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Targeting cancer-initiating cell drug-resistance: a roadmap to a new-generation of cancer therapies?

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The occurrence of drug resistance in oncology accounts for treatment failure and relapse of diverse tumor types. Cancers contain cells at various stages of differentiation together with a limited number of 'cancer-initiating cells' able to self-renew and divide asymmetrically, driving tumorigenesis. Cancer-initiating cells display a range of self-defense systems that include almost all mechanisms of drug-resistance. Different molecular pathways and markers, identified in this malignant sub-population, are becoming targets for novel compounds and for monoclonal antibodies, which may be combined with conventional drugs. These interventions might eliminate drug-resistant cancer-initiating cells and lead to remission or cure of cancer patients.

Cancer-initiating cells and drug resistance

Though of clonal origin, tumors contain cells at various stages of differentiation, which differ in their phenotypic markers and proliferation ability. This heterogeneity has been recently ascribed to a hierarchical organization of cancer, similar to that of normal tissues, with a limited number of 'cancer stem cells' (CSCs) driving tumorigenesis. In analogy with their normal counterpart, CSCs are often quiescent, able to self-renew and may divide asymmetrically giving rise to more differentiated cells, which represent most of the tumor mass. CSCs, firstly described in acute myeloid leukemia [1], have been successfully isolated from different cancers such as breast, brain, colon, head and neck, ovary, pancreas and other solid tumors [2–8]. CSCs are mainly identified by their ability to replicate the original tumor heterogeneity (phenocopy) upon xenotransplantation in immunodeficient mice and are therefore defined as cancer initiating cells (CICs). However, because the murine microenvironment may differ from that of the original tumor, the engraftment might be limited and selection of cancer cells may occur [9,10]. In addition, the residual natural immunity present in nude and non-obese diabetic (NOD)/SCID mice may influence the growth of xenografts. Interleukin-2 receptor-γchain null NOD/SCID (NOG) mice, which have a broad defect of both specific and natural immunity, represent a more suitable animal model for studying CIC xenografts [9].

Despite several controversial aspects, substantial evidence for CIC relevance in the biology of cancer and in the clinical setting has been obtained [11]. Conventional anti-cancer treatments are mainly directed to the highly proliferating cells, whereas the great majority of CICs are mostly quiescent and poorly responsive [12]. Indeed, several reports indicate that CICs, displaying stem cell-like markers, are intrinsically resistant to chemotherapy or radiotherapy [13,14]. These observations suggest that CICs may not be adequately targeted by the conventional anti-cancer treatments and are involved not only in resistance to therapy but also in tumor relapse. Different cellular mechanisms of drug resistance have been identified such as increased DNA damage detection and repair, overexpression of ATP-binding cassette (ABC) transporters, alterations of cell cycle checkpoints and impairment of apoptotic pathways [15,16]. Additional mechanisms are related to the peculiar acidic and hypoxic environment of the niches where CICs reside. Several developmental signaling pathways, such as NOTCH, Wnt/β-catenin, Sonic hedgehog, BMI-1, SOX-2, and OCT-4 play an important role in normal stem cell as well as CIC's survival and/or self-renewal. These pathways have been the object of recent reviews and will not be discussed here [16,17], although some of the clinical trials using drugs that target these pathways are listed in Table 1.

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TABLE 1

Molecules expressed in CICs which are targeted by anti-cancer drugs that entered clinical trials

