Review Article

Immune targeting of cancer stem cells in gastrointestinal oncology

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Abstract: The cancer stem cell (CSC) hypothesis postulates that a sub-population of quiescent cells exist within tumors which are resistant to conventional cytotoxic/anti-proliferative therapies. It is these CSCs which then seed tumor relapse, even in cases of apparent complete response to systemic therapy. Therefore, therapies, such as immunotherapy, which add a specific anti-CSC strategy to standard cytoreductive treatments may provide a promising new direction for future cancer therapies. CSCs are an attractive target for immune therapies since, unlike chemotherapy or radiotherapy, immune effector cells do not specifically require target cells to be proliferating in order to effectively kill them. Although recent advances have been made in the development of novel systemic and targeted therapies for advanced gastro-intestinal (GI) malignancies, there remains an unmet need for durable new therapies for these refractory malignancies. Novel immunotherapeutic strategies targeting CSCs are in pre-clinical and clinical development across the spectrum of the immune system, including strategies utilizing adaptive immune cell-based effectors, innate immune effectors, as well as vaccine approaches. Lastly, since important CSC functions are affected by the tumor microenvironment, targeting of both cellular (myeloid derived suppressor cells and tumor-associated macrophages) and sub-cellular (cytokines, chemokines, and PD1/PDL1) components of the tumor microenvironment is under investigation in the immune targeting of CSCs. These efforts are adding to the significant optimism about the potential utility of immunotherapy to overcome cancer resistance mechanisms and cure greater numbers of patients with advanced malignancy.

Keywords: Cancer stem cells (CSCs); aldehyde dehydrogenase (ALDH); CD24; CD44; CD133; immunotherapy; T cells; natural killer (NK) cells; vaccines; EpCAM

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Overview of cancer stem cells (CSCs)

CSCs, also known as tumor-initiating cells or tumor-repopulating cells, are a subset of cancer cells within the bulk tumor mass. This sub-population of cells exhibits a unique phenotype that mirrors that of embryonic or pluripotent stem cells, namely the capacity to self-renew, to differentiate (or repopulate bulk tumor mass), and to maintain homeostatic control (i.e., balance self-renewal and differentiation) (1,2). In extensive pre-clinical studies, the CSC phenotype has been modelled principally by colony engraftment in long term culture and by tumor formation in immune-compromised mice. Increasing studies have validated the presence of CSC subpopulations in nearly all human malignancies (3-5), and landmark tracking studies of genetically modified cells in intestinal adenomas, among other solid neoplasms, have identified a hierarchy of asymmetric cell division and tumor repopulation, providing the highest level of evidence to date that CSCs are clinically and biologically relevant (6-8).

Experimentally, the identification and characterization of CSCs has been predicated on the expression of cell

surface markers such as CD24, CD44, and CD133 as well as the expression of the intracellular enzyme aldehyde dehydrogenase (ALDH) (9). CD24 is a cell surface glycoprotein anchored by a glycosyl-phosphotidyl-inositol tail (10). It is heavily glycosylated and is involved in both cell-cell and cell-matrix interactions. Although CD24 has been shown to have preferential expression on CSCs, it has also been identified on differentiated cancer cells (non-CSCs) in numerous malignancies as well as hematopoietic and neuronal cells (10). In addition, tissue-specific and epigenetic patterns of glycosylation suggest that CD24 affects diverse physiological functions, some of which remain incompletely characterized. These features underscore the plasticity of CSCs and the markers which identify them (9).

CD44 is a transmembrane glycoprotein which is expressed in normal cells as well as numerous cancer cells (9,10). It functions primarily as a hyaluronic acid receptor. In this way, it promotes and regulates cell migration. CD44 has also been identified as a key protein in cell adhesion, survival, differentiation, and interaction with the tumor microenvironment. Similar to CD24, CD44 has pleotropic effects, including roles in multiple signaling cascades, so the precise mechanism by which it fosters the CSC phenotype is not well defined (9).

