinical trials must be designed with competitive biological and clinical endpoints with the aim of providing highly effective therapies in patients with all stages of cancer.

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Author Contributions

M.A.: conception and design, manuscript writing, collection and/or assembly of data and final approval of manuscript; K.C.: conception and design and final approval of manuscript; R.B.-J. and L.V.D.: collection and/or assembly of data and final approval of manuscript; A.S.N.: conception and design, manuscript writing, collection and/or assembly of data, final approval of manuscript, financial support, and administrative support.

Disclosures of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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