Target	Drug	Cancer type	Clinical trial
ALDH1*	Disulfiram, disulfiram plus copper gluconate, disulfiram plus arsenic trioxide	Refractory solid tumors of liver, prostate and metastatic melanoma	I/II (NCT00742911)
ABCG2§	Dofequidar fumarate (MS-209)	Breast cancer	III
ABCB1*	Tariquidar (XR9576)	Different solid cancers	I/II (NCT00069160, NCT00001944)
γ-Secretase*	MK-0752	Stage IV pancreatic cancer that cannot be removed by surgery	I/II (NCT01098344)
γ-Secretase*	RO4929097	Advanced solid tumors, melanoma stage IV	I/II (NCT01198535)
Hypoxia	PR-104 and docetaxel or gemcitabine	Solid tumors	I (NCT00616213, NCT00616213)
Hypoxia	PR-104 and docetaxel	NSCLC	II (NCT00862134)
Hypoxia	Indisulam (E7070)	Kidney neoplasms carcinoma, renal cell adenocarcinoma, CRC	II (NCT00059735)
Hypoxia	Monoclonal antibody G250	Kidney cancer	III (NCT00087022)
Hypoxia (TrxR)	TH-302	Advanced solid tumors	I (NCT00495144)
Hypoxia (TrxR)	TH-302 and doxorubicin	Sarcoma	I/II (NCT00742963)
Hypoxia (TrxR)	TH-302 and gemcitabin	Pancreatic adenocarcinoma	II (NCT01144455)
Wnt*	Resveratrol	Colon cancer	I/II (NCT00256334)
Smo*	IPI-926 and gemcitabine	Metastatic pancreatic cancer	I/II (NCT01130142)
Smo*,^	BMS-833923 (XL139)	Advanced or metastatic cancer	I (NCT00670189)
Smo*	LDE225	Advanced solid tumors	I (NCT00880308)
Shh plus γ-secretase*	GDC-0449 and RO4929097	Advancer breast cancer	I (NCT01071564)
PKCι*	Myochrysine (aurothiomalate)	Lung cancer	I (NCT00575393)
AMPK/mTOR/S6K1	Metformin	Lymphoma and solid tumors	I (NCT00659568)
mTOR*	Sirolimus and vinblastine	Recurrent or refractory solid tumors including central nervous system tumors	I (NCT01135563)
Topoisomerase II*	Banoxantrone (AQ4N), temozolomide and radiotherapy	Glioblastoma	I/II (NCT00394628)
$CD3^+$T cells on EpCAM positive tumors	Catumaxomab	Ovarian cancer, malignant ascites, EpCam positive cancers and CNS	II/III (NCT01065246, NCT00822809)

* See: http://clinicaltrials.gov.
§ Ref. [34].
^ American Society for Clinical Oncology (ASCO) 2010 (see: http://www.asco.org).

In the present article, we will briefly review different approaches to override the drug-resistance mechanisms of CICs and for increasing the efficacy of current cytotoxic treatments. We will focus our attention to the new anti-CICs based therapies that have entered clinical trials (Table 1). In addition, we will discuss the possible role of CICs in tumor immune-escape as well as their targeting by immunotherapy strategies.

DNA repair and detoxifying enzymes

Resistance to chemotherapy may be due to the overexpression of cellular DNA repair and detoxifying enzymes. Various DNA repair inhibitors combined with chemotherapy have shown promising results in preclinical studies and clinical trials are in progress [17].

For instance, the elevated expression of the O6-methylguanine DNA methyltransferase (MGMT) in CICs protects them from the effects of alkylating agents such as temozolomide and bis-chloronitrosourea (BCNU) [18]. O(6)-Benzylguanine (O6-BG) is an irreversible inactivator of MGMT, which sensitizes cancer cells to the antitumor effects of alkylating agents including the orally active temozolomide used for glioma treatment. Clinical trials are ongoing to evaluate the ability of O6-BG to efficiently inactivate MGMT and potentiate the antitumor effects of temozolomide. However, emerging results are not conclusive yet and need to be substantiated by additional data [19].

Aldehyde dehydrogenase-1 (ALDH1) is a detoxifying enzyme, which catalyzes the irreversible oxidation of intracellular aldehydes, thereby mediating self-protection and resistance to some alkylating agents used in cancer therapy [20]. Moreover, ALDH1 is implicated in the biology of normal as well as cancer stem cells, as it plays a role in the metabolism of retinol to retinoic acid, which initiates a program of cellular differentiation [21]. Previous results suggested that the use of specific inhibitors of ALDH1, such as disulfiram and cyanamide (antialcoholism drugs) in association with cyclophosphamide, overcomes chemoresistance [22] and disulfiram alone or associated with copper is presently tested in several clinical trials (Table 1).