CD133 (prominin-1) is a transmembrane glycoprotein which was initially described as a marker of human hematopoietic progenitor cells (9,11). CD133 was subsequently discovered on primitive neural tissue, and seminal studies subsequently linked CD133 expression to tumor initiation and propagation in immunodeficient mice (12). Subsequent investigations demonstrated CD133+ CSCs in diverse GI malignancies, including pancreas, biliary, gastric, and colorectal (9). Investigators have also observed that CD133 expression is critical to the production of the plasma membrane, frequently in combination with cholesterol (11). As a result, CD133 has been referred to as the "organizer" of the plasma membrane. However, a complete understanding of the ligands for CD133 nor its downstream targets has not been fully clarified, leaving some ambiguity regarding its biological functions.

ALDH represents a class of enzymes important to numerous biochemical and metabolic cellular processes, including detoxification of enzymes and retinoic acid synthesis. Elevated ALDH activity is closely linked with the CSC phenotype (9,13). Although investigators have demonstrated other cell surface markers to correlate with the CSC phenotype (notably EpCAM in pancreatic cancer) and the expression of CSC markers has been shown to vary depending on experimental conditions and tumor type, these markers have, nevertheless, been consistently identified as CSC markers in multiple gastrointestinal (GI) malignancies. Furthermore, enriched CSC populations are predictive for worse oncologic outcome in numerous cancers, including GI (4,14-19). Although the mechanism by which ALDH and other cell surface markers confers a CSC phenotype is not definitively known, over-expression of these molecules has been associated with the CSC phenotype, while knockout or inhibition has been associated with loss of the CSC phenotype in multiple pre-clinical cancer models, including pancreatic and upper GI malignancies (20-23).

Similarly, epithelial-to-mesenchymal transition (EMT) is a process by which cells acquire an increased invasive and mutable phenotype. In fact, accumulating evidence indicates that EMT enables tumors to acquire a metastatic phenotype. Although controversial, there is emerging evidence that CSCs may promote the development of EMT. For example, in a model of pancreatic cancer exposure to TGF-beta upregulated CSC markers, leading to decreased E-cadherin expression, increased invasion *in vitro*, and increased metastases *in vivo* (24). Similarly, Su *et al*. showed that TGF-beta exposure in pancreatic cancer increased stem cell markers and features of the CSC phenotype via the SMAD4 pathway (25). Other authors have maintained that CSCs share the activation of common pathways with EMT, but may represent two distinct phenomena (26).

Traditional anti-cancer therapeutic strategies target proliferating cells through cytotoxic effects or targeted inhibition of pro-proliferative signaling pathways. The significantly reduced proliferative state of CSCs appears to impart these cells with intrinsic chemoresistance, and anti-proliferative therapies such as chemotherapy and radiotherapy have been shown to enrich for CSCs (12,27-31). As a result, CSCs are able to survive and remain viable in a quiescent state, and ultimately, this capacity allows CSCs to promote relapse and demise at a subsequent date, even after a period of so-called remission.

CSC biology in GI malignancies

In the past decade, there have been significant advances in the field of CSC biology (2). The emerging evidence has demonstrated that CSCs play critical roles in drug resistance, invasion, and metastasis. Furthermore, although CSCs and non-CSCs within the same tumor share similar

genetic fingerprints, there are distinct transcriptional patterns observed between CSCs and non-CSCs, highlighting the importance of plasticity and epigenetic modifications in regulating CSCs and non-CSCs. Furthermore, the activation of disparate pathways, such as hedgehog, TGF-β, and Wnt/β-catenin, between CSCs and non-CSCs suggests that effective therapy may require selective targeting of these distinct cell populations (32).

