Concise Reviews: Cancer Stem Cell Targeted Therapies: Toward Clinical Success

AMAR DESAI, a,b YAN YAN, a,b STANTON L. GERSON a,b

Key Words. Cancer stem cells • Epigenetics • Immunotherapy • Metastasis

ABSTRACT

Cancer stem cells (CSCs) are a subpopulation of cells within tumors that possess the stem cell characteristics of self-renewal, quiescence, differentiation, and the ability to recapitulate the parental tumor when transplanted into a host. CSCs are correlated with poor clinical outcome due to their contribution to chemotherapy resistance and metastasis. Multiple cell surface and enzymatic markers have been characterized to identify CSCs within a heterogeneous tumor, and here we summarize ongoing preclinical and clinical efforts to therapeutically target these cells and improve patient outcomes.

INTRODUCTION

Cancer stem cells (CSCs) are a small subpopulation found within the heterogenous bulk of solid and liquid tumors. They are broadly characterized as demonstrating the stem cell properties of asymmetric division, possessing the ability to reconstitute a differentiated tumor upon transplantation, participating in the epithelial-mesenchymal transition via induction of genetic programs, and contributing to resistance to traditional chemotherapeutic regimen due to upregulation of DNA repair components and drug efflux transporters. Additionally, CSCs are believed to play a critical role in the onset of tumor relapse and metastasis. As described below, CSCs have been reported in a variety of tumor types including those of leukemia, breast, brain, colon, and lung, and although the markers and driver pathways vary among tumor types, the general stem cell characteristics and their roles in therapy resistance appear conserved. CSCs often demonstrate the re-expression of embryonic factors including Sox2, Oct4, Nanog, and Dnmt1, display distinct metabolic profiles from terminally differentiated tumor cells, and reside in specialized hypoxic microenvironments that contribute to long-term maintenance [1–4]. CSC specific therapies have long been proposed in conjunction with traditional chemotherapeutic regimen to kill both differentiated and CSC populations and prevent subsequent relapse (Fig. 1). As such, a number of clinical trials are underway to determine the efficacy of CSC specific therapeutics (Table 1, adapted from [5]). Here, we describe various therapeutic approaches toward targeting CSC populations to potentially affect tumor relapse and metastasis.

LEUKEMIA STEM CELLS

CSCs were first described by the lab of John E. Dick almost 25 years ago via transplantation of human acute myeloid leukemia (AML) cells into SCID mice and observing these cells homed to the bone marrow niche and proliferated to reproduce disease similar to that seen in the original patient. Limiting dilution studies identified that the frequency of these leukemia stem cells (LSCs) was one engraftment unit in 250,000 and that these cells expressed the same markers as normal human adult stem cells (CD34+/CD38−), indicating a normal cell was the target of leukemic transformation [6]. Further work has led to a general consensus that LSCs...
The concept of the cancer stem cell (CSC). Tumor cells are heterogeneous which contain a majority of cells are non-/poorly tumorigenic, and a small subset of CSCs. The CSCs can be functionally distinguished from other populations by their ability to reconstitute a differentiated tumor upon transplantation into an immunocompromised mouse. Based on this model, CSC specific therapies are proposed in combination with conventional chemotherapies to kill both CSC and other differentiated populations and prevent subsequent relapse.

CSCs arise from the transformation of hematopoietic stem or progenitor cells toward a LSC capable of self-renewal and differentiation, and while the surface markers have changed considerably over time. There are several works demonstrating that true AML LSCs are some combination of CD90(−)/CD117(−)/CD123(+)/TIM3(+)/CD47(+)/CD96(+)/CLL-1(+)/IL1RAP(+) [7-14]. In most instances, they appear distinguishable from normal hematopoietic stem cells.

