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REVIEW ARTICLE

Promising Targets in Anti-cancer Drug Development: Recent Updates

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Abstract: Cancer is a multifactorial disease and its genesis and progression are extremely complex. The biggest problem in the anticancer drug development is acquiring of multidrug resistance and relapse. Classical chemotherapeutics directly target the DNA of the cell, while the contemporary anticancer drugs involve molecular-targeted therapy such as targeting the proteins possessing abnormal expression inside the cancer cells. Conventional strategies for the complete eradication of the cancer cells proved ineffective. Targeted chemotherapy was successful in certain malignancies however, the effectiveness has often been limited by drug resistance and side effects on normal tissues and cells. Since last few years, many promising drug targets have been identified for the effective treatment of cancer. The current review article describes some of these promising anticancer targets that include kinases, tubulin, cancer stem cells, monoclonal antibodies and vascular targeting agents. In addition, promising drug candidates under various phases of clinical trials are also described. Multi-acting drugs that simultaneously target different cancer cell signaling pathways may facilitate the process of effective anti-cancer drug development.

Keywords: Anticancer, tubulin inhibitor, kinases, cancer stem cells, multi-drug resistance, multi-targeting agents.

1. INTRODUCTION

Cancer is caused by the uncontrolled growth of cells and is a major cause of death throughout the world. It has more than 200 distinct types affecting over 60 human body organs. The major challenge in the anticancer drug development is to design a drug that targets cancer cells with high selectivity and specificity. It is difficult to identify most of the tumors at the primary level and at later stages metastasis of tumor may lead to cancer-related deaths [1-5]. Various strategies employed for the treatment of cancer includes surgery, chemotherapy and radiation therapy used either alone or in combination. Classical chemotherapeutics directly target the DNA of the cell, while the contemporary anticancer drugs involve molecular-targeted therapy such as targeting the proteins possessing abnormal expression inside the cancer cells. These molecular-targeted drugs selectively kill the cancer cells with decreased toxicity towards the normal cells in comparison to the traditional anticancer drugs [6]. Targeted drugs inhibit specific cell signaling pathways that contribute to the malignant phenotype of cancer cells. Chemotherapy has gradually improved with the development of novel antitumor drugs. The treatment of some malignancies with the targeted chemotherapy was successful however, development of drug resistance and toxicity to the normal tissues and cells are the major limitations. Drug transporters are overexpressed in many of the cancer cells and these reduce intracellular drug concentrations. In addition, the evolution of point mutations may be responsible for stronger drug resistance. Multidrug resistance (MDR) is a major obstacle in the effective treatment and complete eradication of cancer.

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The success rate and effectiveness of the anticancer drug development is very low as compared to the investment. Cancer is a multifactorial disease and most of the anticancer drug development centered on a promising target. A lot of efforts have been put in by the scientific community for the total and permanent eradication of the cancer. However, limited success has been achieved due to the complex etiology of the disease. Reasons for the drug failure in most of the human malignancies can be attributed to the incomplete understanding of the pathogenesis of the disease and over rating of the target over expression. The putative role of cancer stem cells in the failure of various cancer chemotherapeutics need to be seriously investigated.

Many drug targets have been identified for the treatment of cancer. Most of the molecularly targeted agents proved ineffective due to either efficacy or toxicity concerns. Recent research work in the field of molecular biology and a better understanding of the pathology of cancer at the molecular level have challenged researchers to focus on the drug targets that can help in the complete eradication of the disease. It has been observed that around 30% of the anticancer drug discovery efforts are focused on finding or validating the kinase inhibitors (Source: Kinexus BioInformatics Corporation). Similarly, a search on clinicaltrialsgov.com and PubMed databases showed that tubulin/microtubule, cancer stem cells and MDR pathways are other hot targets in anticancer drug development. In addition, monoclonal antibodies and multi-targeting anticancer agents have attracted the attention of researchers for developing effective drugs for the complete eradication of disease. A number of potential drug candidates are in various stages of clinical trials. In the current review article following hot targets are described in detail.

2. KINASES AS TARGETS

Kinase inhibitors are the class of anti-cancer drugs that directly interact with the active site of the target enzyme and inhibit kinase function. There are about 2000 kinases estimated in the human genome that are either serine/threonine or tyrosine specific and closely related with one another. A number of small molecule kinase inhibitors have been explored as antitumor agents and about 80 inhibitors are in advanced stage of clinical trials [7, 8]. Protein kinases involved in most of the signal transductions in cells, reversibly phosphorylate the proteins in post translational modifications, and regulate most of the cellular activities. These play role in cell proliferation, apoptosis, differentiation, metabolism of various substrates, and in number of other cellular processes involved in the cell survival [9]. Protein kinases are ATP-dependent phosphotransferases that transfer one phosphoryl group from γ-position of ATP to the hydroxyl group of serine, threonine and tyrosine. The process is generally catalyzed by Mg^{2+} ion which also helps in the ATP binding [10, 11]. In 1978, Ray Erikson reported that the protein kinase activity is associated with the avian sarcoma virus src gene product [12]. Thereafter a total of 538 human kinases (divided into 7 typical families and 7 atypical families) and 900 genes encrypting proteins with kinase activity have been confirmed [13, 14]. Although there are large differences in the amino acid sequencing of the protein kinases however, most of these have similar 3D protein structure [15]. Some of the important kinases and their role in anticancer drug development are described below:

2.1. Tyrosine Kinases

Tyrosine kinases (TKs) play crucial role in the regulation of cell proliferation and survival of cancer cells. TKs catalyze the transfer of phosphate from ATP to tyrosine residues in polypeptides [16]. Tyrosine kinases consist of around 30 different families including EGFR, VEGFR and NGF. In human genome, 90 tyrosine kinases and 43 tyrosine kinase like genes are identified which can be divided into two subclasses naming receptor tyrosine kinases and non-receptor tyrosine kinases [17]. A receptor tyrosine kinase consists of an N-terminal extracellular ligand-binding domain (LBD), a C-terminal intracellular domain with tyrosine kinase activity, and a transmembrane domain. The ATP-binding site of tyrosine kinases can be further divided into three sub regions i.e. adenine region, phosphate-binding region, and the sugar region [18]. Stimulation of tyrosine kinases leads to activation of number of signaling pathways. The binding of a phosphorylated TK receptor with a Src homology 2 (SH_2) domain-containing protein leads to phosphorylation. The activation of this effecter protein further phosphorylates a range of kinases. Phosphorylation of mitogen-activated protein kinase (MAPK) and extracellular signal regulated kinase (ERK) by protein kinase C (PKC) activates the p42/44 MAPK pathway. Phosphorylated MAPK activates various transcription factors and regulates cell proliferation [18, 19]. Some other tyrosine kinase activated pathways like PI_3K, Raf, Ras, p38 MAPK and FAK are also responsible for the cell migration and reorganization which in turn is responsible for the metastasis of tumors (Fig. **1**) [20, 21]. The role of tyrosine kinases has been well established in different cancers

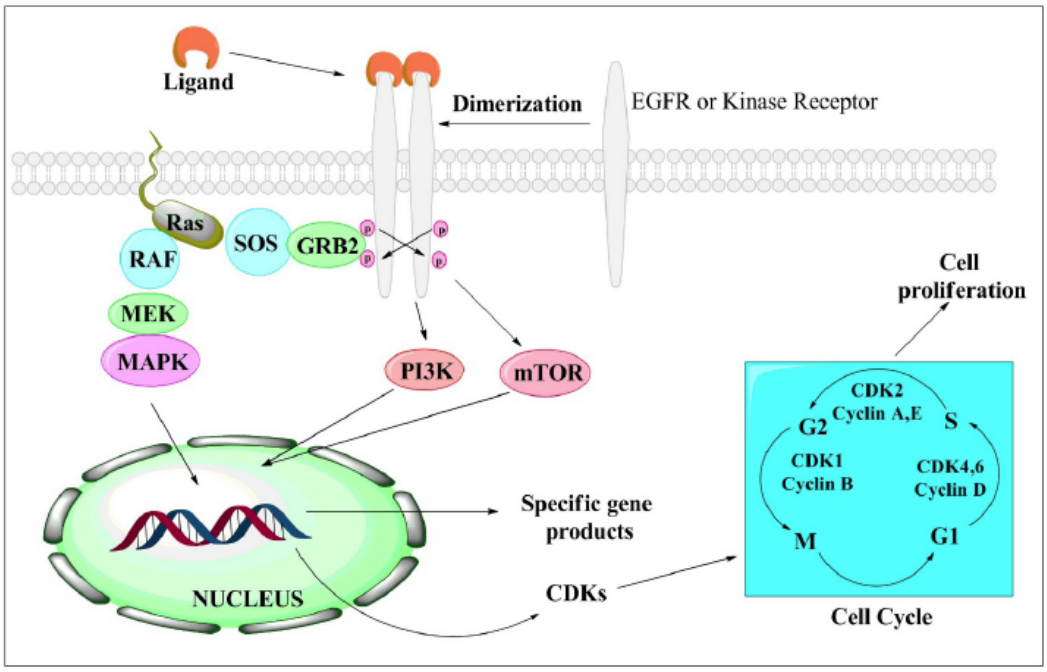

Fig. (1). Mechanism of action of kinase receptors and activation of different kinase dependent pathways by TKs and CDKs

and these are explored as important target for the anti-cancer drug development.