The clinical relevance of CSC populations has been demonstrated in numerous GI malignances, including pancreatic, gastro-esophageal, colon, and biliary (33-37). For example, Rasheed *et al*. performed a detailed analysis of pancreatic cancer xenografts (38). These authors demonstrated that ALDH-positive cells were significantly more clonogenic *in vitro* and *in vivo* compared with unsorted or ALHD-negative cells. These ALDH-positive CSCs expressed genes consistent with a mesenchymal state and had substantially greater *in vitro* migratory and invasive behavior. Using ALDH, as well as CD24+CD44+EpCAM+ cells, other investigators have similarly identified pancreatic cancer cells that have CSC and mesenchymal features (39,40). The enhanced clonogenic growth and migratory properties of these stem-like pancreatic cancer cells (ALDH and/or EpCAM-positive) suggest that they play a key role in the development of metastatic disease and oncologic outcome of patients with pancreatic adenocarcinoma. Although these cells have been phenotypically and functionally well characterized, we still know very little about their genetic and epigenetic aberrations. Further analyses should reveal CSC-specific oncogenes and tumor suppressor genes.

Similarly, CSC behavior has been identified in hepatocellular carcinoma (HCC), and key studies have demonstrated the plasticity and epigenetic regulation of CSCs and non-CSCs. For example, Yimlamai *et al*. demonstrated that inactivation of the Hippo pathway, a regulator of cell proliferation, fostered the de-differentiation of adult hepatocytes into cells with progenitor characteristics and CSC features (41). Villaneuva *et al*. identified increased Notch activation in human HCC samples, suggesting that this pathway is triggered in HCC development. Furthermore, pre-clinically, the authors observed that activation of Notch signaling correlated with biliary cancer formation via insulin-like growth factor 2, and this process was inhibited by novel γ-secretase inhibitors which inhibit the Notch pathway (42). Ultimately, the high penetrance of CSCs in this tumor model will allow for a better understanding of their biological features such as the regulation of proliferation and progression to metastases.

CSCs have been shown to be the source of treatment resistance and eventual progression of disease. Even the recent development and introduction of targeted therapies such as tyrosine kinase inhibitors (TKIs) is associated with temporary tumor response and the subsequent development of resistance (43). Furthermore, CSCs display increased levels of the DNA checkpoint kinases, such as Chk1 and Chk2, which may play a further role in their resistance to genotoxic stress (44). These findings are not surprising given that these therapies predominantly rely on DNA damage to induce mitotic cell death (45,46). Overall, the emerging data support the concept that CSCs are important to cancer biology. Therefore, it will be important to design strategies to target CSC subsets within tumors to prevent relapse and advance multidisciplinary cancer therapy.

T cell immunotherapy

T cells, particularly cytotoxic T cells, form the principal immune effector cell of the adaptive immune system. The fundamental properties of a cytotoxic T cell response (including antigen specificity, clonal expansion, and memory) have made CD8+ T cells essential features of successful immune-based strategies toward cancer (47). Since studies indicate that CSCs are the reservoir of differentiated tumor cells and the putative source of metastases, attention has focused on using T cell therapies to specifically target CSCs.

Visus *et al*., for example, demonstrated that $ALDH^{high}$ cells derived from human cancer cell lines, including pancreatic, could be used to induce a CD8+ T cell response. $ALDH^{high}$ cells were sorted by fluorescence-activated cell sorting (FACS) and exposed to CD8+ T cells *in vitro* along with dendritic cells isolated from HLA-A2-restricted healthy volunteers. In some experiments, an additional step of an artificial, engineered antigen-presenting cell was also used. These CD8+ T cells were then adoptively transferred into tumor-bearing mice, and the authors observed that this strategy inhibited tumor growth and metastasis formation while survival was prolonged. This study is a notable demonstration of the concept that CSCs, in general, and ALDH1A1, in particular, are potential therapeutic target for T cell immunotherapy to selectively target CSCs in solid tumors (48). Luo *et al*., utilizing a similar approach, sorted $ALDH^{high}$ cells and then co-cultured them with dendritic cells to stimulate CD8+ T cells with specificity to $ALDH^{high}$ CSCs (49). Subsequent CD8+ T cells were found to recognize and lyse $ALDH^{high}$ CSCs. The authors further

demonstrated significant reductions in tumor growth and improvements in survival in a mouse model. It should be noted, however, that the authors did not demonstrate that the CD8+ T cells were mediating their anti-tumor effects *in vivo* by eliminating $ALDH^{high}$ CSCs.