ABC transporter inhibitors

ABC transporters are a large family of transmembrane proteins and are classified in seven subclasses (A–G) based on sequence and structural homology. The functional unit of these molecules contains two transmembrane and two ATP-binding domains, which provide ATP energy for the efflux of different molecules [23]. Some members of this family act on a wide range of substrates, while others have a more limited specificity. Five ABC transporters have been described in different CICs such as ABCA2 [24], ABCA3 [25], ABCB1 [24], ABCB5 [8], ABCC1 [15,26], and ABCG2 [6,24].

High expression of ABC transporters on CICs corroborates the hypothesis of an intrinsic drug-resistance present in the tumors since its origin. It is of note that CICs from different cancers may display diverse types of ABC transporters and therefore be endowed with resistance to a different and not always overlapping spectrum of drugs. Attempts to reverse MDR with transporter inhibitors disclosed interesting effects *in vitro* but quite disappointing results in the clinical setting [27]. First generation inhibitors of ABCB1 such as cyclosporine, verapamil and nifedipine showed some benefit in clinical phase I/II trials [28], but no efficacy in subsequent studies [29]. Valspodar (PSC-833), a second generation ABCB1 inhibitor, displayed elevated toxicity due to pharmacokinetic interactions with anti-cancer drugs [30]. The third generation ABCB1 inhibitor Tariquidar (XR9576), an anthranilic acid derivative, had no significant side effects and pharmacokinetic interactions [31], but a phase II study showed limited clinical activity in restoring sensitivity to anthracycline or taxane in advanced breast cancer [32]. Results of phase III trials in non-small cell lung cancer (NSCLC), combining Tariquidar with conventional anticancer drugs, are not available yet. Recently, dofequidar fumarate (MS-209) an inhibitor of the ABCG2 transporters sensitized breast CICs to chemotherapeutic drugs [33]. A phase III clinical study of dofequidar in advanced or recurrent breast cancer reported a prolonged progression-free survival, which was significant in pre-menopausal patient who did not receive prior therapy or were stage IV with an intact primary tumor [34].

More recently, salinomycin, a monocarboxylic polyether antibiotic, was found to specifically target CICs, by using a high-throughput screening method [35]. Salinomycin, besides acting as a potent inhibitor of ABCB1 [36], interferes with normal potassium channel regulation and activates a distinct apoptotic pathway in cancer cells (independent of p53 and caspase activation) [37]. Moreover, salinomycin reduces CICs by >100-fold as compared to paclitaxel, a standard drug frequently used in breast cancer treatment. In this perspective, salinomycin should be regarded as a potential anti-CICs compound to be tested in association with standard chemotherapy in preclinical and clinical studies.