Because conventional chemotherapeutic strategies appear unable to completely eradicate LSCs, the identification of the cell surface markers listed above has led to a number of promising therapeutic candidates to specifically target and kill the LSC populations. Multiple groups have targeted the alpha chain of the interleukin 3 receptor (IL-3RA or CD123) due to its presence on blasts and LSCs of many hematopoietic malignancies including AML, myelodysplastic syndrome, B-cell acute lymphoblastic leukemia, Hodgkin’s lymphoma, and others. Busfield et al. describe the promise of the monoclonal antibody CSL362 which targets CD123 and demonstrates in vivo efficacy in AML mouse xenografts and single IV dose in cynomolgus monkeys depleted dendritic cells (DCs) and basophils [15]. He et al. report the first-in-human trial (NCT00401739) of a CD123 monoclonal antibody, CSL360, in high-risk AML patients but only demonstrated limited anti-leukemic activity [16]. This study spurred an interest in adopting multiple modalities to block CD123 in hematological neoplasms, such as SL-401 a fusion protein composed of human IL-3 and truncated diptheria toxin that directly target CD123 [17], cell depleting strategies by bispecific antibodies [18, 19], and more recently CART123 [20]. All these preclinical studies appear similarly promising as novel anti-leukemia approaches by targeting CD123.

In addition to surface marker therapies, other strategies involve manipulation of the bone marrow microenvironment to disrupt the LSC niche and communication with bone marrow cells. It is believed that by disrupting this niche, the LSC populations will become sensitive to traditional chemotherapy treatment. One approach involves targeting the CXCR4/CXCL12 interaction which is critical for LSC homing to the bone marrow niche using the CXCR4 antagonist Plerixafor. This has shown to be safe in treating AML in phase 1/2 trials in combination with etoposide or cytarabine therapy [21, 22]. Additional LSC specific therapies have demonstrated preclinical success including delivery of parthenolide nanoparticles to the bone marrow niche to inhibit NF-kB activity [23], and novel combination therapies that include targeting the mTOR, and PI3K pathways [24]. Together, these preclinical findings provide strong evidence that targeting LSCs will have significant promise for clinical trials treating primary and relapsed disease.

**Table 1.** Ongoing clinical trials of CSC-targeted agents

| Trial               | Target                                                                 | Status               |
|---------------------|------------------------------------------------------------------------|----------------------|
| NCT02753127         | BBI-608 (STAT3 inhibitor) + FOLFIRI I metastatic colorectal cancer       | Enrolling phase 3    |
| NCT01190345         | Bevacizumab (anti VEGF) + conventional therapy in breast cancer         | Phase 2 complete     |
| NCT01579812         | Metformin (Type 2 anti-diabetic) + conventional therapy in ovarian, fallopian tube, and primary peritoneal cancer | Phase 2              |
| NCT01624090         | Mitomycin (RNA synthesis inhibitor) in lung, esophageal, mesothelioma, breast cancer | Phase 2              |
| NCT01861054         | Reparixin (inhibitor of CXCR1 and CXCR2) in breast cancer               | Phase 2 (terminated) |
| NCT01195415         | Vismodegib (Hedgehog inhibitor) + Gemcitabine in advanced pancreatic cancer | Phase 2 complete     |
| NCT00645333         | MK-0752 (γ-secretase inhibitor) + Docetaxel in metastatic breast cancer | Phase 2 complete     |
| NCT01088815         | GDC-0449 (Hedgehog inhibitor) + conventional therapy in metastatic pancreatic cancer | Phase 2              |
| NCT02370238         | Paclitaxel + Reparixin in metastatic triple negative breast cancer       | Phase 2              |
| NCT02279719         | BBI608 (STAT3 inhibitor) + Sorafenib or BBI503 (Nanog inhibitor) + Sorafenib in advanced hepatocellular carcinoma | Phase 2              |
| NCT01951690         | VSO6063 (FAK inhibitor) in KRAS mutant non-small cell lung cancer        | Phase 2 complete     |

Adapted from [5].