Huang *et al*. engineered an anti-CD3/anti-CD133 bispecific antibody (BsAb) linked to cytokine-induced killer cells (50). In both *in vitro* and *in vivo* models of pancreatic and biliary cancer, the authors observed enhanced tumor killing and loss of CD133 positive cells with their BsAb. Despite these impressive results, it remains to be seen whether this novel therapy will have similar effects in models where CD133 is not expressed at such high levels, especially in unmanipulated primary tumors where CSC populations are frequently a small minority of the overall bulk tumor population.

The tremendous advances in the treatment of hematological malignancies using engineered T cells transduced with chimeric antigen receptors (CARs) has created substantial interest in using this cell-based immunotherapy for solid cancers (51). Following the collection of a patient's T cells, the cells are genetically engineered to express CARs specifically directed towards antigens on the patient's tumor cells. These modified T cells are then infused back into the patient. Adoptive transfer of T cells expressing CARs is a promising anti-cancer therapeutic as CAR-modified T cells can be engineered to target virtually any tumor-associated antigen. Given the experience in hematologic malignancies, there is great potential for this approach to improve patient-specific cancer therapy in a profound way.

Given the lack of meaningful treatment options for patients with advanced/refractory GI malignances, these cancers appear to be optimal candidates for the application of CAR therapy. However, a key feature of CAR therapy is selection of the target antigen to maximize selectivity to the tumor and minimize off-target effects/toxicity. In pre-clinical models, CAR T cells have been designed to target CD133+ (52), chondroitin sulfate proteoglycan 4 (with structure and function similar to CD24) (53), and epidermal growth factor receptor variant III (which is preferentially expressed on glioma stem cells) (54). Although these studies demonstrated proof-of-concept that CAR T cells could be engineered and expanded to recognize CSC targets, they were limited by their reliance on *in vitro* and *ex vivo* experimental designs.

Although adoptive transfer of CAR-modified T cells is a unique and promising cancer therapeutic, there are significant safety concerns as well as questions regarding the sustainability and affordability of this technology. Particularly in solid cancers where there is overlap in the expression of target antigens between healthy and neoplastic tissue, clinical trials have revealed toxic effects of CARs, including CAR-mediated recognition of target antigens in normal tissues. In some cases, the toxicities have paralleled those observed with graft-versus-host disease, and importantly, rare cases of fatal adverse events have been reported (55). These toxicities highlight the need for well-designed and rigorously conducted pre-clinical and early stage clinical trials to evaluate CAR therapy in the immune targeting of CSCs since these CSC markers are also present on normal stem cells in diverse tissues.

A potential solution to the toxic side effects of CAR T cells is engineering a suicide gene into the modified T cells (56). When activated, the suicide gene triggers apoptosis in the CAR T cells, thereby reining in potential immune-related toxicity. Adoption of suicide gene therapy to the clinical application of CAR-modified adoptive T cell transfer has potential to alleviate toxicity, but concerns exist about the ability to optimally control and decouple the anti-tumor effects of the treatment while minimizing the toxicity. Nevertheless, clinical trials using CAR technology have been initiated in pancreatic cancer targeting both CEA and mesothelin, and results from these trials are eagerly awaited (57).

Natural killer (NK) cell immunotherapy

Characterized by the expression of CD56 and a lack of T cell markers, such as CD3 or the T-cell receptor (TCR), NK cells are efficient effector cells of the innate immune system. They are able to recognize and kill virally-infected and malignant cells, primarily because of modulations in MHC-I and MHC-Ib molecules on target cells. Two distinct immunotherapy strategies utilizing NK cells have evolved: one which harnesses endogenous NK cells by administering NK stimulants or targeting agents, and one which uses exogenous NK cells via adoptive cell transfer. Each of these approaches is under investigation in the immune targeting of CSCs.

