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REVIEW

A review on various targeted anticancer therapies

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Abstract Translational oncology aims to translate laboratory research into new anticancer therapies. Contrary to conventional surgery, chemotherapy, and radiotherapy, targeted anticancer therapy (TAT) refers to systemic administration of drugs with particular mechanisms that specifically act on well-defined targets or biologic pathways that, when activated or inactivated, may cause regression or destruction of the malignant process, meanwhile with minimized adverse effects on healthy tissues. In this article, we intend to first give a brief review on various known TAT approaches that are deemed promising for clinical applications in the current trend of personalized medicine, and then we will introduce our newly developed approach namely small molecular sequential dual targeting theragnostic strategy as a generalized class of TAT for the management of most solid malignancies, which, after optimization, is expected to help improve overall cancer treatability and curability.

Keywords Targeted anticancer therapy · Inhibitors · Monoclonal antibody · Radiotherapy · Small molecules

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Introduction

Along with human longevity is the fact that cancer becomes a commonest disease and a major cause of human suffering and death. A recent report from the International Agency on Cancer indicates that in 2008 alone, 12.4 million cases of cancer were diagnosed worldwide with 7.6 million cancer deaths; by 2030, there will be 27 million incident cases of cancer, and deaths from cancer are projected to continue to rise to over 11 million in the world [1]. The rapid increase in cancer cases and social burdens represents a real crisis for public health and health systems worldwide [1, 2].

Meanwhile, rapid progress in our knowledge on cancer at sub-cellular and molecular levels has been made during the last 50 years. Now it is widely accepted that disruption of the normal regulation of cell-cycle progression and division leads to cancer [3, 4]. Multifarious factors, such as oncogenes, viruses, cytokines, hormones, bacteria, and carcinogens have been identified to impose crucial effects on the initiation and promotion of cancer. Sub-cellular mechanisms that drive hyper-proliferation, invasion, angiogenesis, and metastasis of cancer have been explored (Fig. 1a). Moreover, the structure of entire human genome and at least some of those genes that mediate tumorigenesis become quite apparent now [4]. In spite of tremendous increase in our understanding about cancer, the war against cancer over

decades has experienced an awkward imbalance between the input and output with limited improvement of overall cancer mortality.

Translational cancer research aims to translate scientific discoveries into new methods of cancer treatment and is, therefore, overwhelmingly important for cancer control. Relative to conventional surgery, chemotherapy, and radiotherapy, targeted anticancer therapies (TATs) utilize cutting-edge translational research findings either from the unique characteristics of molecules, antibodies, proteins, and peptides or from structures, metabolisms, and other phenotypic properties of cancer to destroy cancer cells more precisely and therefore may significantly improve cancer treatability. Thus, TATs bear the most expectations by the researchers and clinicians to be an integral part of state-of-the-art cancer therapies.

TATs refer to drugs with particular mechanisms that specifically act on a well-defined target or biologic pathway that, when activated or inactivated, causes regression or destruction of the malignant process [5]. Anticancer antibodies, especially conjugated with cytotoxic drugs, radioisotopes or poisons, are widely considered as typical TAT agents that seek out and kill malignant cells bearing the target antigens [6]. Besides, small molecular inhibitors of protein kinases have also emerged as viable drugs for TATs. Moreover, multifarious agents such as pro-apoptotic agents, PARP-inhibitors, vascular disrupting agents (VDAs), angiogenesis inhibitors, radiolabeled peptides, radiolabeled meta-iodo-benzyl-guanidine (MIBG), immunoconjugates, as well as antisense strategies, immunologic therapies, etc., can selectively target cancer cells, stroma, or parenchyma, hence falling into the category of TAT in its broad sense (Fig. 1b–d).

Ideally, cancer targets are expected to be macromolecules that are crucial to malignant phenotypes but not significantly expressed in normal organs and tissues [6]. Biologic relevance of such targets can be measured reproducibly [7]. Besides, when interrupted or inhibited, significant clinical outcomes can present in targeted patients, but no or less response occurs in patients whose tumors do not express such a target. By interfering with the ability of cancer cells to grow, divide, repair, and intercellular communicate, TATs that focus on specific molecular and cellular changes may bring relatively high therapeutic index and are currently in a very active research area [8]. In addition, TATs that can achieve an optimally effective treatment at a dose below the maximal tolerated dose may improve treatability with fewer side effects.