2.2. Cyclin Dependent Kinases

Cyclin-dependent kinases (Cdks) are heterodimeric proteins that composed of a Cdk catalytic subunit and a regulatory cyclin subunit. Cdks play critical role in the cell cycle progression and cellular transcription, and in apoptotic pathways. The mammalian genome has twelve types of Cdks and five of them *i.e.* Cdk1, Cdk2, Cdk3, Cdk4 and Cdk6 are directly involved in driving the cell cycle [22]. Cdk1 play role in the mitotic phase while all other cdks are involved in interphase of cell cycle [23]. It has been established that during cell division Cdk4/cyclin D and Cdk2/cyclin E/A favour the passage through G_1 and S phases, whereas Cdk1/cyclin B modulate transition through late G_2 and mitosis (Fig 1). Inhibition of Cdk1 before mitosis results in G2 phase arrest and quick exit from the mitosis without cytokinesis [24]. Although Cdk1 is an important target for the cell cycle arrest in tumor cells however, unavailability of specific molecular tools for the designing of reversible inhibitors of Cdk1 is the major limitation. It is perceived that a permanent blockade of Cdk1 could result in various toxic effects in normal cells. The subunits of Cdk/cyclin complexes are found mutated in various types of cancers. For example cyclin D1 is found overexpressed in leukaemia, lymphoma [25], neoplasia [26], colorectal, esophageal squamous cell cancer [27], head/neck cancer [28], lung, kidney, breast and prostate cancer [29]. Cdk1/cyclinB complex transiently and incompletely phosphorylates the Bcl-x_L and Bcl-2 proteins during normal mitosis but in cancer states Bcl-x_L and Bcl-2 were found highly phosphorylated [30]. Hence, cdks emerged as an attractive set of targets for the anti-cancer drug development. Several molecules that inhibit cell cycle kinases have been developed and are under clinical investigation as potential anti-cancer agents (Fig. **3**).

2.3. Kinase Inhibitors Under Clinical Development

BCR-AbL was the first kinase targeted for the treatment of chronic myeloid leukemia (CML) using a small-molecule inhibitor imatinib **(1)** [31]. Consequently, a large number of imatinib derivatives (Fig. **2**) have been synthesized and evaluated to develop next generation kinase inhibitors and to investigate their mechanisms of inhibition. Till date, 30 small molecular inhibitors and seven therapeutic antibodies kinase inhibitors have received US Food and Drug Administration approval for the treatment of different cancers. More than half of these are approved in the last three years [9, 32]. Majority of these kinase inhibitors target the highly conserved ATP binding pocket of the catalytic kinase domain. Ibrutinib **(2)** (15 January 2015), palbocicnib **(3)** (3 February 2015), lenvatinib **(4)** (13 February 2015), gefitinib **(5)** (13 July 2015), cobimetanib **(6)** (10 November 2015), osimertinib **(7)** (13 November 2015) and alectinib **(8)** (11 December 2015) are some of recently approved protein kinase inhibitors

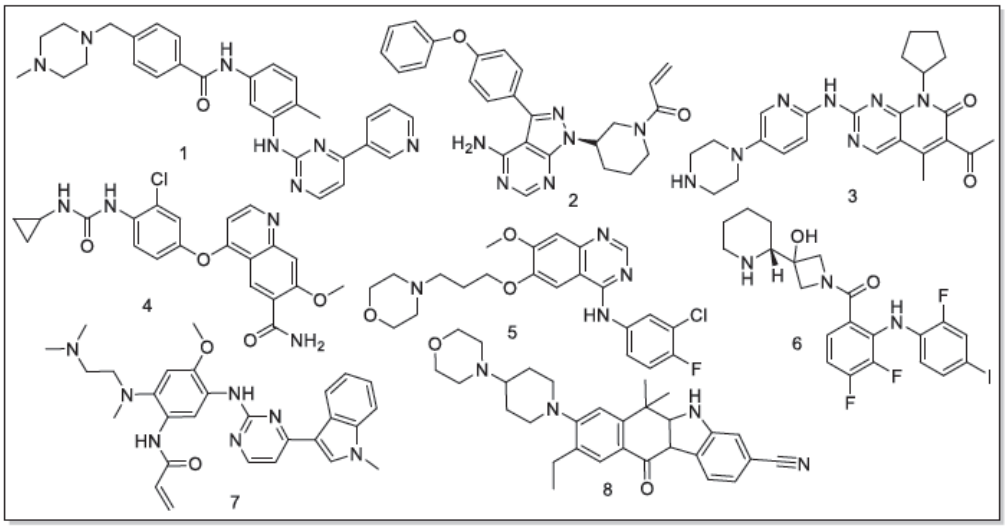

Fig. (2). Kinase inhibitors approved as drugs for the treatment/management of cancer.

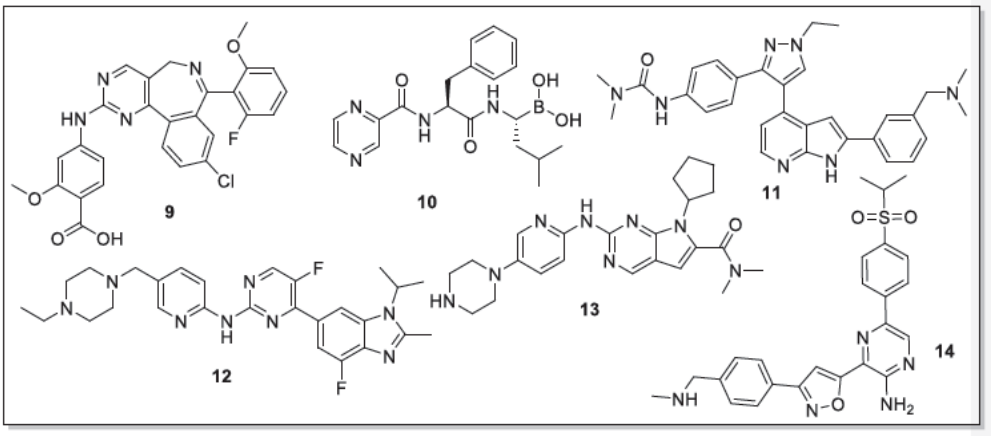

Fig. (3). Some promising kinase inhibitors in advance stage of clinical development.

for the treatment of various cancers [33]. Imatinib is the most successful kinase inhibitor owing to its multi-targeted nature. After its approval for the treatment of chronic myelogenous leukemia, it has been approved for various other cancers including gastrointestinal stromal tumors (GIST) (targeting KIT and PDGFR) [34, 35], recurrent and/or metastatic fibrosarcoma protuberans (targeting PDGFR) [36], hypereosinophilic syndrome (targeting PDGFR) [37] and myeloproliferative diseases (targeting KIT, and/or ABL1).

Currently, about 150 protein kinase inhibitors are in advanced stage of clinical trials and more than 500 are in pre-clinical stages (Fig. **3**). Aurora Kinase Inhibitor MLN8237 (**9)** and Bortezomib (**10**) are under phase I/II clinical trials for the treatment of patients with relapsed or refractory multiple myeloma (NCT01034553). Aurora B/C kinase inhibitor GSK1070916A (**11**) (GlaxoSmithKline) completed phase I clinical trial [38] for its safety, tolerability in advanced solid tumor patients (NCT01118611). Abemaciclib (**12)** (Eli Lilly) and Ribociclib (**13**) (Novartis/Astex) are under phase III clinical trials targeting cyclin dependent kinase 4 and 6 [39]. X-82 a dual VEGFR/PDGFR kinase inhibitor is under phase-I clinical trials for its enzyme blocking potential in unspecified solid tumors (NCT02146222) and undergoing phase-II trial for treating patients with pancreatic neuroendocrine tumors (NCT01784861). VX-970 **(14)** was the first ATR kinase inhibitor to enter clinical trials [40] for treating patients with solid tumors (NCT02595931). It is also undergoing safety studies in advanced solid tumors (NCT02157792).

Only a small number of kinases are targeted for the anti-cancer drug development and majority of the kinases still need to be explored. Understanding the 'switch on' and 'switch off' mechanism of the kinase enzymes can allow the rational designing of kinase inhibitors and may help in the development of protein kinase inhibitors that could be developed as promising drug candidates for the complete eradication of cancer.

3. TUBULIN/MICROTUBULE AS A TARGET

Cancer cells divide and grow very fast in comparison to the normal cells. Since one of the key components required for the cell division and cell growth is the microtubule, therefore microtubules-targeting agents are explored for anti-cancer drug development [41, 42]. Microtubules are universal cytoskeletal structures formed by the association of α and β tubulin heterodimer. These play important role in the development and maintenance of cell shape, in cell division, cell reproduction, cell signaling and cellular movement [43]. Microtubules and α, β-tubulin heterodimers exists in a dynamic equilibrium. Both the monomer units (α and β) of tubulin contains a GTP molecule which bound at the dimer interface of the α-subunit in an irreversible way and is non-hydrolysable while, GTP molecule of the β-subunit of tubulin bounds in a reversible way and is exchangeable with GDP molecule. α,β-tubulin heterodimer acts as a monomer unit and β-polypeptide of one tubulin monomer unit get attached to the α-polypeptide of another tubulin monomer in head to tail manner, and this arrangement results in the formation of protofilaments. Thirteen protofilaments arrange in the parallel fashion to give rise to a cylindrical microtubule wall.