There are several key advantages to harnessing NK cells (58). First, NK cells are antigen non-specific and therefore do not require the expression of a specific antigen on a given HLA allotype. In contrast, therapies targeting a specific antigen are dependent on the presence of that antigen. While antigen-specific therapies may be

highly effective and achieve long-term responses in many cases, antigen-shedding and escape variants can limit the effectiveness of this approach. Second, NK cells can be easily isolated and expanded *ex vivo* which allows for their use in adoptive cell therapies. Third, NK cells have a shorter lifespan than T cells. Whereas T cell adoptive therapies, such as CARs, often require a suicide vector to prevent the sequela of over-expansion of the transferred cells, NK cells, unless genetically altered, have a lifespan on the order of one month or less which effectively eliminates the risk of chronic toxicities which has been observed with CAR T-cell therapy.

CSCs have recently been demonstrated to be highly susceptible to NK cell attack, suggesting that NK cells may be useful as part of a combined modality approach capable of targeting CSC and non-CSC populations. Tseng *et al*., for example, demonstrated in both human and mouse models that stage of differentiation for both malignant and embryonic cells predicted their sensitivity to NK cell lysis (59). These authors also reported that inhibition of differentiation or reversion of cells to a less-differentiated phenotype by blocking NFkappaB or targeted knock down of COX2 significantly increased NK cell effector functions. Tallerico *et al*. demonstrated that freshly purified allogeneic NK cells can recognize and kill colorectal carcinoma-derived cancer-initiating cells (CICs) whereas the non-CIC counterpart of the tumors was less susceptible to NK cells (60). This difference in the NK cell susceptibility was correlated with higher expression on CICs of ligands for NKp30 and NKp44 in the natural cytotoxicity receptor group of activating NK receptors. In contrast, CICs were shown to express lower levels of MHC class I on their surface than do the "differentiated" tumor cells, and MHC class I molecules are known to inhibit NK recognition and function.

The results of human clinical trials using autologous NK cells as monotherapy to treat advanced cancers have largely been disappointing, leading some investigators to conclude that autologous NK cells, in the setting of active malignancy, are inherently dysfunctional and/or hyporesponsive because of the host's immune environment (61). Accordingly, recent interest has focused on the therapeutic potential of allogeneic NK cells, primarily because of increasing evidence that NK cells become maximally activated and cytotoxic when they recognize cells lacking self MHC molecules (i.e., the "missing self" hypothesis). The selective targeting of the CSC population with NK immunotherapy (after or in combination with initial tumor debulking using cytotoxic therapies) is a novel and innovative approach which our lab and others are using to overcome the previous limitations of adoptive NK transfer. Our laboratory is actively studying the capability of *ex vivo*-activated autologous NK cells to target CSCs in a combination approach, and we hypothesize that NK targeting of CSCs in the appropriate multimodality setting will translate to durable anti-tumor effects. Although questions remain regarding how to best optimize expansion, activation, delivery, and homing of NK cells, should the targeting of CSCs by NK immunotherapy prove to be feasible for even a select subset of GI cancer patients, then this approach will have significant clinical impact

Vaccines

The best source of tumor antigens may be autologous, self-renewing CSCs that are proliferating in cell culture (62). As they proliferate in cell culture, such cells increasingly express phenotypic markers that are associated with invasiveness and "stemness". The efficiency of such cell cultures can be enhanced by utilizing specialized culture conditions which leads to spheroid formation, a marker of stem-like cells. Yin *et al*. observed decreased expression of MHC class I molecules on pancreatic CSCs in culture consistent with previous observations that CSCs are able to evade antigen-specific immune attack (63). However, these authors pulsed DCs with these *in vitro* CSC lysates and found the DCs were able to stimulate an effective cytotoxic effect against both CSCs and bulk tumor cells in their model. Similarly, in a breast cancer model, Mine *et al*. pulsed immature DCs (iDCs) with a Numb-1 peptide, a membrane-bound protein which plays an important role in asymmetric cell division and regulates Notch, a highly conserved regulator of cell differentiation and homeostasis (64). The authors then exposed these iDCs to non-adherent peripheral blood mononuclear cells and observed an expansion of antigen-specific CD8+ cells. However, despite this novel finding, the authors did not demonstrate the translation of these immunological effects into improved anti-tumor therapy. In contrast, Duarte *et al*. used FACS to isolate $ALDH^{high}$ colon cancer cells in a rat syngeneic model (65). Immediately after sorting, cells were seeded in culture, lysed by freeze-thaw, and injected intraperitoneally with CpG as an immune adjuvant. Using this CSC-based vaccine approach, animals demonstrated a significant reduction in tumor growth and metastasis.