As illustrated in Fig. 2, TATs comprise a variety of direct and indirect approaches. Direct approaches hit targets of tumor cells to alter their molecular pathways by either mAbs or small molecular inhibitors. Indirect approaches target tumor stroma to disrupt tumoral vasculartur, inhibit angiogenesis, interrupt tumor fibroblasts macrophages, and contaminate tumoral micro-environment by monocolonal antibodies (mAbs) or peptide ligands or radiolabeled chemicals specific to tumor interstitia [9]. Among many possible TATs, small molecular inhibitors, mAbs and antivascular agents are currently the top three that have received the most attentions (Fig. 1b–d). The emerging therapies based on the mechanisms involving critical molecular pathways or various mechanisms of malignancies have given rise to considerable interests [8].

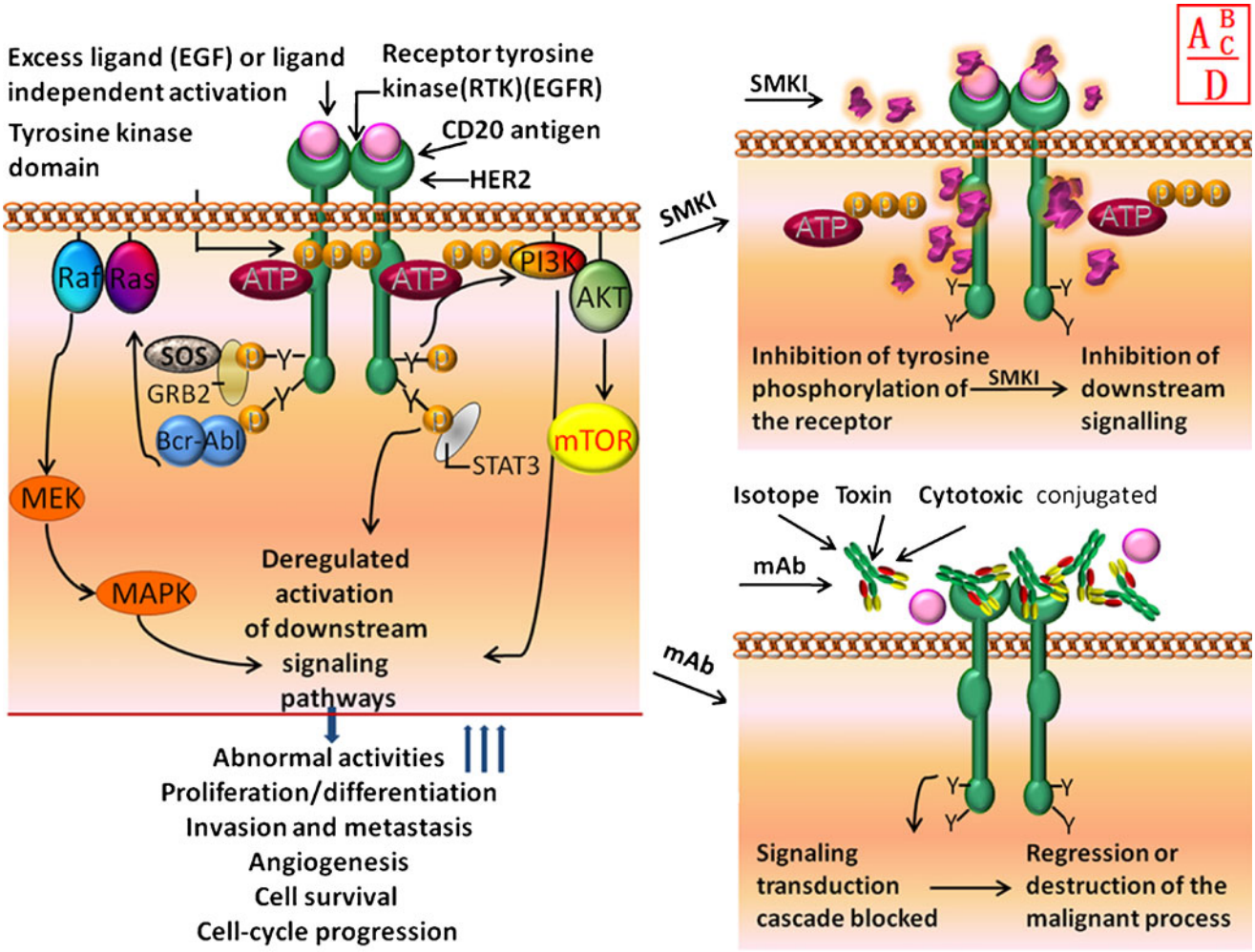
In this review article, we intend to first give a brief overview on various TAT approaches that are deemed promising for clinical applications in the trend of personalized medicine, and then introduce our newly developed approach SMSDTTS as a promising generalized class of TAT for the management of most solid malignancies [10].