In the polymerization cycle of the microtubule, β-subunit of tubulin should be in the GTP-bound state. After attachment to microtubule, GTP of β-subunit irreversibly hydrolyzed to GDP and majority of the β-tubulin in the microtubule converts to GDP-bound form. Microtubule is capped with GTP-bound β-tubulin at the plus end. For the next cycle of tubulin addition, the GTP of capped β-tubulin is hydrolyzed to GDP and the exposed GDP β-tubulin leads to conformational changes which in turn initiate rapid depolymerization of microtubule with the release of GDP-tubulin unit [44]. Microtubules dynamics mainly consist of stochastic phases of growth and shrinkage due to polymerization and de-polymerization of tubulin dimers. This pattern of non-equilibrium behavior is known as dynamic instability [45]. Dynamic instability of microtubules consists of phases describing growth and disassembly. The transition from growth to disassembly is called *catastrophe* while transition from disassembly to growth is known as *rescue*. Any alteration to the dynamic instability of microtubules halts the cell division process and may lead to apoptosis (Fig. **4**).

The three-dimensional structure of the α,β-tubulin heterodimer has been well explored [46] and therapeutic potential of tubulin has been reviewed by number of research groups [47-53]. A number of structurally diverse small molecules attach to the tubulin at four different binding sites: taxane/epothilone, laulimalide, vinca alkaloid and colchicine binding site. Microtubule binding agents interact with these sites on the tubulin and disrupt microtubule dynamics by either stabilizing or destabilizing the polymerized state. The taxoid binding site is located on the polymerized tubulin i.e. microtubule and its ligands like paclitaxel stabilizes microtubule. Similarly, laulimalide can also promote the tubulin-polymerization but binds to a different site on the microtubules [54]. Vinca and colchicine domains present on the microtubule and monomeric α,β-tubulin respectively, and are microtubule polymerization inhibitors. During cell division, microtubule dynamics play crucial role for the proper attachment and movement of chromosomes [55]. Disruption of the microtubule dynamics in a cell by an inhibitor, blocks the cell division at mitosis leading to cell death. Hence, the modulation in microtubule dynamics represents an important target for the development of anti-cancer drugs.

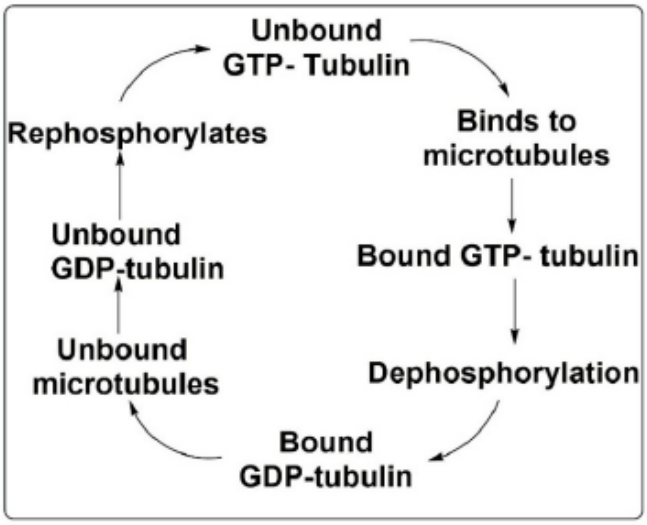

Fig. (4). Tubulin-microtubule dynamic instability: Microtubule undergoes different phases of growth and shrinking. In rescue phase microtubules grow by addition of tubulin unit and after monomer incorporation GTP of β-subunit hydrolyse to GDP inside the tubulin. In catastrophe phase microtubules undergo depolymerization and it shrinks in size.

3.1. Microtubule Stabilizing Agents and their Mechanism of Action

Microtubule stabilizing agents are the chemical agents that bind to the tubulin in polymeric microtubule form and prevent the depolymerization process. These agents promote the addition of tubulin monomer units to microtubule even when tubulin is in the GDP-bound state. Microtubule stabilizing agents bind with one of the two binding sites (taxoid binding site and lauli-

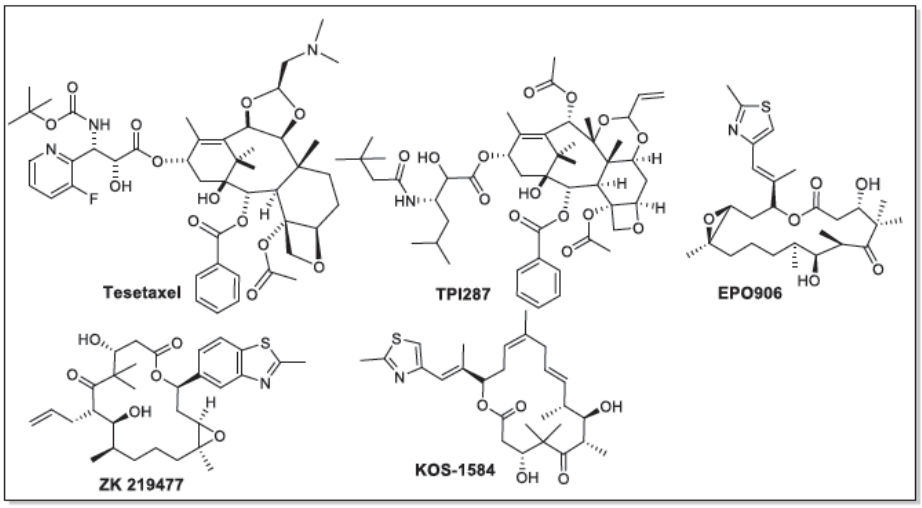

Fig. (5). Structures of various microtubule-stabilizing agents under clinical trials as described in Table **1**.

malide/peloruside (LAU/PEL) binding site) that promote its stabilization and stop depolymerization process. This leads to disturbance in the spindle dynamics during cell division and cell arrest at G_2/M phase [56]. The ligands binding to the taxoid site strongly influence the interactions at three regions in the tubulin; H3, S3 and M-loop [57-59]. Binding of a taxoid binding ligands causes increased interactions of M-loop in the β-subunit with H1-S2 and H2-S3 loops of the neighboring protofilaments and hence stabilizes the microtubule [60]. In addition, paclitaxel pushes the M-loop downwards which results in enhanced interactions with loops in the adjoining monomer unit. Conversion of GTP to GDP in β-subunit of tubulin destabilizes the microtubule and is responsible for its depolymerization. But, paclitaxel binding leads to some structural changes at the nucleotide binding site that compensate for the GTP hydrolysis [61, 62]. Microtubule stabilizing agents also bind to the LAU/PEL binding site, distinct from the taxoid binding site [63]. The exact location of LAU/PEL binding site is still not clear. Pineda *et al*. through computational studies proposed that the LAU/PEL binding site present on the α-subunit of tubulin and localized to the S9-S10 loop region [64]. In contrast, Huzil *et al.* in 2008 reported that peloruside A binds with the β-tubulin instead of α-tubulin [65]. Hydrogen-deuterium exchange mass spectrometry (HDX-MS) was used to elucidate the bonding pattern, and it was proposed that peloruside binding site was situated on the exterior surface of the β-tubulin subunit [65].

3.2. Microtubule Destabilizing Agents and their Mechanism of Action

Chemical agents that bind to the colchicine and vinca domain destabilize microtubule and act as tubulin polymerization inhibitors. The colchicine binding site is located at the interface between β and α-tubulin and majority of the interactions are restricted to the β-tubulin monomer [66]. Colchicine forms a complex with the tubulin and then adds to the microtubule and induces many conformational changes [67]. These conformational changes make microtubule polymerization process energetically unfavourable and prevent the microtubule polymerization by sterically hindering further addition of tubulin dimers at the ends [68]. The vinca binding pocket is present on the longitudinal interface between two tubulin heterodimers and is close to the GTP-exchangeable site of the β-subunit [69]. Various compounds such as vinblastine (VBL), vincristine (VCR), vindesine (VDS) and vinorelbine (VRL) bind to the vinca domain [70]. Unlike colchicine, vinca alkaloids bind directly with the microtubules without forming a complex with the soluble tubulin. Their binding can bring a conformational change in tubulin and stabilize tubulin-tubulin longitudinal associations into spiral assemblies, protofilaments, oligomers or paracrystals at the expense of microtubule growth. Besides inhibition of microtubule dynamics, vinca alkaloids are found to interfere with the nucleotide cycle of tubulin and inhibit its GTPase activity.

Tubulin has emerged as one of the important targets for the anti-cancer drug development. Therefore, a number of tubulin targeting agents (Table **1**) have been synthesized and structure-activity relationship studies have been performed for the discovery and development of safer and more potent drug candidates [71, 72]. It has been well documented that the alteration in β-tubulin isotypes expression and/or mutation in tubulin genes may lead to drug resistance [73]. Most common mechanism for resistance to tubulin binding agents is

Table 1. Tubulin binding agents under different phases of clinical trials.