Tanida *et al*. demonstrated notable anti-tumor effects *in vivo* including improved mouse survival using a polyvalent vaccine designed to express α-gal epitopes (66). This study

demonstrated important translational relevance since the authors' vaccines were derived and engineered from clinical samples of primary pancreatic cancers. However, an important limitation of their study was the *in vivo* evaluation of their vaccine in α 1,3-galactosyltransferase knockout mice. This approach raises concerns that the specificity of their CSC vaccine for tumor antigens may be falsely elevated in this knockout model with the potential for less efficient targeting of CSCs where non-neoplastic α-gal is expressed. In addition, the use of knockout mice deficient in homologous antigens may mask potential toxicity.

Consequently, as with all vaccine-based approaches, there remains a concern that vaccines targeting CSCs will stimulate an immune response against non-neoplastic host tissues which express comparable antigens important for host functions. As with CAR therapy, this could lead to toxicity as well as the potential for auto-immunity, particularly since CSCs share similar antigens to healthy stem cells. Some authors have also questioned the effect of the mode of delivery of vaccines on outcome. The local delivery of vaccines may be limited by the immunosuppressive nature of the tumor microenvironment, while the systemic delivery of vaccines may be limited by the ability of primed immune effector cells to home/traffic to their targets in the tumor. A combined approach using both systemic and local delivery of vaccines may produce stronger anti-tumor responses, but this raises the possibility of greater toxicity.

Nevertheless, vaccine-based approaches targeting CSCs have the potential to evoke long term antigen specific memory to both treat advanced GI malignancies and prevent their recurrence. The ideal CSC vaccine would integrate and activate both the innate and adaptive arms of the immune system.

Tumor microenvironment

It has long been recognized that not all tumor cells are capable of propagating tumors in pre-clinical models of cancer. Although CSCs have been implicated to account for the heterogeneity identified within tumors, it has also been established that the tumor microenvironment directly interfaces with developing tumors and contributes to local immunosuppression as well as the CSC phenotype (67). Increasing studies are pointing to the importance of the tumor microenvironment to CSC maintenance, EMT transition, and oncologic outcomes. For example, Yamashina *et al*. demonstrated that CSCs were a source of immunosuppressive cytokines (GM-CSF among others), and the elaboration of these cytokines generated myeloid-derived suppressor cells (MDSCs) and M2 macrophages, both of which were associated with chemoresistance (68). As noted above, Wang *et al*. introduced TGF-β into pancreatic cancer models and observed increased invasiveness, angiogenesis, and metastasis formation as well as cells with a CSC phenotype (69). Lin *et al*. observed that IL-6 promoted CSC proliferation in colon cancer CSCs through a STAT3 dependent pathway (70). They further observed that inhibition of IL-6 or its receptor was able to counteract these effects, suggesting that immune modulation of the tumor microenvironment may be an effective strategy for CSC targeting.