© 2018 The Authors. Published by Wiley Periodicals, Inc. on behalf of AlphaMed Press.
first identification of a CSC population in solid tumors. These cells were able to repopulate tumors in mice with as few as one hundred cells, whereas hundreds of thousands of cells with alternate phenotypes were incapable of forming tumors. These BCSCs were able to recapitulate the cellular heterogeneity of the original tumor and remained tumorigenic after multiple rounds of transplantation, suggesting this population contains the self-renewal and differentiation properties of normal stem cells [25]. Multiple theories exist regarding the origin of BCSCs including the accumulation of mutations that transform normal stem cells to CSCs [26], the “misplacement” of normal tissue stem cells that do not undergo transformation into connective tissue stroma [27], failure of the mitochondrial respiratory chain resulting in transformation [28], or the acquired phenotype of increased quiescence and stemness via alteration of DNA repair or other signaling pathways [29]. Consistent with CSC characterization, a number of signaling pathways have been associated with the therapy resistance phenotype of BCSCs including Notch, Hedgehog, and Wnt which promote apoptosis evasion, maintenance of a stem cell niche, and increased invasion capacity [30–32].

Many therapeutic approaches to specifically target the BCSC populations have yielded intriguing preclinical results. Doherty et al. recently published that treating triple negative breast cancer (TNBC) cells with interferon beta represses the CSC properties, including decreased expression of mesenchymal proteins, reduced migration, and tumor sphere formation, with re-expression of CD24 promoting an epithelial phenotype [33]. Lu et al. described the role of chemotherapy-induced secretion of glutathione S-transferase omega 1 which increases intracellular calcium levels, activates STAT3 signaling, and enriches the BCSC microenvironment. They describe the therapeutic potential of GSTO1 knockdown to specifically target the BCSC populations and reduce tumor invasiveness and metastasis [34]. Shi et al. illustrated the use of the type 2 diabetes therapy Metformin to reduce the frequency of BCSCs in TNBC by targeting KLF5 for degradation and preventing the activation of its downstream target genes Nanog and FGF-BP1. This FDA approved drug could have enormous potential in the TNBC population [35]. Many additional approaches have yielded success in BCSC targeted therapies including breaking the CSC reliance on autophagy to sensitize the cells to chemotherapy [36], targeted delivery of iron oxide nanoparticles to CD44+ cells for the selective killing of BCSCs using conventional chemotherapy [37] and understanding the role of miRNA expression in contributing to chemoresistance, with miRNA targeting as a potential future therapeutic direction [38]. Nonetheless, no agents are yet approved for CSC targeted therapy in breast cancer.

**Brain Cancer Stem Cells**

Brain tumors are aggressive cancers that account for the leading cause of cancer death in children and continue to have a poor prognosis with the median survival time 12–18 months for glioblastoma multiforme (GBM). The cellular heterogeneity is a hallmark of brain tumors with an increasingly well-defined population of CSCs being established. The first description of brain CSCs was provided by Ignatova et al. when comparing the ability of clinical specimen of glial tumors and normal brain sections to generate neurosphere clones under anchorage and serum withdrawal in methylcellulose media. They identified that glial tumors possessed a population of cells with similar characteristics to normal brain neural stem cells that could contribute to the hyperplastic growth of these tumors [39]. Since this finding, multiple groups have identified characteristic markers enriching for brain tumor CSCs including CD133 [40], integrin alpha 6 marker CD49 [41], CD36 [42], and L1CAM [43] among others. These markers are primarily used to distinguish adult brain CSCs, although the expression varies and markers alone are not indicative of a CSC population. Despite pediatric and adult brain tumors are dramatically different diseases, the markers CD133 and CD49f have been found to be expressed on CSC populations in both tumor types. Thus, therapeutically targeting CD133 in an adult tumor may have significantly different results than doing the same in a pediatric one.