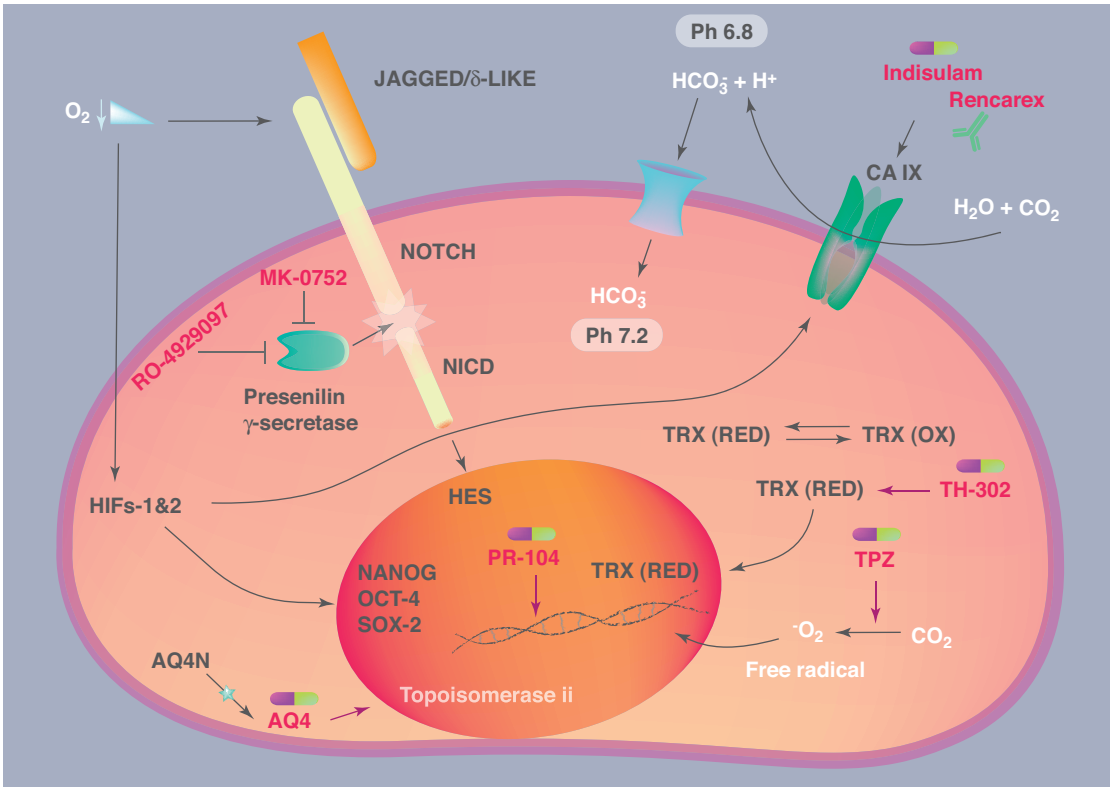

FIGURE 1

Schematic representation of targeted pathways involved in hypoxia-induced drug resistance of cancer-initiating cells. Hypoxia induces HIFs translocation to the nucleus, resulting in up-regulation of stem-cell associated Nanog, OCT-4 and SOX-2 gene expression. Concomitantly, hypoxia significantly increases signaling mediated by NOTCH, a transmembrane receptor, which is cleaved by γ-secretase upon interaction with specific ligands. The truncated NOTCH intracellular domain (NCID) crosses the nuclear membrane and induces a cascade of gene activation. γ-Secretase inhibitors are currently investigated in clinical trials. Drug names are in red. Drugs targeting the hypoxia-inducible molecules Thioredoxin (TRX) and carbonic anhydrase type IX (CA IX) are also illustrated. Tirapazamine (TPZ), Banoxantrone (AQ4N) and PR-104 are hypoxia activated pro-drugs with different targets.

The role of hypoxia in CICs

Hypoxia plays a central role in normal development and in a variety of pathological conditions, including solid tumors where it has been involved in resistance to radiotherapy and chemotherapy. Indeed, hypoxic niches may regulate survival and differentiation of CICs within tumors, as well as that of stem cells in normal tissues. Low oxygen levels, commonly found at the perivascular region and surrounding necrotic tissue of solid tumors, may also induce a fraction of cells to acquire a stem-like condition that sustains tumor growth [38,39].

Cells respond to hypoxia through hypoxia-inducible factors (HIFs) that induce the expression of CICs markers such as CD133 [40] and stemness related genes such as NANOG, OCT-4 and SOX-2 [39]. Furthermore hypoxia potentiates the biological effect of Notch, a signaling molecule involved in stemness maintenance in lung adenocarcinoma cell lines and, at the same time, makes tumor cells more susceptible to the action of γ-secretase inhibitors, which affect the Notch pathway [41]. Phases I and II trials of the γ-secretase inhibitors MK-0752 and RO4929097 are ongoing in different solid cancers (Table 1).

Several strategies have been undertaken to overcome resistance in tumor hypoxic areas such as oxygen delivery enhancers, hypoxic radiosensitizers (agents that mimic the radiochemical effects of oxygen) and pro-drugs preferentially activated by hypoxia-dependent metabolic pathways (Fig. 1).

Tirapazamine (TPZ) is the most widely studied hypoxia-activated pro-drug. In preclinical investigations TPZ improved the cytotoxicity of cisplatin and selectively killed hypoxic cells [42]. However, TPZ in combination with cisplatin/radiotherapy failed to improve overall survival in a recent phase III trial for advanced head and neck cancer [43].