Zoglmeier *et al*. evaluated CpG treatment on MDSC phenotype and function in a mouse model of gastric neoplasia (71). Their results indicated that TLR9 activation via CpG significantly decreased MDSC suppressive function in tumor-bearing mice. Although the authors did not assess for CSC-specific effects of their MDSC-targeting strategy, the authors suggested this mechanism as an avenue for further study. Wang *et al*. demonstrated that IL6 ligand and receptor expression contributed to CSC growth and survival in a glioma model (72). Furthermore, they showed that inhibition of IL6 ligand and receptor expression in CSCs increased survival of mice bearing orthotopic human xenografts. Although similar studies have not been performed in GI malignancies, there is enthusiasm that CSC targeting via IL6 antagonism may offer therapeutic benefit for advanced cancer patients.

The recent development of immune checkpoint inhibitors has demonstrated the untapped, and previously unharnessed, power of the immune system to reject malignancies and lead to sustained, long term responses (73). Yet, despite the excitement surrounding immune checkpoint inhibitors, novel approaches are needed to deliver the promise of immunotherapy to greater numbers of cancer patients (74). Recent impressive results in clinical trials of PD-1 and PDL-1 inhibitors have generated notable enthusiasm surrounding these therapies. Since quiescent/dormant CSCs must develop a mechanism of immune escape to avoid elimination by immune surveillance, it is plausible to postulate that immune checkpoint inhibitors may preferentially target CSCs and the CSC niche. This hypothesis is supported by several key publications showing that mesenchymal stem cells utilize the PD-1/PDL-1 axis to suppress inflammation and inhibit the immune response (75,76). However, as yet, there is

little pre-clinical or clinical evidence to support the notion that the impressive clinical efficacy of immune checkpoint inhibitors is acting via an anti-CSC mechanism.

Potential limitations

Immune targeting of CSCs in the stem cell niche and tumor microenvironment poses inherent challenges which may limit its potential clinical translation. Studies have shown that CSCs are less immunogenic than non-CSCs, and CSCs may downregulate many tumor-associated antigens, thereby limiting the ability of the adaptive immune system to recognize and mount an antigen-specific response to CSCs. In addition, although potentially limited by using a strictly *in vitro* model, Volonté *et al*. demonstrated that colon cancer CSCs express both membrane-bound and soluble IL-4 (77). This CSC-mediated inhibitory signaling could negatively downregulate anti-tumor T cells responses designed to target CSCs *in vivo*. Similarly, IL-4 levels have observed to promote tumor proliferation, invasion, and metastases in pre-clinical models of cancer, suggesting an important role of this cytokine in the immunosuppressive phenotype which is potentially preferentially regulated by CSCs (78,79).

In addition, key studies have observed evasion of immunosurveillance through shedding of MICA and MICB by CSCs and apparent CSC recruitment of regulatory T cells to promote an immune privileged state (80,81). Furthermore, in a notable study with important translational implications, Kryczek *et al*. observed that IL-22 promoted a CSC phenotype in both pre-clinical and patient-derived models (82). These authors then determined that CD4+ T cells were a source of IL-22 secretion, and that a higher concentration of IL-22 was associated with a worse oncologic outcome. Collectively, the findings of Kryzczek *et al*. highlight a fundamental point of the immune system with respect to CSCs or any other target cell, namely that it can be primed both for and against immune targeting. It will be important to recognize and address these potential limitations to ensure that the optimal results from these novel approaches are achieved.

Summary and conclusions

Accumulating evidence suggests that CSCs exist as a sub-population of quiescent cells within the dominant tumor bulk of heterogeneous tumor cells (1,2). These typically dormant cells are considered resistant to standard anti-cancer therapies such as chemotherapy and RT. They also are capable of self-renewal and differentiation (28-31), suggesting that CSCs are responsible for tumor repopulation after bulk tumor has been destroyed (8). Targeting the CSC population will be critical to additional meaningful advances in cancer treatment, especially for difficult to treat GI malignancies. There is significant optimism that a multimodality approach using immunotherapy in combination with cytotoxic treatments to simultaneously eradicate CSCs and non-CSCs will lead to more complete and durable cancer eradication. Immune targeting of CSCs holds significant promise in the ultimate goal of overcoming cancer resistance and curing more patients with cancer.

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Footnote

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