Current efforts at targeting the CSC populations in brain tumors have demonstrated some preclinical success. The Hif-1alpha and Jak1/2-Stat3 pathways have recently been exploited due to the idea that the hypoxic microenvironment of glioblastoma stem cells enhances the self-renewal capacity of these CSCs, with VEGF playing a supportive role in maintaining the stemness of these cells. Brefeldin A and EHT-1864, two agents that prevent the secretion of VEGF, were found to decrease CSC self-renewal potential and inhibit tumor growth in part by decreasing the hypoxic gene expression signature of these cells [44]. Jin et al. describe the role of the glioblastoma CSC niches and characterize a proneural CSC that resides in vascular spaces and a mesenchymal CSC that resides in hypoxic regions. The proneural CSCs displayed activated EZH2 while the mesenchymal CSCs expressed high BMI1 levels, with each population demonstrating sensitivity to inhibition of the two proteins, and combination therapy showing the highest killing effect in cells and in mice. This study suggests that more detailed understanding of the heterogeneity of the CSCs themselves will lead to more effective therapeutic strategies. Recently, Talukdar et al. describe the role of protective autophagy in glioblastoma stem cells, which prevents anoxia mediated cell death in nonadherent conditions, and the importance of MDA-9/Syntenin in maintaining this protective autophagy effect. The authors propose that MDA-9 promotes EGFR signaling which inhibits autophagy markers and together with BCL2 promotes survival of the CSC populations. Loss of MDA-9 resulted in elevated autophagy and CSC cell death due to disrupted EGFR signaling and downregulated BCL2. This study suggests that disrupting protective autophagy could be a valuable tool to specifically target glioblastoma CSCs.

In addition of targeting signaling pathways, different formulations of DC-based immunotherapy that specifically target against glioblastoma CSCs have been studied in multiple clinical trials and more are underway. In a clinical trial, GBM patients were administered with ICT-107, a patient-specific DC vaccine developed by pulsing with six synthetic peptides derived from tumor associated antigens present on brain CSCs [45]. The study showed promising results with favorable safety and all patients expressed at least three of the immunizing antigens in the therapy of ICT-107 vaccine (NCT01280552). This phase 1 clinical trial ensures a 10-year follow-up demonstrated 19% of 16 patients with disease free for 8 years and a median overall survival of 38.4 months. In another study, patients were treated with autologous DCs transfected with whole CSC-mRNA prepared from brain tumor biopsies (NCT 00846456). T cells specific to hTERT- or survivin-derived peptides
were found in all patients after this vaccine strategy and a 2.9-fold increase in progression-free survival was reported in patients with GBM [46]. Studies such as these suggest that vaccination against glioblastoma CSC is well tolerated and may be more effective than conventional therapies which need larger scale investigation.

**Additional Cancer Stem Cell Populations**

**Colon Cancer Stem Cells**

Colon CSCs were originally described by Ricci-Vitiani et al. after identifying the presence of CD133+ cells in freshly dissociated human colon adenocarcinoma cells. These cells constituted approximately 2.5% of the total tumor and serially reproduced the original tumor when transplanted into mice, whereas the CD133− population was unable to do so [47]. Subsequent work has focused on identifying CSC specific therapies in colorectal cancer, in part due to the rising spread of incidence and ~50% mortality rate worldwide [48]. In addition to CD133 targeting that has shown preclinical success via targeted nanoparticle delivery [49], reports have identified CD44 as enrichment for CSC-like properties [50], CD26+ cells capable of initiating tumor formation and facilitating EMT [51], and a potential role for the LGR5+ normal intestinal stem cells serving as part of a colon microenvironment capable of promoting the initial stages of tumorigenesis [52]. In fact, Shimokawa et al. show that LGR5+ cells serve as CSCs in human colon cancers, and that its ablation results in transient tumor regression that yields a higher frequency of reemerging LGR5+ cells. Thus combination therapies targeting both LGR5+ cells and differentiated cancer cell types could prevent tumor resistance and relapse [53]. Additional colon CSC markers are being explored as potential therapeutic targets to improve clinical treatment options, as is the development of culturing patient derived cells as organoids to understand disease progression and identify novel therapeutic strategies [54, 55]. Besides targeting surface and enzymatic CSC markers, blocking CSCs at the point of a more fundamental level is now another emerging approach. For example, key regulators of cancer stemness such as STAT3 is considered as a promising therapeutic target. Napabucasin (BBI608 or BB608) is a small-molecule STAT3 inhibitor known to directly inhibit STAT3-driven gene transcription as well as spherogenesis [56, 57]. In an in vivo mouse model of colon cancer, napabucasin effectively blocked spleen and liver metastases and dampened signaling pathways such as c-Myc, β-catenin, NANOG, and Sox2 that implicated in supporting the stemness of CSCs [37]. Napabucasin demonstrated encouraging anticancer activity in phase 1 and 2 trials (NCT01325441, NCT02024607, and NCT02983578) against multiple cancers as both monotherapy and in combination with standard chemotherapies. Current phase 3 clinical trial of napabucasin (NCT02753127) is ongoing in a combinatory setting with standard chemotherapy FOLFIRI to treat advanced colon cancer. Targeting cancer stemness is a novel approach and may prove to be the next-generation anti-cancer therapy to decrease cancer recurrence.