S. No.	Name of Drug Candidate/ Compound	Clinical Trial Phase and Status	Cancer Disease	Reference (Clinicaltrials.gov)
I. Taxane binding agents				
	Tesetaxel	Phase II, Ongoing	Safety and efficacy study for locally advanced or metastatic breast cancer	NCT01609127
		Phase II, Ongoing	Progressive castration-resistant prostate cancer	NCT01296243
	TPI-287	Phase-I, Completed	Hodgkin disease	NCT00113724
		Phase-I and II, Ongoing	Melanoma	NCT01067066
		Phase-II, Ongoing	Glioblastoma	NCT01933815
	Oraxol (oral paclitaxel)	Phase-I, Completed	Advanced solid tumors	NCT01967043
		Phase-III, Ongoing	Metastatic breast cancer	NCT02594371
	EPO906	Phase-II, Completed	Non-small cell lung cancer	NCT00219297
		Phase-III, Failed	Ovarian cancer	[77]
	ZK 219477	Phase-II, Completed	Melanoma, Prostate and ovarian cancer, NSCLC	NCT00598507 NCT00751205 NCT00359359
		Phase-II, Terminated	Breast cancer, CNS Disease	NCT00496379
	KOS 1584	Phase-II, Completed	Non-Small Cell Lung Cancer	NCT00651508
II. Colchicine site binding agents				
	Fosbretabulin	Phase-II, Completed	Non-small cell lung cancer	NCT00653939
		Phase-I/II, Ongoing	Ovarian neoplasms	NCT02055690
	OXi4503	Phase-I, Completed	Solid tumors	NCT00977210
		Phase-I/II, Ongoing	Acute myelogenous leukemia Myelodysplastic syndromes	NCT02576301
	Ombrabulin	Phase-I/II, Completed	Advanced solid tumors	NCT01293630, NCT01021150, NCT01063946, NCT01907685
	Plinabulin	Phase-I, Completed	Advanced solid tumors or lymphoma	NCT00322608
		Phase-III, Ongoing	Non-small cell lung cancer	NCT02504489
	MPC-6827	Phase-I, Ongoing	Refractory solid tumors	NCT00394446
	BNC105P	Phase-II, Ongoing	Renal cell carcinoma	NCT01034631
		Phase-I/II, Withdrawn	Ovarian cancer	NCT01624493
	CYT997	Phase-I/II, Terminated	Glioblastoma multiforme	NCT00650949
		Phase-II, Terminated	Relapsed and refractory multiple myeloma	NCT00664378

(Table 1) contd….

S. No.	Name of Drug Candidate/ Compound	Clinical Trial Phase and Status	Cancer Disease	Reference (Clinicaltrials.gov)
III. Vinca site binding agents				
	Vinflunine	Phase-II, Completed	Metastatic transitional cell carcinoma of the urothelium	NCT00101608
		Phase-II, Completed	Prostate cancer	NCT00545766
		Phase-II, Ongoing	Urothelial carcinoma, Bladder cancer, Renal pelvis cancer, Ureter cancer, Urethra cancer	NCT02665039
	Romidepsin	Phase-I, Ongoing	Advanced solid tumors	NCT01537744
		Phase-I, Ongoing	Relapsed/refractory lymphoid malignancies	NCT01998035
	Eribulinmesylate	Phase-IV, Ongoing	Locally advanced or metastatic breast cancer	NCT01961544
		Phase-I, Ongoing	Triple negative breast cancers	NCT02120469, NCT02513472
	Vinorelbine	Phase-I, Completed,	Non-Small Cell Lung Cancer	NCT00702182, NCT00870532,
		Phase-III, Completed	Advanced Non-Small Cell Lung Cancer	NCT00737867
		Phase-II, Ongoing	Breast cancer	NCT02144194

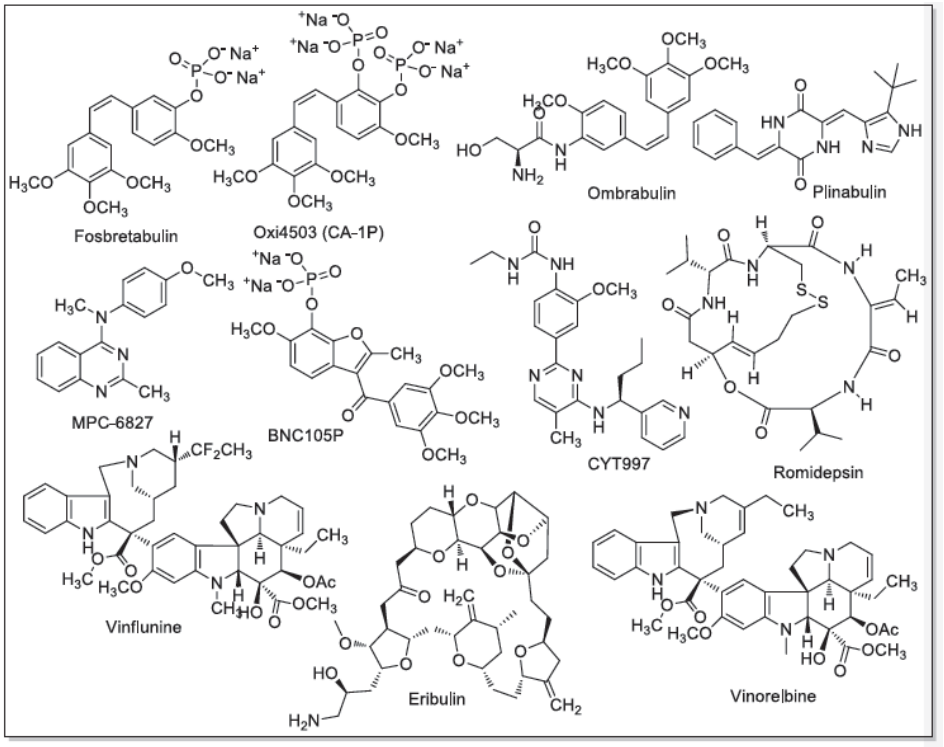

Fig. (6). Structures of various microtubule-destabilizing agents undergoing clinical trials as described in Table **1**.

P-glycoprotein mediated multidrug resistance [74]. In many studies, βIII-tubulin isotype has been implicated in the paclitaxel resistance through decreased stability of the microtubule and negates the effect of paclitaxel [75, 76]. Therefore, the existing drug molecules need to be chemically modified so that these show minimum affinity towards transporting proteins, responsible for the multi-drug resistance. Use of different drug combinations or different formulations of tubulin binding agents in combination with other che-

motherapeutic agents may also reduce the chances of drug resistance. Some formulations of already existing tubulin binding agents like paclitaxel are already under clinical investigation for the development of effective and safe anti-cancer agents.

4. VASCULAR TARGETING AGENTS

Targeting the tumor vasculature is an effective strategy in the treatment of cancer given its accessibility to blood-borne drugs. The tumor cells divide very fast and a constant supply of oxygen and nutrients is required. Hence, the development of blood vessel networks is essential in tumor growth and progression as well as in metastasis. Vascular disrupting agents (VDAs) are capable of interrupting tumor blood flow [78-80]. Unlike anti-angiogenic agents, which prevent the growth of new capillaries, VDAs target established tumor vasculature. Tumor vessels are abnormal and differ significantly from vessels in healthy tissues, being poorly organized, tortuous, leaky and comprising actively proliferating endothelial cells and incomplete basement membrane [78, 81]. These differences create a therapeutic window that could be exploited by VDAs to selectively target the tumor vasculature. Majority of the VDAs in development are microtubule binding agents that work by disrupting the endothelial cytoskeleton, resulting in increased vascular permeability and rapid vessel shutdown [78, 81]. Consequently, side-effect toxicity of such agents is relatively mild. A number of structurally different compounds including combretastatins are being investigated as VDAs [82]. The mechanism of action of VDAs is believed to be related to the induction of cytokines such as tumor necrosis factor-alpha. Different clinical trials so far have shown that VDAs are generally well tolerated and tumor selective. One of the problems associated with the VDAs is that they commonly lead to hypoxia and necrosis at the tumor core, but tumor tissue at the periphery remains unaffected, leaving a viable rim of cells [78]. These cells are well-oxygenated and may be sensitive to the traditional cytotoxic agents. In fact, studies have reported synergistic effects of VDAs combined with the standard chemotherapeutic agents, and such combinations are being investigated in clinical trials [81].

FAA (flavones-8-acetic acid) was the first member of synthetic flavonoid reported as vascular disrupting agent. It was developed as a non-steroidal anti-inflammatory agent but displayed potent anti-cancer activity in pre-clinical studies [83, 84]. Unfortunately, it lacked clinical activity in humans and hence more active FAA derivatives were developed and DMXAA (5, 6-dimethylxanthenone-4-acetic acid) was found to be the most potent derivative of FAA [85]. DMXAA **(15)** can directly disrupt the tumor vasculature by inducing apoptosis of endothelial cells (Fig. **7**). Further, in randomized phase II clinical trials in patients with non-small cell lung carcinoma (NSCLC), it has been observed that when carboplatin and paclitaxel were used together with DMXAA (NCT00832494) there was no additional toxicity [86]. A second randomized phase II study evaluated the action of DMXAA when combined with docetaxel on castration refractory metastatic prostate cancer. In phase-III study, DMXAA was found well tolerated and no increase in cardiac and vascular toxicity was detected however, it failed to improve the anti-tumor activity in advanced NSCLC [85].