PR-104 is a 'pre-prodrug', which becomes activated to a dinitrobenzamide nitrogen mustard cytotoxin by nitroreduction in hypoxic regions of tumors. PR-104A penetrates into hypoxic tumor tissue more efficiently than TPZ and is activated, at tenfold lower oxygen concentrations than for TPZ. PR-104 showed marked antitumor activity in xenograft models, both as monotherapy and in combination with radiotherapy or chemotherapy [44]. Clinical trials evaluating the effectiveness of PR-104 alone or in combination with docetaxel or gemcitabine in patients with solid tumors are currently ongoing (Table 1). In addition, a randomized, multicenter, open-label, study of PR-104 *vs* PR-104/docetaxel in NSCLC has been terminated and results are awaited.

Banoxantrone (AQ4N) is a prodrug that, within hypoxic tissues, is enzymatically converted to AQ4, a topoisomerase II inhibitor and DNA intercalator [45], which was designed to specifically target the treatment-resistant fraction of cells in tumor hypoxic regions [46]. In preclinical and clinical studies, AQ4N was well tolerated and increased the antitumor activity of standard radiotherapy and chemotherapy treatments. In view of its ability to cross the blood–brain barrier, it has been included in treatment regimens of primary and metastatic brain cancers [47].

Carbonic anhydrase IX (CA IX) is induced by HIF-1 and Notch signaling and supports breast CIC's survival in hypoxic conditions [48]. CA IX is a membrane-bound enzyme, which catalyzes the hydration of CO_2 to bicarbonate and protons [49]. Thus CA IX, together with increased anaerobic glycolysis, contributes to the generation of an extracellular acidic pH particularly in hypoxic area. The action of CA IX and of proton pumps maintains a relatively alkaline intracellular pH, thus generating a pH gradient across the cell membrane, which hampers the entrance of slightly basic cytotoxic drugs into the cell [50]. As a result, therapeutic strategies aimed at increasing extracellular pH, through proton pump or CA IX inhibitors [49], may improve tumor response to specific anticancer compounds [51] In particular, indisulam, a sulfonamide anti-cancer drug has entered phase II clinical trials and a human–mouse chimeric anti-CA IX monoclonal antibody G250 (Rencarex) [49,52] is currently evaluated in a phase III clinical trial (Table 1).

Thioredoxin reductase (TrxR), is a hypoxia-inducible disulfide reductase that regulates cell proliferation and redox homeostasis in several cell types, including vasculogenic stem cells involved in neoangiogenesis [53]. Indeed, increase in thioredoxin levels has been observed in many human malignancies and contributes to enhance cancer cell growth [54]. Thus, lowering thioredoxin levels might help reverting resistance to some antitumor agents as cisplatin [55]. TH-302 is a promising antineoplastic prodrug, whose effects are associated with both DNA alkylation and modulation of TrxR in a mouse hepatoma model [56]. Clinical studies showed that TH-302 dose-limiting toxicities were oral and gastrointestinal mucositis but not myelosuppression [57]. TH-302 monotherapy showed some activity in refractory small cell lung cancer and in metastatic melanoma. In addition, TH-302 with doxorubicin or with gemcitabine are currently tested in advanced soft tissue sarcoma and in pancreatic adenocarcinoma, respectively (Table 1).

Targeting CICs through immunotherapy

Given the ability of the immune system to recognize tumor-associated antigens (TAAs) and to potentially eliminate tumor cells, specific vaccines have been used in mouse models and clinical trials to stimulate the host anti-tumor response. However tumors, and in particular CICs, seem capable to escape the immune surveillance and to activate a series of immune suppressive mechanisms, which may hamper immunotherapy. For example, melanoma CICs do not express TAAs such as MART-1 or have low expression of HLA molecules, which are required for antigen presentation to T lymphocytes [58]. Other mechanisms such as production of cytokines stimulating M2 immune suppressive-type macrophages, lack of co-stimulatory molecules, presence of inhibitory molecules and ability to stimulate the expansion of regulatory T cells might be involved in CIC's immune escape [59–61].