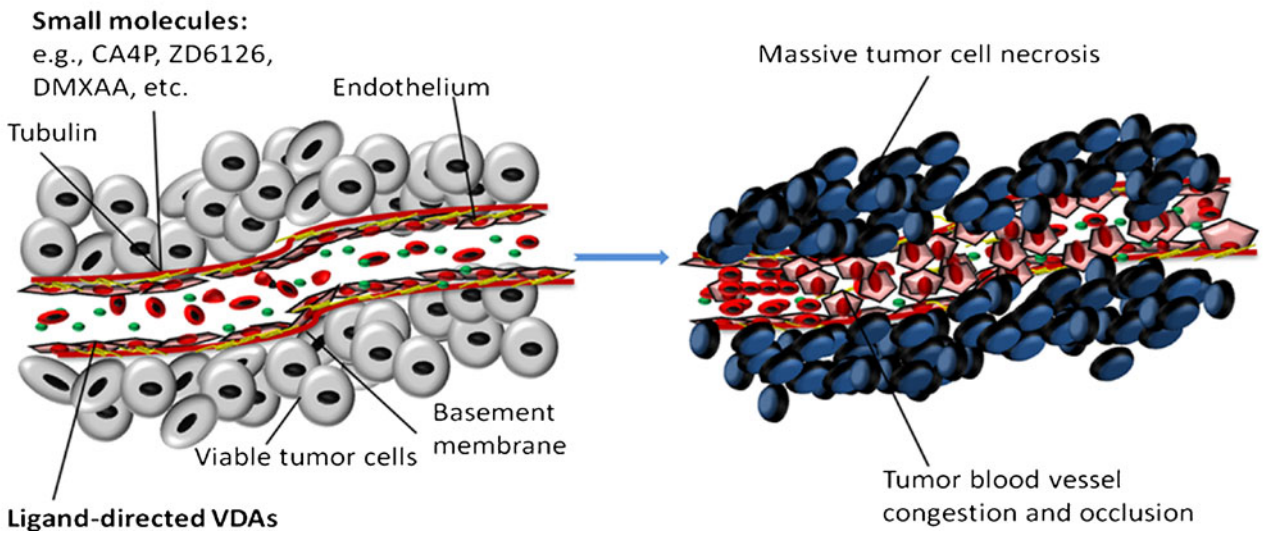
Current targeted anticancer therapies

Targeting mutant kinases by small molecule kinase inhibitors

Structure-based design of anticancer drugs has emerged as a key tool for addressing the challenges of specificity, e.g., a selectivity profile has been identified from the adenosine triphosphate (ATP) binding site of highly conserved nature [11]. Protein tyrosine kinases exist in different molecular and cellular contexts and have different mechanisms of activation. They share a conserved structural similarity in the region of the ATP binding site where most inhibitors interact [12]. In polypeptides, the transfer of phosphate from ATP to tyrosine residues is catalyzed by protein tyrosine kinases [13]. In a variety of cancers, diversified protein kinases are activated and mutated. A series of therapeutic inhibitors have been explored utilizing the fundamental role and structure of protein kinases in progression of malignant cells [14]. Small molecule kinase inhibitors (SMKIs) are a class of chemicals that have been successfully developed by the pharmaceutical industry for the treatment of a number of