Combretastatin A-4P (CA-4P) **(16)** has emerged as a promising vascular disrupting agent and is under clinical study in combination with other chemothera-

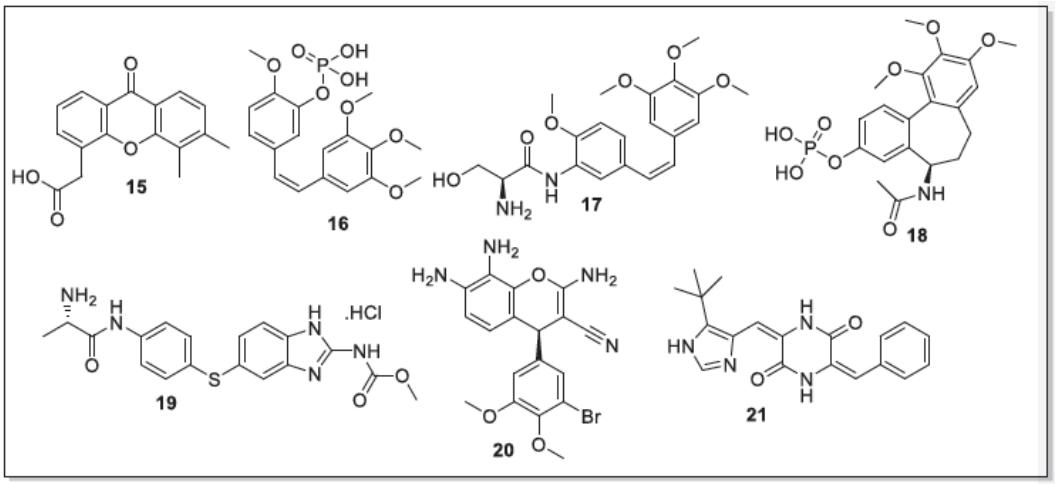

Fig. (7). Some vascular disrupting agents in various stages of drug development.

peutic agents. Three different phase I clinical trials have been completed with CA-4P as a single agent [87]. A significant reduction in blood flow was observed in majority of the patients. Combination studies of CA-4P along with carboplatin or paclitaxel were also performed. It was concluded that the combination therapy was well tolerated and most of the patients showed good response to the tumor [88]. A derivative of CA-4, AVE8062 **(17)** is undergoing phase-I study in combination with docetaxel, cisplatin, carboplatin and bevacizumab [89]. A combination of AVE8062 with taxanes and platinum complexes is under Phase-II clinical trial (NCT01263886). A Phase III clinical trial has been completed using AVE8062 as a single agent in soft tissue sarcoma (NCT00699517).

Similarly, a colchicine derivative ZD6126 **(18)** has completed Phase I clinical trial for the anti-vascular effects [90]. The blood flow in all tumors was reduced to 36–72 % of the baseline value, with strongest affects exerted at the tumor center. Recently, ZD6126 has undergone a Phase II clinical trial for the treatment of metastatic renal cell carcinoma. In a combination therapy with oxaliplatin, 5-fluorouracil, and leucovorin it has been evaluated against metastatic colorectal cancer. However, these trials have been terminated due to the toxicity issues (NCT00065117). Similarly as vascular disrupting agents, MN-029 (**19**) (NCT00423410), EPC2407 (**20**) (NCT01240590) and NPI2358 **(21)** (NCT00630110) are undergoing phase-I clinical studies.

5. CANCER STEM CELLS AS A TARGET

Cancer stem cell (CSC) is a cell within the tumor that possesses self-renewing capacity and differentiation ability and unlimited proliferative potential. The CSC can divide asymmetrically and maintain their proliferative potential through self-replication. CSCs can produce daughter cells that can multiply very fast for a limited period of time. These cell can differentiate and produce bulk of cells in the tumor mass that are non-CSCs. CSCs are responsible for the tumor initiation and maintenance of population of highly proliferating cells in the tumor [91, 92].

The leukemic stem cells (LSCs) were the first to be described as CSCs in human acute myeloid leukemia (AML)[93]. In the breast cancer cell study, it was found that a new tumor initiating capability remains largely in a very small subpopulation of self-renewing breast tumor cells with primitive surface immunophenotype and differentiation potential [94]. The CSCs have now been identified and characterized in many different tumors [95] including brain [96-99], prostate [100-102], melanoma [103], colon [104, 105], lung [106], ovarian [107, 108] and chronic myelogenous leukemia [109-111]. Thus, it is believed that different types of cancer contain a subset of stem-like tumor cells and tumor-initiating and progressing capacity exists largely or exclusively within the stem-like fraction.

Targeting CSCs population is critical for an effective anti-cancer drug development. CSCs can have the proliferation capability for the long term and are crucial for the tumor progression and metastasis. In order to permanently arrest the disease progression, the CSC population must be completely eradicated. However, it is very difficult to target the CSCs population. Just like the normal tissue stem cells, CSCs may inherent a number of characteristic features that make them resistant to many of the conventional anti-cancer therapies. These cells show low proliferation rate, improved DNA damage repair, and over expression of anti-apoptotic proteins and multidrug resistance transporters [112]. In fact, higher radio resistance [113-117], chemo resistance [118-122], and over expression of multidrug resistance-associated proteins [123] have been observed in CSCs in a variety of cancers. In addition, the protective effects provided by the niche microenvironment make it difficult to target CSCs. A number of strategies for targeting CSCs are under investigation (Fig. **8**; Table **2**) [124]. Majority of the CSCs targeting therapies involve interruption of cell signaling pathways that are critical for the survival and functioning of the CSC population. Targeting the CSC niche may provide an opportunity to indirectly block various functions of CSCs and hence circumventing many aspects of CSC-associated intrinsic drug resistance. Disruption of the supportive vascular niche may also sensitize CSCs to the effects of conventional cytotoxic radiotherapy or chemotherapy, and may also potentiate the effects of other CSC-targeted therapies.

5.1. Notch Pathway

Notch receptors are important in determining the cell fate of various ancestries in different organisms. Generally mammals contains four Notch members and all of these have an extracellular region containing multiple epidermal growth factor repeats [125]. Notch signaling pathway is initiated on binding of transmembrane ligands of one cell to the Notch receptor present on an adjacent cell. It leads to the release of the γ-secretase-mediated proteolytic of the Notch intracellular domain (NICD) [125, 126]. NICD then moves into the nucleus and interacts with CBF1 cofactor and acti-

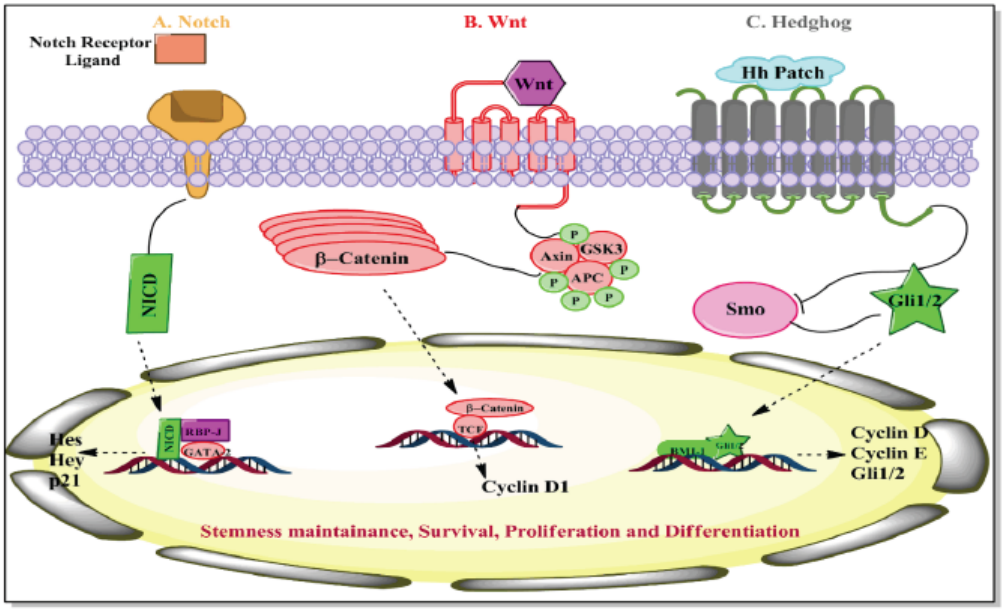

Fig. (8). Various cancer stem cell pathways: A) Notch pathway, B) Wnt pathway and C) Hedghog pathway.

Table 2. Different strategies to target cancer stem cells.

Targets for cancer stem cells	
Surface markers CD33 CD44 CD90 CD133	**Signal cascade** Notch Hedgehog Wnt NFkB
ABC cassette MS-209 VX-710 Verapamil Tariquidar	**Tumor microenvironment** Tumor vascular CXCL12/CXCR4 VEGF/VEGFR

vates the HES and HEY target genes (Fig. **8**). The interaction between the extracellular region of the Notch and its ligand triggers cell signaling that that either hamper differentiation of stem or progenitor cells or induces differentiation into specific lineages. Notch ligands and receptors have been found overexpressed in many cancerous organs including breast [127], cervix, renal, pancreas carcinoma, leukemia [128], neuroblastoma, myeloma [129], and medulloblastoma [130]. In most of the tumor types increased Notch activity has been observed which promotes tumor growth. Thus, targeting Notch signaling is a promising therapeutic target for different types of cancers.

5.2. Hedgehog Pathway

The Hedgehog (Hh) family of proteins play important role in the development of embryonic stem cells and in the differentiation of many tissues [131, 132]. The Hedgehog gene can be categorized into three subgroups: Sonic Hedgehog (SHh), Indian Hedgehog (IHh) and Desert Hedgehog (DHh). The Hh pathway is activated when Hh protein binds to the cell-surface receptor Patched (Ptch) [133]. Formation of Hh-Ptch1 complex triggers release of Smoothened (Smo) and Glioma (Gli1, Gli2 and Gli3) associated oncogene transcriptional proteins which induces the expression of various context-specific genes that regulate cellular differentiation, proliferation, cell migration, and maintenance of cell stemness properties [132, 134]. The anomalous activation of the Hh pathway may lead to the unregulated growth of tissue cells, deformations in cell structures during cell division and contribute to tumor genesis in various human cancers (Fig. **8**) [135]. The Hh pathway is essential for the maintenance of CSCs in various human cancers including pancreatic cancer, gastric cancer, colorectal cancer [136-138] and

it is responsible for the development of drug resistance of cancer cells. Thus, the small molecules that inhibit the Hh signaling pathway may result in depletion of CSCs and may overcome treatment resistance. Several compounds are under preclinical and clinical studies for targeting Hh pathway (Fig. **10**, Table **3**). Cyclopamine, a SMO signaling inhibitor, has been explored *in vitro* and *in vivo* as an inhibitor of the hedgehog pathway and metastatis of tumor cells in various cancers including breast cancer [139], pancreatic cancer [140], prostatic cancer [141] and glioblastoma [142].