The identification of relevant markers, preferentially expressed on the cell surface of CICs of different types of tumors such as EpCAM [62], CD44 [63] and CD133 [64] has allowed their specific targeting by monoclonal antibodies as biological weapons (Fig. 2). Antibodies targeting CICs' surface antigens may be used alone and as immuno-conjugates with cytotoxic or radioactive compounds. Epithelial cell adhesion molecule (EpCAM) represents the most common antigen in epithelial cancer, as it is expressed by CICs in breast, colon, prostate and pancreatic tumors and is regarded as a good target for immunotherapy [62]. Catumaxomab, is a trifunctional bispecific (anti-CD3-anti EpCAM) chimeric antibody, able to target $CD3^+$ T lymphocytes on EpCAM positive tumor cells and to bind through its Fc portion to FcR^+ effector cells of natural immunity, such as macrophages and natural killer (NK) cells. It

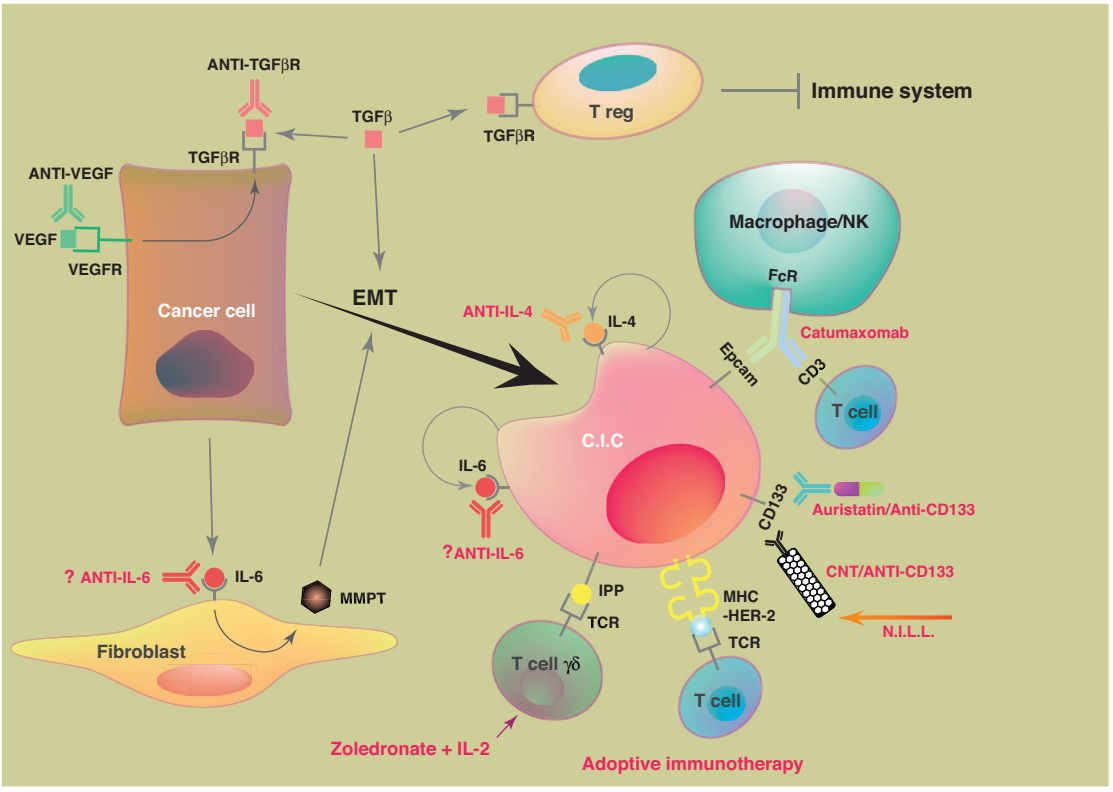

FIGURE 2

CICs targets for antibody or cell-based immunotherapy. Antigens or auto-para-crine loops of CICs mediating survival, EMT, or immune-regulation represent potential targets for antibody or cell-based immunotherapy. Drugs (Zoledronate), antibodies (anti-IL-4, -6, -TGFβ, -VEGF and -CD133), antibody-conjugates (aurisatin/anti-CD133, CNT-anti-CD133) and adoptive immunotherapy (HER-2-specific T cells) are indicated in red characters. NILL (near-infrared laser light) is used to activate thermal lysis of CICs by CNT (carbon nanotubes) delivered to CICs via anti-CD133 mAb. Zoledronate in combination with IL-2 activates TCRγ/δ^{+} lymphocytes to lyse CICs. Catumaxomab is a trifunctional antibody, which simultaneously triggers CD3^{+} T cells and FcR^{+} macrophages/NK cells to kill EPCAM + CICs.

efficiently activated T cell-mediated anti-tumor reactivity and innate immune response *in vitro* and in preclinical studies [65,66]. By intraperitoneal treatment of carcinoma associated with malignant ascites, catumaxomab was able to induce partial tumor response and increase the mean survival time [67]. Catumaxomab has entered several phase II/III clinical trials and was approved in the European Union in April 2009 for the treatment of patients with malignant ascites.

Monoclonal antibodies anti-CD44, a signal-transducing molecule expressed by most CICs, have been also used for CIC targeting [63]. These antibodies are close to entering clinical trials for pancreatic cancer patients [68,69]. Phase I trials were performed with bivatuzumab, a humanized monoclonal antibody recognizing CD44v6, a splice variant of CD44 expressed in squamous cell carcinoma and in normal squamous epithelium. Immunoconjugates with the microtubule inhibitor mertansine were used in head and neck cancer patients [70] and radiolabelled bivatuzumab in patients with early-stage breast cancer [71]. However, both compounds showed serious skin toxicity with some fatal events and these studies were therefore discontinued [72].