**Lung Cancer Stem Cells**

The concept of lung CSCs was first described by Carney et al. who reported that less than 1.5% of tumor cells from lung adenocarcinoma patients were capable of forming colonies in vitro and reconstituting tumors when transplanted into nude mice [58]. Multiple markers, including some that overlap with additional CSC subtypes, have since been identified in lung cancer including CD44 [59], CD166, and ALDH1 [60]. Therapeutic strategies have included the use of the double stranded RNA mimetic of microRNA miR-34A which inhibits clonogenic expansion and tumor regeneration when expressed in CD44+ cells [61], the pan-ALDH1 inhibitor Disulfiram (which is also being clinically tested in other CSC types) [62, 63], a monoclonal antibody DKN-01 which targets dikkopep-1 and showed a promising 6 month survival increase in phase 1 clinical trial [64], and GDC-0449 a sonic hedgehog inhibitor that demonstrated efficacy in multiple cancer types [65]. Importantly, CSC populations have been well described in a number of other cancer types including prostate, renal, skin, and bladder. Although the markers vary between cancer cell types, the take-home message unifying these cell types is that this small population of cells is drivers of tumorigenesis and metastasis, and targeted therapies continue to be an incredibly promising direction with which to more effectively treat disease.

**Potential for Immunotherapy Treatment of CSCs**

In addition to targeted small molecule inhibitors to improve CSC-specific therapies, the onset of immunotherapy has led to exciting developments in exploiting CSC specific antigen presentation to harness the power of the immune system to improve cancer therapies. In addition, antigen nonspecific targeting has shown early success. An example of this is the potential strategy utilizing the notion that CSCs often display lower MHC class 1 molecules than the bulk tumor and thus escape immune targeting by cytotoxic T cells. Certain CSC types, such as those found in glioma and colorectal cancer, have been shown to express NK cell-specified ligands such as poxivirus receptor and Nectin-2 that lead them susceptible to IL2 or IL15 activated NK cell killing [66]. Gamma-delta T cells, which also do not require MHC presentation for activation, have also been studied for their ability to target CSC populations. Chen et al. describe a unique combination therapy in which BCSCs are sensitized to gamma-delta T cells by pre-treatment with zoledronate. The gamma-delta T cells subsequently upregulate MHC1 and CD54 on these cells which results in subsequent sensitization to CD8+ T cells. This combination treatment that targets the CSCs at multiple stages of differentiation may yield promising results in multiple cancers [67].

Specific T-cell priming to target CSC-specific antigens is a promising approach to target the small population of CSCs found in bulk tumors. Miyamoto et al. recently described identification of an antigen ASB4 found specifically on a subset of CSCs in colorectal cancer, but not on the bulk tumor. Adoptively transferred CD8+ cytotoxic T cells specific for ASB4 were able to infiltrate mouse colorectal tumors and prevent growth. It is plausible to hypothesize that combining primed CD8+ cells with traditional chemotherapy could lead to killing of both bulk and CSC populations in tumors [68]. Continuing to identify antigen specific to CSCs over bulk tumors, such as BORIS sf6 in cervical cancer which can be targeted using a BORIS C34_24(9)-specific cytotoxic T cell [69] and DNAJ-B8, an HSP40 family member implicated in the formation of renal CSCs [70] are important future directions for the success of immunotherapy. Other novel approaches include the CD133 DC immuno-therapy ICT-121, which showed promising phase 1 data by
Desai, Yan, Gerson 79