Fig. 1 Schema illustrates the mechanisms of common TATs (e.g., monoclonal antibodies (mAbs), small-molecule kinase inhibitors (SMKIs), and vascular targeting therapies). Deregulated activation in cell signaling lead to abnormal activities in proliferation, differentiation of cells and induce cancer, sub-cellular mechanisms that drive hyper-proliferation, invasion, angiogenesis, and metastasis of cancer have been explored (**a**). SMKIs can pass into the cytoplasm and thereby act on any molecules inside the cell (**b**). Owing to the large molecular size, mAbs cannot pass through the cellular membrane, they can only act on molecules that are expressed on the cell surface or secreted (**c**). Vascular disrupting agents (VDAs) cause tumor-associated endothelial cells to change from a flat to a round shape, which leads to blocking of the blood vessels, hence depriving the tumor of the oxygen and nutrients it needs to survive (**d**)





malignancies. By selectively attaching to the ATP-binding site or adjacent small pocket within the kinase domain, SMKIs generally inhibit enzymatic domains on mutated, overexpressed, or critical proteins inside cancer cells [13–16]. Prominent examples are the tyrosine kinase inhibitors Imatinib and Gefitinib.

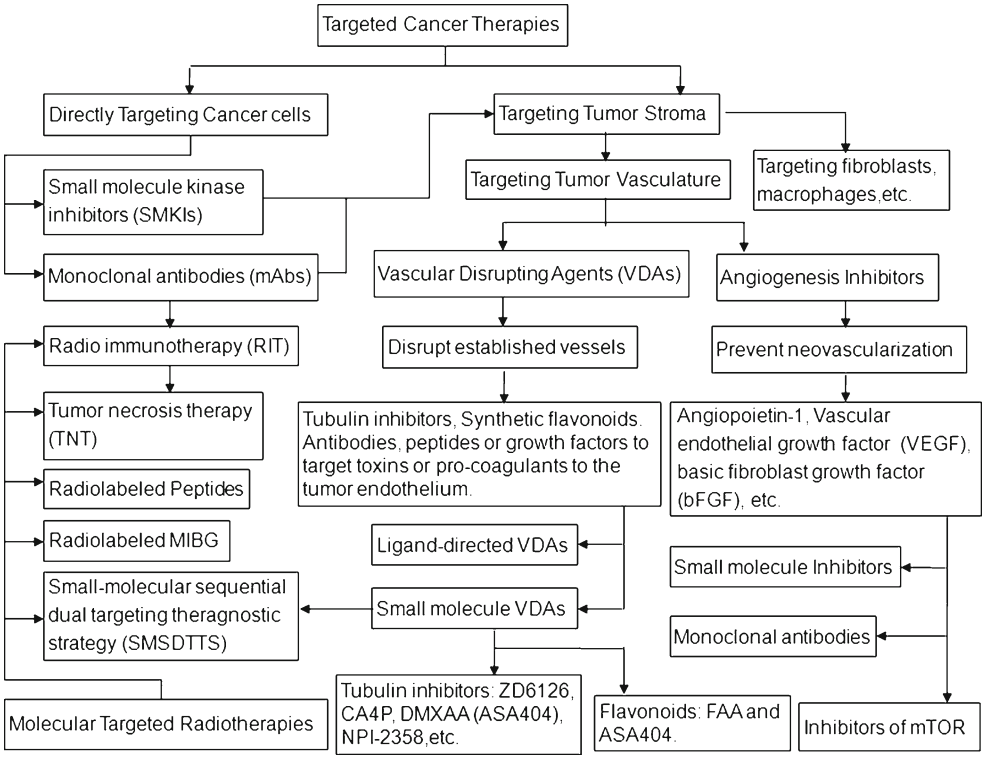


Fig. 2 A scheme listing currently known targeted anticancer therapies (TATs): both small molecular kinase inhibitors (SMKIs) and monoclonal antibodies (mAbs) can directly target cancer cells or indirectly target cancer stroma by inhibiting various cancer molecular pathways. Vascular targeting therapies target cancer stroma by disrupting established tumor vessels or inhibiting neovascularization. Most TATs have been proven to be effective but not thoroughly. Remnant tumor cells always exist and tumor relapse sooner or later. Accordingly, we proposed a new generalized strategy, namely small molecular sequential dual targeting theragnostic strategy (SMSDTTS), which sequentially combines a small molecular VDA and a stroma-targeted radiotherapy, providing a non-overlapping complementary mechanism to most solid tumors. Synergetic anticancer efficacy has been achieved in recent research in rodent tumors

