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CANCER STEM CELLS

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

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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. STEM CELLS TRANSLATIONAL MEDICINE *2019;8:75–81*

SIGNIFICANCE STATEMENT

This concise review summarizes ongoing preclinical and clinical efforts to therapeutically target cancer stem cells, a subpopulation of bulk tumors that have been implicated in therapy resistance and metastasis. This article reviews signaling pathways involved in cancer stem cell maintenance, and recent novel approaches such as epigenetic targeting and immunotherapy that hold promise for improving patient outcomes.

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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, possesing 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 chemotherapy 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

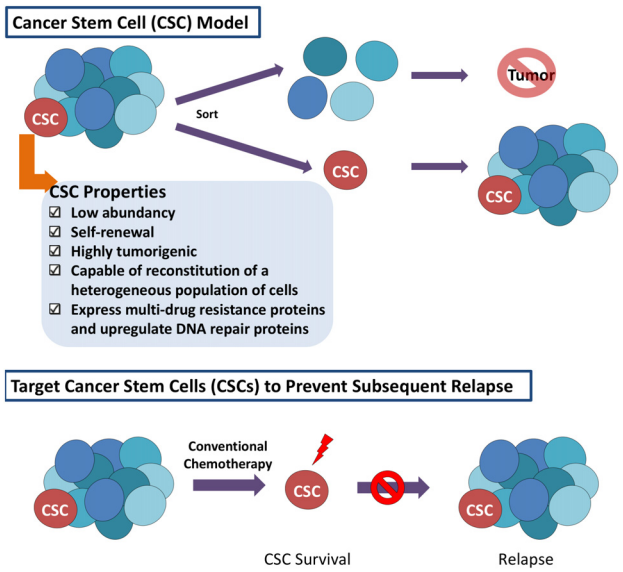

Figure 1. The concept of the cancer stem cell (CSC). Tumor cells are heterogeneous which contain a majority of cells are non/-poorly tumorigenic, and a small subset of CSCs. The CSCs can be functionally distinguished from other populations by their ability to reconstitute a differentiated tumor upon 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.

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 diphtheria 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-κB 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.

Breast Cancer Stem Cells

Breast cancer stem cells (BCSCs) were characterized as CD44 (+)/CD24(low)/lineage(−) by Al-Haji et al. This finding was the

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
NCT01553851	GSK1120212 (MEK1/2 inhibitor) in oral cavity squamous cell cancer	Phase 2 complete
NCT01190345	Bevacizumab (anti VEGF) + conventional therapy in breast cancer	Phase 2
NCT01579812	Metformin (Type 2 anti-diabetic) + conventional therapy in ovarian, fallopian tube, and primary peritoneal cancer	Phase 2
NCT01624090	Mithramycin (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	VS-6063 (FAK inhibitor) in KRAS mutant non-small cell lung cancer	Phase 2 complete

Adapted from [5].

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 microRNA 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 anoikis 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 administrated 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 dikkopf-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-specific ligands such as poliovirus 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 DNAJB8, 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 immunotherapy ICT-121, which showed promising phase 1 data by

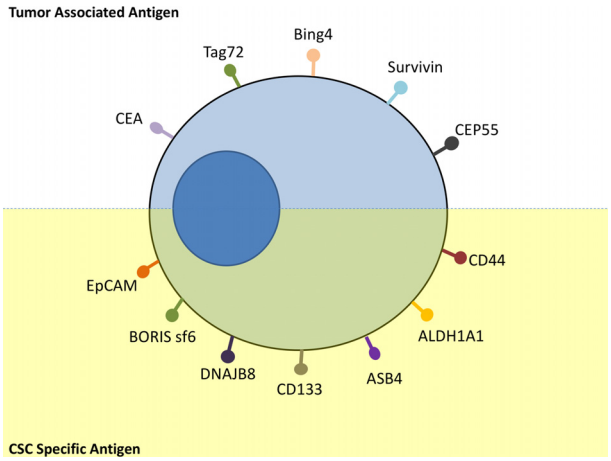

Figure 2. Antigens found specifically on CSCs versus those on differentiated tumors. Examples of antigen identified in the bulk tumor and CSC populations. Simultaneously targeting both types of antigen is emerging as a successful approach to maximize immune responses to a variety of cancers.

mounting a cytotoxic T-cell response against CD133+ CSCs, as well as ALDH1^{high} presenting DCs which have demonstrated promising results in preclinical melanoma models [71]. It is feasible to assume that utilizing immunotherapy approaches to target CSCs and conventional chemotherapy to target the remaining bulk tumor will yield synergistic killing effects in a number of cancers [71] (Fig. 2).

Additional Therapeutic Strategies

Notch Signaling

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 regimen. 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.

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