immune responses to a variety of cancers. The Notch signaling pathway is one that has been extensively explored as a CSC target for multiple tumor types. The pathway is activated upon ligand binding to the Notch receptor, which is subsequently cleaved by ADAM family proteases and γ-secretase to release the Notch intracellular domain (NICD). The NICD translocates to the nucleus, binds to the transcription factor CSL, and converts the complex from a repressor to an activator of Notch genes. Notch activation has been proposed as vital to CSC populations by maintaining stemness, enhancing therapy resistance, and promoting a hypoxic niche [72]. Therapeutic potential using γ-secretase inhibitors and antibodies directed toward the receptor have demonstrated clinical success, as demonstrated by ~15 completed clinical trials examining γ-secretase inhibitors in multiple cancers including breast, pancreatic, colorectal, and renal cell carcinoma [73].

Epigenetics
The role of epigenetics in CSC formation, regulation, and function has begun to emerge. During oncogenic transformation, alterations in DNA methylation and chromatin can be modulated by both intrinsic and extrinsic cues, and such modifications have been shown as drivers of CSC formation. For instance in mixed lineage leukemia (MLL)-associated leukemia, chromosomal rearrangements in the histone methyltransferase KMT2A/MLL have been implicated in the formation of LSCs [74]. The DNA methyltransferase family of DNMT1, DNMT3A, and DNMT3B, which are responsible for methylating CpG dinucleotides, are mutated in ~25% of AML patients and lead to the expansion of leukemic stem cells, as does loss of function of the TET proteins which antagonize the DNMT family, suggesting that any disruption of steady state methylation patterns can affect CSC formation. A plethora of other examples implicating epigenetic regulators as drivers of CSCs exist such as H1.0, EZH2, BMI1, and DOT1L [75]. Because of the reversible state of epigenetic mechanisms, there is enormous therapeutic potential of targeting enzymes responsible for DNA and chromatin modifications. Of interest is the preclinical success of inhibitors of bromodomain and extra-terminal motif proteins which have demonstrated selectivity to tumor cells by preferentially binding superenhancer regions, and have potential to exert CSC specific effects in combination with other therapies [76].

CONCLUSION
The volume of preclinical and clinical evidence pointing to the importance of CSCs in cancer progression, relapse, and metastasis suggests that targeted therapies may be the best approach toward a comprehensive treatment regime. We believe the vast number of publications, only a few highlighted above, show that the field is certainly progressing in the right direction and that clinically approved CSC-targeted therapies for the treatment of a number of cancer types is within sight.

AUTHOR CONTRIBUTIONS
AD and SLG: wrote and finalized the manuscript; YY: research support, editing, and contributed to writing sections of the manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST
A.D. declared consultancy role for Rodeo Therapeutics. The other authors indicated no conflicts of interest.

REFERENCES
1 Putzer BM, Solanki M, Herchenroder O. Advances in cancer stem cell targeting: How to strike the evil at its root. Adv Drug Deliv Rev 2017;120:89–107.
2 Wang A, Qu L, Wang L. At the crossroads of cancer stem cells and targeted therapy resistance. Cancer Lett 2017;385:87–96.
3 Dragu DL, Necula LG, Bleotu C et al. Therapies targeting cancer stem cells: Current trends and future challenges. World J Stem Cells 2015;7:1185–1201.
4 Batle E, Clevers H. Cancer stem cells revisited. Nat Med 2017;23:1124–1134.
5 Annett S, Robson T. Targeting cancer stem cells in the clinic: Current status and perspectives. Pharmacol Ther 2018;187:13–30.
6 Lapidot T, Sirard C, Vormoor J et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994;376:645–648.
7 Askmyr M, Agerstam H, Hansen N et al. Selective killing of candidate AML stem cells by antibody targeting of IL1RAP. Blood 2013;121:3709–3713.
8 Blair A, Sutherland HJ. Primitive acute myeloid leukemia cells with long-term proliferative ability in vitro and in vivo lack surface expression of c-kit (CD117). Exp Hematol 2000;28:660–671.
9 Bruserud O, Aasebo E, Hernandez-Valladares M et al. Therapeutic targeting of
leukemic stem cells in acute myeloid leukemia—The biological background for possible strategies. Expert Opin Drug Discov 2017;12:1035–1045.