Imatinib is currently used in chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs), and a number of other malignancies [14, 17]. CML is driven by the mutant kinase fusion protein breakpoint cluster region/the Abelson tyrosine (Bcr-Abl), which displays constitutive activation of the Abl kinase in the pathogenesis of the disease process [17, 18], whereas GIST is caused by activating point mutations in the c-Kit or the platelet-derived growth factor receptor (PDGFR)-α kinases [13, 19]. Imatinib binds to the site of kinases, blocks their activity effectively, and therefore produces dramatic prevention effect that correlates precisely with the presence of these mutations in the tumor [19, 20]. By inhibiting Bcr-Abl kinase activity, Imatinib thus blocks the proliferation signal within leukemic progenitor cells and induces apoptotic cell death in cells expressing this activated kinase, and leads to rapid and selective death of CML cells [17]. Imatinib has shown clinical efficacy against at least three different cancers as well as a favorable safety profile [11, 17, 18]. Clinical effectiveness of Imatinib has been demonstrated in large-scale of clinical trials, in 454 patients with chronic CML who were either refractory or intolerant to IFN-α, a complete hematological remission, a major cytogenetic response, and a complete cytogenetic remission were achieved in 95%, 60%, and 41% of the patients, respectively, when treated with Imatinib [18, 21].

Activation of epidermal growth factor receptor (EGFR) is a key factor in cell proliferation and has been shown to play an important role in growth of many solid tumors, i.e., EGFR effects on cell motility, adhesion, invasion, survival, and angiogenesis [22]. Approximately 70–80% of metaplastic breast carcinomas overexpress the EGFR [23]. Gefitinib effectively binds to the ATP-binding site of EGFR tyrosine kinase, thus the function of the EGFR tyrosine kinase in activating the Ras signal transduction cascade is hindered and thereby malignant cells are inhibited [24]. In clinical and preclinical evaluations, significant variability in patients' response to Gefitinib has been identified due to the presence or absence of mutations in the ATP binding site of the EGFR [25–27]. Non-small cell lung cancer (NSCLC) with somatic mutations in the kinase domain of EGFR is highly responsive to Gefitinib [25–28]. According to the results of a phase III trial in 230 NSCLC patients who were selected on the basis of EGFR mutations, significantly higher response rates and longer progression-free survival have been achieved in patients who received Gefitinib comparing with patients who received

standard chemotherapy (73.7% vs. 30.7% and 10.8 vs. 5.4 months, respectively) [28]. Meanwhile, a tolerable toxicity profile including less hematologic toxicity and neurotoxicity was observed comparing with chemotherapy.

Patients with sensitive EGFR mutations are also very responsive to Erlotinib treatment. Besides, a handful of the best-characterized kinases, i.e., PDGFR, c-KIT, mTOR, BCR-ABL, VEGF, etc., have been successfully targeted. Crizotinib was recently approved by the US Food and Drug Administration (FDA) for treatment of locally advanced or metastatic NSCLC that is anaplastic lymphoma kinase (ALK) positive [29, 30]; Vemurafenib was newly approved as an inhibitor of BRAF kinase for the treatment of patients with unresectable metastatic melanoma with the BRAF V600E mutation [31, 32]. Larger numbers of SMKIs are currently under investigation in different stage of clinical trials, and so far, over ten of them have been approved by the FDA for clinical use (Table 1).