Antibodies directed to CD133 conjugated to a cytotoxic drug, auristatin, effectively inhibited the growth of Hep3B hepatocellular cancer *in vitro* and *in vivo* upon transplantation in SCID mice [64]. Carbon nanotubes (CNTs), coupled to antibodies targeting tumor cells, can selectively deliver drugs, even though further studies to verify their potential toxicity are needed. Recently Wang *et al*. showed that single-walled CNTs functionalized with CD133 monoclonal antibody selectively destroyed CD133 + glioblastoma stem cells upon irradiation with near-infrared laser light *in vitro* and in a xenotransplant model [73].

Recent evidence indicates that the high tumorigenic potential and metastatic properties of CICs may relate to paracrine cytokine networks and support their therapeutic targeting through neutralizing antibodies or other inhibitory molecules. In fact, auto- or para-crine production of interleukin (IL)-4 was reported to confer resistance to apoptosis in breast, thyroid, colon, lung and pancreas cancer CICs, which express IL-4 receptor (R) [74]. Indeed, neutralizing antibodies to IL-4 partially restored sensitivity to chemotherapeutic drugs, *in vitro* and in preclinical models, in colon-rectal carcinoma CICs releasing IL-4 [75]. Other cytokine/receptor autocrine loops involving IL-6/IL-6R [76], stem cell factor (SCF)/c-kit [77] or IL-8/CXCR1 [78] play an important role in survival and proliferation of CICs in several cancers and may represent suitable targets for antibody-based therapy.

Cytokines have also been involved in the induction of epithelial mesenchymal transition (EMT), a process inducing loss of polarity

of epithelial tumor cells and gain of invasive properties. EMT is governed in breast, alveolar, ovarian, esophageal, liver cancer cells by a variety of cytokines and growth factors including transforming growth factor-β (TGF-β), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), Wnt ligands and vascular endothelial growth factor (VEGF) [79–84]. EMT inducers are presently evaluated as new molecular targets in the attempt to inactivate drug resistance and eradicate CICs.

In a prostate cancer model [85], IL-6 produced by cancer cells induced an activated phenotype of fibroblasts and secretion of several metalloproteinases, which elicited EMT through disruption of E-cadherin [86]. In another prostate model, an increased autocrine expression of VEGF during the transition from VEGF-R$^+$ prostate intraepithelial neoplasia (PIN) to invasive carcinoma induced TGFβ-RII expression on malignant cells and induction of EMT [87]. Moreover TGF-β1 increased the expression of VEGF in an autocrine loop mode [88]. Therapeutic inhibition of VEGF/TGF-β1 network might impair tumor angiogenesis and early dissemination of malignant cells.