10 Hosen N, Park CY, Tatsumi N et al. CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. Proc Natl Acad Sci U S A 2007;104:11008–11013.

11 Jaiswal S, Jamieson CH, Pang WW et al. CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. Cell 2009;138:271–285.

12 Jin L, Lee EM, Ramshaw HS et al. Monoclonal antibody-mediated targeting of CD123, IL-3 receptor alpha chain, eliminates human acute myeloid leukemic stem cells. Cell Stem Cell 2009;5:31–42.

13 Kikushige Y, Shima T, Takayanagi S et al. TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells. Cell Stem Cell 2010;7:708–717.

14 van Ravenen A, van Dongen GA, Kelder A et al. The novel AML stem cell associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells. Blood 2007;110:2659–2666.

15 Busfeld SJ, Biondo M, Wong M et al. Targeting of acute myeloid leukemia in vitro and in vivo with an anti-CD123 mAb engineered for optimal ADCC. Leukemia 2014;28:2213–2221.

16 Lee M, Wicha MS, Benito-Hernandez A et al. Dissecting the origin of breast cancer subtype stem cell and the potential mechanism of malignant transformation. PLoS One 2011;6:e1965001.

17 Bozorgi A, Khazaie M, Khazaie MR. New findings on breast cancer stem cells: A review. J Breast Cancer 2015;18:303–312.

18 de Sousa, E. M. F., and Vermeulen, L. (2016) Wnt signaling in cancer stem cell biology. Cancers (Basel)8:1–18.

19 Mamaeva V, Niemi R, Beck M et al. Inhibiting notch activity in breast cancer stem cells by glucose functionalized nanoparticles carrying gamma-secretase inhibitors. Mol Ther 2016;24:926–936.

20 Cochrane CR, Szczepny A, Watkins DN et al. Hedgehog signaling in the maintenance of cancer stem cells. Cancers (Basel) 2015;7:1554–1585.

21 Doherty MR, Cheon H, Junk DJ et al. Interferon-beta represses cancer stem cell properties in triple-negative breast cancer. Proc Natl Acad Sci U S A 2017;114:13792–13797.

22 Lu H, Chen I, Shimoda LA et al. Chemotherapy-induced Ca(2+) release stimulates breast cancer stem cell enrichment. Cell Rep 2017;18:1946–1957.

23 Shi P, Liu W, Tala et al. Metformin suppresses triple-negative breast cancer stem cells by targeting KEFS for degradation. Cell Discov 2017;3:17010.

24 Bousquet G, El Bouchtaoui M, Sophie T et al. Targeting autophagic cancer stem-cells to reverse chemoresistance in human triple negative breast cancer. Oncotarget 2017;8:35205–35221.

25 Aires A, Ocampo SM, Simoes BM et al. Multifunctionalized iron oxide nanoparticles for selective drug delivery to CD44-positive cancer cells. Nanotechnology 2016;27:065103.

26 Sun X, Li Y, Zheng M et al. MicroRNA-223 increases the sensitivity of triple-negative breast cancer stem cells to TRAIL-induced apoptosis by targeting MAX-1. PLoS One 2011;6:e1062754.

27 Ignatova TN, Kukkovek VG, Laywell ED et al. Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro. Glia 2002;39:193–204.

28 Hemmati HD, Nakano I, Lazzareff JA et al. Cancerous stem cells can arise from pediatric brain tumors. Proc Natl Acad Sci U S A 2003;100:15178–15183.

29 Lathia JD, Gallagher J, Heddleston JM et al. Integrin alpha 6 regulates glioblastoma stem cells. Cell Stem Cell 2010;6:421–432.

30 Hale JS, Otvos B, Sinyuk M et al. Cancer stem cell-specific scavenger receptor CD36 drives glioblastoma progression. Stem Cells 2014;32:1746–1758.

31 Bao B, Ahmad A, Li Y et al. Targeting CSCs within the tumor microenvironment for cancer therapy: A potential role of mesenchymal stem cells. Expert Opin Ther Targets 2012;16:1041–1054.