Targeting specific antigen by monoclonal antibody

Due to the high binding specificity to targeted antigens on the surface of cancer cells, mAbs have been extensively applied as important therapeutic agents for the treatment of increasing numbers of human malignancies [33]. Such mAbs targeting cancer cells by disrupting and blocking the downstream signaling (either anti-apoptotic or pro-mitotic) triggered by the overactive receptors. A wide range of targets have been involved in mAb therapies including cell-surface proteins in both solid tumors and individual circulating malignant cells, antigens either on tumoral vasculature or associated with the stroma, and ligands that support tumor growth, etc. [34]. Cytotoxicity effects with mAbs can be achieved through various mechanisms, either by antibody-dependent, complement-mediated cytolysis, cell-mediated cytotoxicity, or by the focused delivery of radiation or cellular toxins [35–37]. Furthermore, mAbs may act as sole agents, or they can be conjugated to radioisotopes, small-molecular cytotoxic drugs, or protein toxins to improve the therapeutic efficacy [5]. Several mAbs have been developed and approved by the FDA (Table 1). Particular examples of such therapeutic mAbs are the anti-human epidermal growth factor receptor 2 (HER2) antibody Trastuzumab for breast cancer [38] and the anti-CD20 antibody Rituximab used for a variety of B-cell malignancies [39, 40].

HER2, the membrane receptor, is one of the most promising targets for immunotherapy [41]. HER2 overexpresses and/or amplifies in 20–30% of breast cancers and appears to

Table 1 FDA approved SMKIs and mAbs for use of cancer therapy

Name	Targets	Oncology uses
Small molecule inhibitors for cancer		
Dasatinib	BCR-ABL, SRC family, c-KIT, PDGFR	Chronic myeloid leukemia (CML), acute lymphocytic leukemia
Erlotinib	EGFR	Non-small cell lung cancer(NSCLC), pancreatic cancer
Gefitinib	EGFR	NSCLC
Imatinib	BCR-ABL, c-KIT, PDGFR	Acute lymphocytic leukemia, CML, Gastrointestinal stromal tumor
Lapatinib	HER2/neu, EGFR	Breast cancer
Sorafenib[a]	BRAF, VEGFR, EGFR, PDGFR	Renal cell carcinoma(RCC), Hepatocellular carcinoma
Sunitinib[a]	VEGFR, PDGFR, c-KIT, FLT3	RCC, gastrointestinal stromal tumor
Temsirolimus[a]	mTOR, VEGF	RCC
Pazopanib[a]	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-a/β, and c-kit	RCC
Nilotinib	BCR-ABL	CML
Crizotinib	ALK, HGFR	NSCLC
Vemurafenib	BRAF	Late-stage melanoma
Monoclonal antibodies for cancer		
Alemtuzumab	CD52	Chronic lymphocytic leukemia
Bevacizumab[a]	VEGF	Colorectal cancer, NSCLC, RCC
Cetuximab	EGFR	Colorectal cancer, head and neck cancer
Gemtuzumab Ozogamicin	CD33	Relapsed acute myeloid leukemia
Ibritumomab Tiuxetan	CD20	Non-Hodgkin's lymphoma (NHL) (with yttrium-90 or indium-111)
Panitumumab	EGFR	Colorectal cancer
Rituximab	CD20	NHL
Tositumomab	CD20	NHL (with Iodine-131)
Trastuzumab	HER2/neu	Breast cancer with HER2/neu overexpression
Ipilimumab	CTLA-4	Late-stage melanoma

[a] Agents with antiangiogenic mechanism

be strongly associated with poor prognosis in breast carcinomas [42–45]. Trastuzumab is an unconjugated monoclonal anti-HER2 antibody that can selectively bind to HER2 protein and therefore inhibits proliferation of human tumor cells and suppresses angiogenesis, which in turn, prolongs the survival of patients with breast cancer [46]. Trastuzumab can be used alone, in combination with standard chemotherapy, or in adjuvant settings to reduce relapses and prolong disease-free and overall survival period in high-risk patients after definitive local therapy for breast cancer [47]. Clinical efficacy and safety of Trastuzumab have been investigated in a few large phase III adjuvant trials (NSABP B-31, NCCTG N9831, HERA, and BCIRG 006) for 1 or 2 years. The addition of 1 year of Trastuzumab to adjuvant chemotherapy significantly improved disease-free survival and overall survival in these trials [46–51].