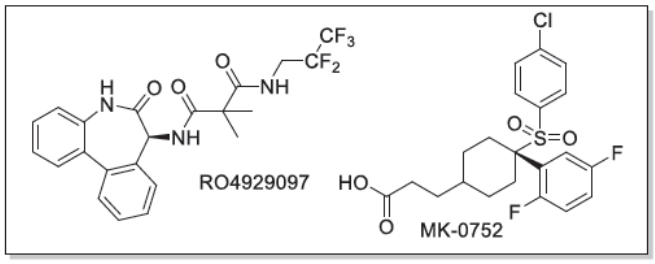

Fig. (9). Structure of Notch pathway inhibitors under clinical development.

5.3. Wnt Pathway

The Wnt signaling pathway plays important role in embryonic and cell development, cell proliferation, and survival [143]. The canonical Wnt/β-catenin signaling pathway involved in self-renewal and maintenance of the stem cells and cancer stem cells [144]. It is initiated when a Wnt ligand binds to the cell membrane co-receptors and activate target genes [145]. The Wnt family of genes encodes 19 cysteine-rich secreted glycoproteins that bind frizzled (Fzd) receptors [146]. Oncogenic mutations of β-catenin, or APC tumor suppressor results in the dysregulation of Wnt/β-catenin pathway that causes proliferation of cancer stem cells in various cancer types including chronic myeloid leukemia (CML) [147], gastric cancer [148], colorectal cancer (CRC) [149]. The canonical Wnt pathway maintains the stem cell niche in conjunction with bone morphogenetic protein (BMP) and Notch signaling [150], and regulates self-renewal.

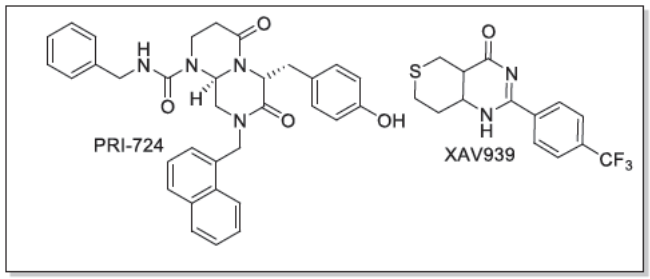

Fig. (11). Structures of Wnt pathway inhibitors under clinical development.

Small molecule CBP/β-catenin inhibitor ICG-001 was found to target and eliminate drug-resistant leukemic stem cells both *in vitro* and *in vivo* [151]. In addition, nonsteroidal anti-inflammatory drugs (NSAID) or vitamins which targets cyclooxygenease 2 (Wnt target enzyme, Aspirin) and E-cadherin (Vitamin A and Vitamin D) [151, 152] act as inhibitors to the Wnt signaling pathway. A number of ligands are in various stages of clinical trials (Fig. **11**, Table **3**) to find a potent and efficient inhibitor for Wnt cascade.

5.4. NF-kB Pathway

Nuclear factor-kappa B (NF-kB) exists in the cytoplasm of the cells as homo- or heterodimers of structurally similar proteins [153]. It is a transcription factor that stimulates the expression of target genes in response to stimuli such as viral and bacterial antigens, UV radiation and cytokines (IL-2 and TNF-α). Till date, around 400 NF-kB targeted genes, such as cytokines, chemokines, oncogenes, pro-/antiapoptotic proteins, growth factors, and cell adhesion molecules have been identified [154, 155]. Within the cell, the NF-kB

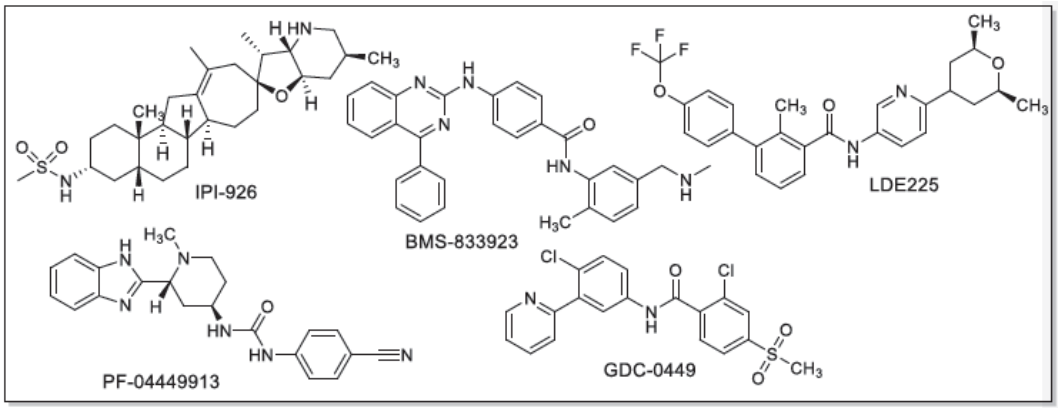

Fig. (10). Structures of various Hedghog pathway inhibitors under clinical development.

molecule form a complex with an inhibitory protein, IkB and it mask the nuclear localization signal of NF-kB and prevents its nuclear translocation. Activation of IkB kinases causes phosphorylation of IkB and it dissociates from the NF-kB dimer through proteasome degradation. Thereafter NF-kB dimer translocate to the nucleus, attaches to the kB binding pocket and stimulates the production of specific proteins [156].

The nuclear factor play important role in the cell proliferation, embryonic and neuronal developments and apoptosis. Dysregulation of NF-kB pathway is linked with the diseases like chronic inflammation, immunodeficiency and cancer [157]. Constitutive activity of NF-kB pathway in tumor cells is responsible for proliferation, angiogenesis, blocking apoptosis, and metastasis [158]. Thus, inhibitor of NF-kB pathway [156] may be developed as effective therapeutic agents for the treatment of cancer.

One of the major issues involved with the CSCs therapy is cytotoxicity towards normal stem cells. During treatment, any damage to the normal stem cells may prove fatal to the patients. Some chemical compounds targeting various CSCs signals cascades are under different phases of clinical trials for their efficacy and safety studies (Table **3**).

6. MONOCLONAL ANTIBODIES AND ANTI-CANCER THERAPIES

Antibodies also known as immunoglobulins, are Y-shaped proteins which help in the identification and removal of foreign antigens such as viruses and bacteria. These are produced by the immune system in response to the presence of an antigen. Antibodies are heterodimers containing two light chains and two heavy chains. Each light chain is bounded to the long chain by a disulfide bond, and heavy chains are connected to each other by multiple disulfide bridges [161, 162]. The idea to treat and diagnose cancer using monoclonal antibodies (mAb) started in late nineteenth century. Now it has become one of the most successful therapies for the treatment of solid tumors. Generally, in the healthy individuals, antibodies identify and label foreign harmful particles found in the body as first step in the destruction of these foreign pathogens or abnormal cells. Following this, various other components of the immune system of body attack the targets tagged by antibodies and destroy them [162, 163]. The specificity of antibodies for a particular antigen depends on the sequence of amino acid located within the variable region [164]. Antibodies target tumor cells through various mechanisms which includes: (i) direct action of the antibody i.e. receptor blockade, induction of apoptosis, or delivery of cytotoxic agent to target receptor; (ii) immune-mediated cancer cell death i.e. regulation of T cell function and antibody-dependent cellular cytotoxicity (ADCC) and (iii) specific effects of an antibody on tumor vasculature [165-168]. ADCC is the major mechanism operating in mAb-mediated immunotherapies. ADCC mechanism involves binding of Immunoglobin G (IgG) antibody to its antigen-binding site on the target tumor cells, and then the Fc domain (portion that defines interactions with antibody) of antigen is recognized by the specific Fcγ receptors (FcγR) on the effector cells [162]. FcγRs contain activation domains that stimulate immune cells *via* Src-family protein tyrosine kinases. ADCC mainly affects the Natural killer (NK) cells *via* Fcγ receptors IIc (FcγRIIc) and Fcγ receptors IIIa (FCγRIIIa). The role of FcγRIIc activation in NK cells is still not clear however, activation of FcγRIIIa induces ADCC and cytokine production. Release of cytokines and chemokines leads to the inhibition of cell proliferation and angiogenesis [169, 170].

After some initial disappointments with the monoclonal antibody therapies, now a number of monoclonal antibodies (mAbs) have been approved by the USFDA for clinical use. Rituximab was the first monoclonal antibody approved for the cancer therapy in November 1997 [171]. These antibodies are used against different types of cancers such as (i) Rituximab for non-Hodgkin's lymphoma (ii) Trastuzumab for breast cancer (iii) Bevacizumab for colorectal cancer (iv) Alemtuzumab for chronic lymphocytic leukemia (v) Cetuximab for colorectal cancer and (vi) Panitumumab for colorectal cancer [172]. Similarly, some unconjugated monoclonal antibody therapies such as humanized monoclonal antibody (CD33), radioisotope-antibody conjugates (ibritumomab tiuxetan and tositumomab) are also approved for anti-cancer therapy. Due to high success rate of this type of therapy, a number of mAbs are in various phases of clinical trials for the treatment of different types of cancer (Table **4**).