32 Alimron Bonnin DA, Havrda MC, Lee MC et al. Secretion-mediated STAT3 activation promotes self-renewal of glioma stem-like cells during hyposia. Oncogene 2015;34:1107–1118.

33 Phuphanich S, Wheeler CJ, Rudnick JD et al. Phase I trial of a multi-epitope-pulsed dendritic cell vaccine for patients with newly diagnosed glioblastoma. Cancer Immunol Immunother 2013;62:125–135.

34 Vik-Mo EO, Nyakas M, Mikkelsen BV et al. Therapeutic vaccination against autologous cancer stem cells with mRNA-transfected dendritic cells in patients with glioblastoma. Cancer Immunol Immunother 2013;62:1499–1509.

35 Ricci-Vitiani L, Lombardi DG, Pilozzi E et al. Identification and expansion of human colon-cancer-initiating cells. Nature 2007;445:111–115.

36 Hatano Y, Fukuda S, Hisamatsu K et al. Multifaceted Interpretation of Colon Cancer Stem Cells. Int J Mol Sci 2017;18:1–14.

37 Ning ST, Lee SY, Wei MF et al. Targeting colorectal cancer stem-like cells with anti-CD133 antibody-conjugated SN-38 nanoparticles. ACS Appl Mater Interfaces 2016;8:17793–17804.

38 Cheng C, Yaffe MB, Sharp PA. A positive feedback loop couples Ras activation and CD44 alternative splicing. Genes Dev 2006;20:1715–1720.

39 Pang R, Law WL, Chu AC et al. A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell 2010;6:603–615.

40 Schnaper RH, Spence JE, Stange DE et al. Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. Science 2012;337:730–735.

41 Shimokawa M, Ohta Y, Nishikori S et al. Visualization and targeting of LGR5(+) human colon cancer stem cells. Nature 2017;545:187–192.

42 Miyoshi H, Maekawa H, Kakizaki F et al. An improved method for culturing patient-derived colorectal cancer spheroids. Oncotarget 2018;9:21950–21964.

43 Drost J, Clevens H. Organoids in cancer research. Nat Rev Cancer 2018;18:407–418.

44 Wang SW, Sun YM. The IL-6/JAK/STAT3 pathway: Potential therapeutic strategies in treating colorectal cancer (Review). Int J Oncol 2014;44:1032–1040.

45 Li Y, Rogoff HA, Keates S et al. Suppression of cancer relapse and metastasis by inhibiting cancer stemness. Proc Natl Acad Sci U S A 2015;112:1839–1844.

46 Carney DN, Gazdar AF, Bunn PA Jr et al. Demonstration of the stem cell nature of clonogenic tumor cells from lung cancer patients. Stem Cells 1982;1:149–164.

© 2018 The Authors. Stem Cells Translational Medicine published by Wiley Periodicals, Inc. on behalf of AlphaMed Press
Leung EL, Fiscus RR, Tung JW et al. Non-small cell lung cancer cells expressing CD44 are enriched for stem cell-like properties. PLoS One 2010;5:e14062.

Zakaria N, Satar NA, Abu Halim NH et al. Targeting lung cancer stem cells: Research and CLINICAL Impacts. Front Oncol 2017;7:80.

Cortez MA, Valdecanas D, Niknam S et al. In vivo delivery of miR-34a sensitizes lung tumors to radiation through RAD51 regulation. Mol Ther Nucleic Acids 2015;4:e270.

MacDonagh L, Gallagher MF, Ffrench B et al. Targeting the cancer stem cell marker, aldehyde dehydrogenase 1, to circumvent cisplatin resistance in NSCLC. Oncotarget 2017;8:72544–72563.

Duan L, Shen H, Zhao G et al. Inhibitory effect of disulfiram/copper complex on non-small cell lung cancer cells. Biochem Biophys Res Commun 2014;446:1010–1016.

Leon G, MacDonagh L, Finn SP et al. Cancer stem cells in drug resistant lung cancer: Targeting cell surface markers and signaling pathways. Pharmacol Ther 2016;158:71–90.