Besides antibody-based therapies targeting CICs' surface antigens and cytokine-growth factor loops, several strategies are being developed to stimulate CIC's recognition and killing by immune effector cells. Adoptive transfer strategies were developed in glioblastoma multiforme (GBM) patients to target CICs, aggressive and resistant to conventional therapies. Stimulation of GBM patients' T lymphocytes with HER2 + autologus tumor cells resulted in the generation of HER2-specific T cells able to kill autologous CICs expressing HER2 and xenografted in SCID mice [89].

Interestingly, a previous report showed that ALDH1-deriving epitopes are recognized by antigen-specific T cells in human ALDH1$^+$ lung cancer cells [90]. It would be therefore important to determine whether ALDH1-specific T cells may be suitable to target the CICs' compartment in other tumors.

Recently it was shown that the bisphosphonate zoledronate, approved for treating bone metastases, stimulates the proliferation and activation of Vγ9Vδ2 T lymphocytes, which recognize and lyse colon CSCs. In addition, zoledronic acid sensitizes CICs to the T cell-mediated cytotoxicity [91]. Interestingly, a phase I clinical trial of zoledronate in combination with IL-2, which further supports Vγ9Vδ2 T lymphocyte expansion, showed good tolerability, disease stabilizations and a decrease of PSA levels in some metastatic prostate cancer patients [92].

A clinical phase I/II trial is evaluating the association of adoptive T cell immunotherapy with IL-2 and zoledronic acid (Table 1) in kidney cancer and lung metastases patients. To more efficiently amplificate Vγ9Vδ2 T lymphocyte population, a new protocol, which includes the use of autologous dendritic cells pretreated with zoledronate has been developed [93].

In a different phase I clinical trial, currently recruiting participants, patients intradermally receive autologous brain tumor stem cell (BTSC) mRNA-loaded DC vaccine. Nonetheless, further studies are required for a better understanding of the role of central nervous system immune privilege and of glioma-mediated immunosuppression to optimize the procedure of DC generation, loading and administration [94].

In general it is hoped that a better knowledge of CICs antigenic profile and of their mechanisms of tumor immune-evasion, may allow the development of a more efficient immune intervention through the combined use of immune-enhancing approaches, such as vaccines, together with agents which selectively target immune-escape mechanisms.

Conclusion

Cancer-initiating cells display a rich repertoire of self-defense systems, which include almost all known mechanisms of cancer-drug-resistance. It seems therefore conceivable that targeting this subpopulation of tumor cells might result in the eradication and cure of cancer. However, the definite identification and characterization of CICs in different types of solid tumors is still far from being accomplished. Each type of tumor may contain CICs exhibiting different molecular properties and markers, which need to be extensively investigated and validated. At the moment, the concept of CICs is mainly operative: it is largely dependent on the potential ability of a limited number of tumor cells to grow in animal models.

Nevertheless, despite several difficulties and controversies, the introduction of the cancer stem cells concept in cancer biology has contributed to find ways for a new philosophy of anti-cancer therapy. To this end, relevant molecules expressed by CICs such as ABC transporters are being evaluated as therapeutic targets, though more efforts are required to increase ABC inhibitors specificity and to avoid toxicity against normal stem cells.

As the cross-talk of CICs with the microenvironment contributes to their survival and resistance, new pro-drugs specifically activated in low oxygen environment are being developed to target their hypoxic niches. The identification of new autocrine/paracrine cytokine and growth factor loops supporting CICs' proliferation within their niche also provides new candidate targets for antibody-mediated blockade. In addition, the identification of CICs' antigenic markers may further allow the development of immunotherapy strategies. Finally, as CICs may display several mechanisms of drug resistance and immune-evasion, a range of multiple drugs and approaches will be necessary to eliminate this highly malignant cell population and achieve tumor eradication or long-term remission.

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