7. CANCER AND MULTI DRUG RESISTANCE

Multi-drug resistance (MDR) is one of the major limitations with most of the chemotherapeutic agents for the treatment of cancer. Newer molecular targets and chemotherapeutic agents are constantly explored to make the anti-cancer treatment more effective. Resistance to chemotherapy can occur in two ways [173]. The primary or intrinsic resistance results from the genetic alterations existing before treatment. Second type of resistance is induced by the drug during treatment and is called secondary or acquired resistance. Both

Table 3. Cancer stem cells targeting agents under various phases of clinical trials.

S. No.	Name of Drug Candidate/ Compound	Clinical Trial Phase and Status	Cancer type	Reference (clinicaltrials.gov)
I. Hedgehog pathway inhibitors				
1.	IPI-926	Phase-I/II, Completed	Neoplasms, Metastatic pancreatic cancer, Primary myelofibrosis	NCT00761696, NCT01130142, NCT01371617
		Phase-I, Recruiting	Pancreatic cancer	NCT01383538
2.	BMS-833923	Phase-I/II, Completed	Basal cell carcinoma, Leukemia,	NCT00670189, NCT01218477, NCT01357655
		Phase-I, Ongoing	Basal cell nevus syndrome	NCT02100371
3.	LDE225	Phase-I, Completed	Pancreatic cancer	NCT01487785
		Phase-I/IB, Recruiting	Prostate cancer, Recurrent ovarian cancer	NCT02111187, NCT02195973
4.	PF-04449913	Phase-I, Completed	Solid tumors	NCT02110342, NCT01286467
		Phase-II, Recruiting	Acute lymphoblastic leukemia, Myelodysplastic syndrome,	NCT01841333, NCT02367456,
5.	GDC-0449	Phase-I/II, Completed	Unspecified adult solid tumor, Basal cell nevus syndrome, Gorlin syndrome, Ovarian cancer	NCT00607724, NCT00957229, NCT00959647
		Phase-I/II, Ongoing	Adult solid neoplasm, Metastatic pancreatic cancer,	NCT00878163,NCT01088815
II. Notch Signaling Pathway Inhibitor				
6.	RO4929097	Phase-I, Completed	Adult anaplastic astrocytoma, Adult gliosarcoma, Unspecified adult solid tumor,	NCT01131234, NCT01096355
		Phase-II, Ongoing	Adult glioblastoma, Adult gliosarcoma,	NCT01122901,
7.	MEDI0639	Phase-I, Recruiting	Solid tumors	NCT01577745
8.	MK-0752	Phase-I/II, Completed	Metastatic breast cancer, Advanced cancer, Pancreatic cancer, Advanced breast cancer	NCT00645333, NCT01295632, NCT01098344, NCT00106145
		Phase-I, Ongoing	Breast cancer	NCT00756717
9.	OMP-21M18	Phase-I, Completed	Solid tumors,	NCT00744562
		Phase-I, Ongoing	Non-Small Cell Lung cancer, Pancreatic cancer	NCT01189968, NCT01189929
		Phase-I/II, Recruiting	Primary peritoneal carcinoma, Locally advanced or metastatic solid tumors	NCT01952249, NCT02722954
10.	OMP-131R10	Phase-Ia/b, Recruiting	Advanced relapsed tumors, Refractory solid tumors	NCT02482441
11.	OMP-52M51	Phase-I, Recruiting	Relapsed or refractory lymphoid malignancies and Solid tumors	NCT01703572, NCT01778439
III. Wnt signaling pathway inhibitors				
12.	CGX1321	Phase-I, Recruiting	Solid tumors, Colorectal adenocarcinoma, Gastric adenocarcinoma	NCT02675946

(Table 3) contd…

S. No.	Name of Drug Candidate/ Compound	Clinical Trial Phase and Status	Cancer type	Reference (clinicaltrials.gov)
13.	PRI-724	Phase-I, Completed	Advanced pancreatic cancer	NCT01764477
		Phase-I/II, Ongoing	Acute and chronic myeloid leukemia	NCT01606579
		Phase-II, Starting	Colorectal adenocarcinoma, Stage IVA colorectal cancer	NCT02413853
14.	XAV939	Phase-I	Neuroblastoma	[159, 160]

Table 4. Monoclonal antibodies under different phases of clinical trials.

S. No.	Name of Drug Candidate/ Compound	Clinical Trial Phase and Status	Cancer Disease	Reference (Clinicaltrials.gov)
	Daratumumab	Phase-II, Ongoing	Multiple myeloma	NCT00574288, NCT01985126, NCT01615029
	Denosumab	Phase-I, Ongoing	Advanced cancers	NCT01624766
		Phase-III, Ongoing	Metastatic breast cancer	NCT02051218
	Dinutuximab	Phase-I,II Ongoing	Recurrent neuroblastoma	NCT01711554, NCT01767194
		Phase-IV, Ongoing	Neuroblastoma	NCT02693171
	Elotuzumab	Phase-I,II Ongoing	Multiple myeloma	NCT02252263, NCT02655458, NCT02719613
	Ibritumomab tiuxetan	Phase-II, Completed	Multiple myeloma	NCT01207765
		Phase-I/II Ongoing	Lymphoma	NCT00372905, NCT00732498
	Necitumumab	Phase-II, Completed	Non-small Cell Lung Cancer, Malignant solid tumor	NCT01788566, NCT01606748,
		Phase-II, III Ongoing	Non-small Cell Lung Cancer	NCT01769391, NCT00982111
	Nivolumab	Phase-I/II Ongoing	Lukemia, Metastatic brain cancer, Bladder cancer	NCT02464657, NCT02696993, NCT02845323
	Obinutuzumab	Phase-I, Completed	Lymphocytic leukemia	NCT01680991
		Phase-II, Ongoing	Leukemia	NCT02629809, NCT02225275
	Pembrolizumab	Phase-II, Ongoing	Advanced cancers, Small Cell Lung Cancer, Melanoma	NCT02721732, NCT02551432, NCT02706353
	Pertuzumab	Phase-I/II, Ongoing	Breast cancer, Esophageal carcinoma	NCT02598427, NCT02120911

types of resistances are caused by mutations in the genome of cancer cells and/or to epigenetic changes. Drug resistance not only appears for the conventional chemotherapy but it is also common to the targeted therapies such as kinase inhibitors [174].

Pathogenesis of different cancer cells is of complex nature due to their clonal evolution and genomic instability [175]. Cancer cells are formed from a sequence of mutations in a particular subset of genes that trigger unregulated proliferation [176]. Cancer cells may contain complex chromosome rearrangements and hundreds to thousands of mutations. Furthermore, different patients may have different mutations in the same gene that may results in different types of cancer [177]. Many times, it has been observed that different patients suffering with the same type of cancer may respond differently to the same drug regimen.

A number of mutations in the cancer cells, over expression of the ATP-binding cassette (ABC) drug transporters and enhanced DNA repair are some of the factors responsible for the development of resistance to the chemotherapeutics [178]. Chemotherapeutic resistance can also arise through alterations in the drug pharmacokinetics and metabolism, modification of drug target expression or function, drug compartmentalization in cellular organelles and changes in the apoptotic signaling pathways such as mutated p53 [179, 180]. The high proliferative potential of such cells could therefore result in the rapid regrowth of the resistant tumors. The P-glycoprotein (P-gp) pump and multidrug-resistant protein-1 (MRP1) are the major drug efflux transporters [181]. P-gp is a 170 kDa glycoprotein encoded by the *MDR1* gene. This ATP-dependent membrane transporter pumps large number of structurally diverse chemotherapeutics across the cell membrane and out of the cells that include anthracyclines, taxanes, vinca alkaloids, podophyllotoxins, and antifolates. The exact physiologic role of P-gp is still not clear, but it may shield the normal tissues from toxic products and xenobiotics [179]. These proteins actively pump chemotherapeutic agents out of the cells, thereby reducing their intracellular concentration and decrease cytotoxicity. MRP1 is a member of the ABC drug transporter family (MRP1 to MRP7) [182]. The MRP1 expression is linked with the low survival rate in cancer patients with early-stage disease who received chemotherapy [183].

Thus, chemo resistance is a major obstacle in the total eradication and full proof treatment of cancer. A better understanding of the drug resistance mechanisms at the molecular level is must for the complete eradication of the disease. The development of novel inhibitors for the drug resistance pathways is one of the frontier areas of cancer research (Fig. **12**, Table **5**). However, the ligands developed to target ABC transporters has failed in clinical trials due to their high toxicity [184].

8. MULTI-TARGETING ANTI-CANCER AGENTS

The anti-cancer drug discovery programs are exclusively focused on the design and development of drugs intended to act against a specific target with high potency and selectivity. This single target approach is based on a direct cause-effect relationship between the activity of a gene product and a particular phenotype. Earlier, multi-targeting drugs with a wider and unpredictable spectrum of biological activities were avoided due to its potential cytotoxicity and adverse reactions. However, this simplistic explanation for the mechanism of action of some drugs cannot substantiate the role of drugs used for complex and multifactorial diseases like cancer. The molecular and genetic complexity of advanced-stage cancer suggests that targeting a single cell signaling pathway cannot achieve the desired results [185]. The novel anti-cancer drug development strategies involve specific drug combinations that can simultaneously target and block disease-relevant signaling pathways. The combination drugs may be individually active, may operate through different mechanisms of action, non-overlapping mechanisms of resistance, and distinct toxicities. The combination therapy involves simultaneous administration of two or more drugs while the multi-targeting drugs can achieve the same goal through a single drug molecule able to target multiple oncoproteins.

Designing and development of a single drug molecule that can simultaneously and specifically interacts with the multiple targets is gaining attention in drug discovery [186-188]. Multi-targeting drugs may potentiate efficacy either additively or synergistically and are less prone to the insurgence of drug resistance mutations. A drug active on multiple targets may be characterized by an improved efficacy when compared to a highly selective pharmacological agent. Simultaneous inhibition of multiple kinases is now established as a successful therapeutic strategy. Sunitinib, a promising compound for the treatment of anaplastic thyroid cancer was tested *in vitro* and *in vivo* proving its ability to inhibit Akt and ERK1/2 phosphorylation and down-regulate cyclin D1 [189]. A small molecule, MDG892 was discovered as a dual inhibitor of Hsp90 and tubulin [190]. Tubulin is an Hsp90 client protein and also a prime cancer drug target. Another compound (CDBT,58) targeting Hsp90 and tubulin was discovered through phenotypic screening by observing its activity in non-small-cell lung cancer (NSCLC) cells [191].

The success of one drug multi-target approach will depend on several challenges, most of which still have to be faced. Rational design of multi-target compounds is still in its infancy and need further implementation and methodological development. Major areas of interest include rational design of multi-target drugs, prediction of off-target toxicities and drug repositioning in different therapeutic areas (Fig. **13**, Table **6**).

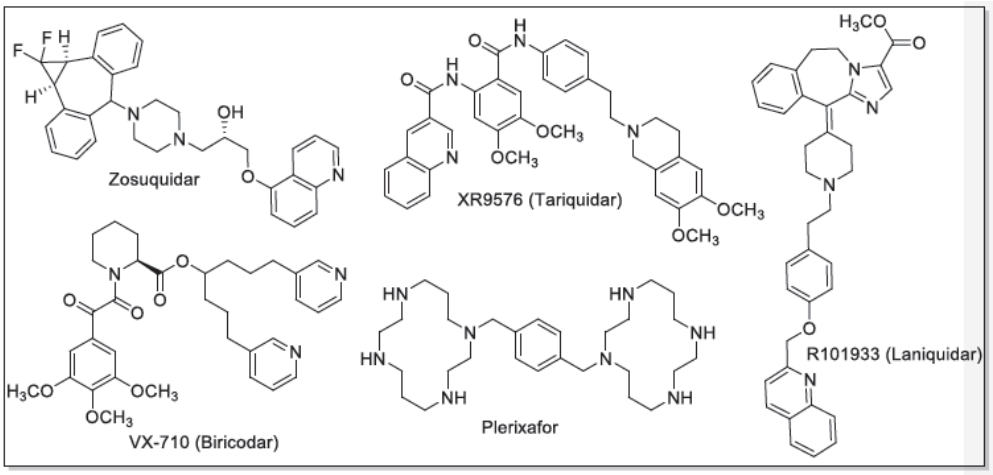

Fig. (12). Structures of various multi-drug resistant pathway inhibitors under different phases of clinical trials as described in Table **5**.

Table 5. Multi-drug resistance pathway inhibitors under different phases of clinical development.

S. No.	Name of Drug Candidate/ Compound	Clinical Trial Phase and Status	Cancer type	Reference (clinicaltrials.gov)
	Zosuquidar	Phase-I/II/III, Completed	Leukemia, Myeloid, Myelodysplastic syndromes	NCT00129168, NCT00233909, NCT00046930
	XR9576 (Tariquidar)	Phase-I/II, Completed	Breast cancer, Lung neoplasms, Ovarian neoplasms, Cervix neoplasms, Renal neoplasms, Adrenal cortex neoplasms	NCT00001944, NCT00069160, NCT00071058
	R101933 (Laniquidar)	Phase-II, Completed	Breast cancer	NCT00028873
	VX-710 (Biricodar)	Phase-II, Terminated	Lung cancer	NCT00003847
	Plerixafor	Phase-I, Ongoing	Acute leukemia	NCT01319864

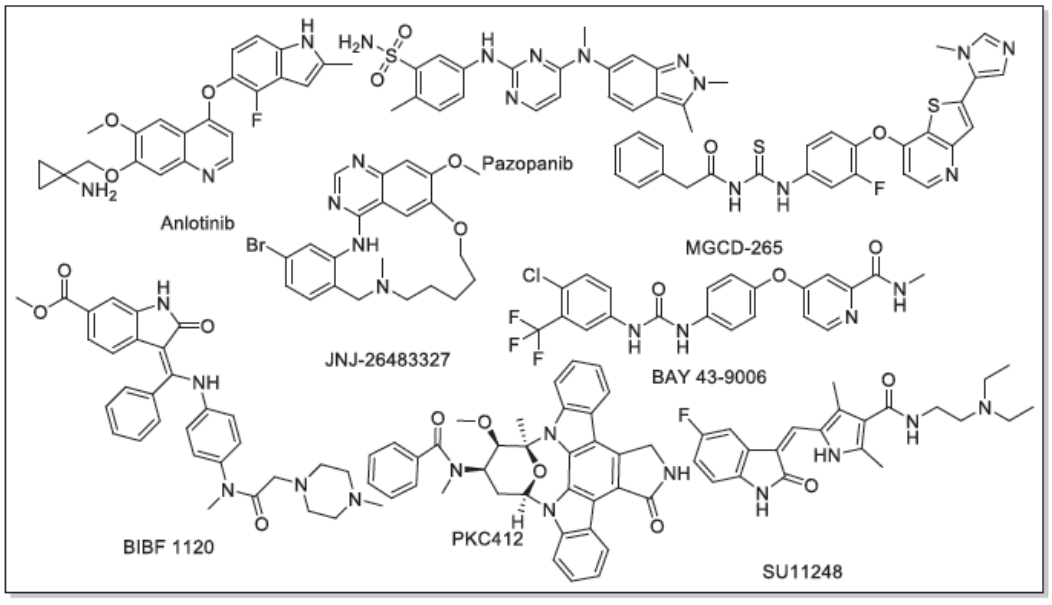

Fig. (13). Structures of multi-targeting anti-cancer agents undergoing clinical trials as described in Table 6.

Table 6. Multi-target anti-cancer agents under clinical trials and development.

S. No.	Name of Drug Candidate/ Compound	Drug Targets	Clinical Trial Phase and Status	Cancer type	Reference (clinicaltrials.gov)
	Anlotinib	VEGFR2, VEGFR3, PDGFRβ and c-Kit	Phase-II, completed	Non-small Cell Lung Cancer	NCT01924195
			Phase-II, ongoing	Cancer	NCT01878448, NCT02072031
	Pazopanib	c-KIT, FGFR, PDGFR and VEGFR	Phase-I/II, completed	Epithelial ovarian cancer	NCT01238770
			Phase-II, ongoing	Progressive desmoids tumors	NCT01876082
	MGCD265	MET, Axl and other receptors	Phase-I, completed	Advanced malignancies	NCT01930006
			Phase-II, ongoing	Non-Small Cell Lung cancer	NCT02544633
	JNJ-26483327	EGFR-1, -2 and -4, VEGFR-3 and Src family	Phase-I, completed	Advanced malignancies, Solid malignancies	NCT00676299
	BAY 43-9006	VEGFR, PDGFR, KIT, FLT3, p38, Raf	Phase-I, completed	Lung Cancer, Kaposi's Sarcoma	NCT00533585, NCT00287495
			Phase-I/II, recruiting	Leukemia, Liver cancer	NCT02530476, NCT01900002
	BIBF 1120	VEGFR, PDGFR, FGFR	Phase-I, completed	Carcinoma, Non-Small-Cell Lung cancer	NCT00979576, NCT00876460
			Phase-I and II, ongoing	Ovarian Neoplasms, Pulmonary Fibrosis	NCT01314105, NCT01170065
	PKC412	PKC, VEGFR, PDGFR, KIT, FLT3, CDK1/cyclin B	Phase-I and II, completed	Acute Myeloid Leukemia, Myelodysplastic syndrome	NCT00977782
			Phase-I/II, ongoing	Acute Myeloid Leukemia, Myelodysplastic syndrome	NCT00819546, NCT01830361
	SU11248	VEGFR, PDGFR, KIT, FLT3, RET	Phase-I and II, completed	Acute Myeloid Leukemia, Metastatic breast cancer	NCT00783653, NCT00270413
			Phase-I, ongoing	Clear cell sarcoma of the kidney	NCT01061411

CONCLUSION

Cancer is a multifactorial disease and so far single target approach for its complete eradication proved ineffective. Cancer cells develop multiple and complex mechanisms to evade the drug induced cytotoxicity. Chemo resistance therefore represents a significant impediment to successful cancer therapy. A better understanding of the resistance mechanisms is crucial for the success of chemotherapy. Combinations of several drugs targeting different signaling pathways may avoid secondary resistance and increase the drug efficacy. The multi-targeting anti-cancer agents were found effective against selected cancer cell lines and resolved the issue of MDR to some extent. Similarly, novel therapies against CSC are encouraging that may kill the CSCs and avoid or minimize the normal tissue stem cell toxicity. The combined therapy using conventional anti-cancer drugs with CSCs-targeting agents, may offer a promising strategy for the management and complete eradication of different types of cancers.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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