CD20, a transmembrane protein, is a signature B-cell antigen that plays an important role in the activation, function, proliferation, and differentiation of B cells [39]. CD20 is overexpressed on approximately 85% of B cell non-Hodgkin's lymphomas (NHL) and to a lesser degree on B cell chronic lymphocytic leukemia (CLL) cells [39, 40]. Rituximab is an unconjugated antitumor mAb that is directed against the CD20 antigen; when it binds to CD20 on surface of B cells, it triggers an immune response that results in destruction and apoptosis of the malignant cells [40]. Mechanism of action of Rituximab comprises direct growth inhibition, induction of apoptosis, as well as increase sensitization of cells to chemotherapy. A significant complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) can also be achieved when binds to certain receptors [52]. Rituximab is indicated for first-line treatment of low-grade or follicular B cell, CD20-positive NHL or for other B cell malignancies such as intermediate grade or diffuse large B cell lymphoma in combination with chemotherapeutic agents (cyclophosphamide, doxorubicin, vincristine, prednisone, also called CHOP) [53]. Clinical efficacy of Rituximab has been demonstrated in patients with various lymphoid malignancies, including indolent and aggressive forms of B cell NHL and B cell CLL [40, 53].

Targeting tumor vasculature

Solid tumors cannot grow beyond a certain size, generally 1–2 mm^3 without an angiogenic phenotype to generate new vessels [54]. Angiogenesis, the recruitment of new blood vessels, is essential for metastatic growth and imperative in the invading of malignant tumor cells into the neighboring host tissues. Angiogenesis involves several processes including proliferation of endothelial cells, proteolytic degradation of the extracellular matrix and migration of endothelial cells, which lead to the formation of a functioning vessel with a lumen [55]. Malignant tumors overexpress various proangiogenic factors through perturbing the local balance of proangiogenic and antiangiogenic factors, so as to stimulate neoangiogenesis for metastatic potential and development [54, 56]. Antiangiogenesis inhibitors prevent the tumor-initiated angiogenic process by interrupting essential aspects of angiogenesis, most notably signaling process among the tumor and endothelial cells as well as endothelial cell function, through which new blood vessel formation is compromised [56, 57].

Meanwhile, as the "lifeline" of solid tumors, tumor vasculature, which delivers nutrition to and transports waste from the tumor, has become a major target for the development of new anticancer approaches. Endothelial cells lining the blood vessels of malignant tumors proliferate rapidly with increased permeability, abnormal morphology, and variable density. Tumor vessels are irregularly shaped, distended capillaries with leaky walls and sluggish flow, and often demonstrate a lack of pericytes [58–60]. All these characteristics lead to adequate phenotypic differences, which provide unique and selective targets for cancer therapies [58]. Given its characteristics of being relatively immature, proliferating, and more permeable and disorganized in comparison to normal vasculature, tumor vasculature has been exploited for developing vascular disrupting agents (VDAs) [61, 62]. VDAs induce direct damage to the pre-existing tumoral endothelium, cause collapse of the vasculature inside solid tumors, prohibit the tumor blood flow and oxygen supply, and lead to rapid hemorrhagic necrosis or tumor cell death [57, 61].

Based on whether to inhibit neovascularization or to damage the established tumor vasculature, vascular targeting therapies are divided into two categories and expanded rapidly with a large number of investigational drugs undergoing clinical evaluations. Both categories are under preclinical and clinical applications for the treatment of a wide range of malignant tumors.

Angiogenesis inhibitors

Angiogenesis and vascular remodeling are key processes for tumor growth and metastasis. Over a dozen of substances, e.g., Angiopoietin-1, basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF), etc., have been identified that promote angiogenesis. They activate formation of new capillaries surrounding the tumor and create convenient routes for nutrients supply. Angiogenesis inhibitors bind to the substances or receptors on the surface of endothelial cells or in the downstream signaling pathways, thereby blocking their angiogenesis activities [63, 64]. Antiangiogenesis agents involved in tumor treatment can be classified into two major types: (1) mAbs directly against specific proangiogenic growth factors and/or their receptors; and (2) SMKIs of multiple proangiogenic growth

factor receptors. In addition, mammalian target of rapamycin (mTOR) inhibitors and other approved antiangiogenic agents may also inhibit angiogenesis through direct or indirect mechanisms [65].

The most successful means of blocking angiogenesis comes from the development of the monoclonal antibody Bevacizumab. Bevacizumab prevents the formation of new blood vessels by blocking the binding of VEGF to their receptors on vascular endothelium [66–68]. The FDA-approved indications of Bevacizumab include first- or second-line treatment of metastatic colorectal cancer when used with standard chemotherapy treatment or in combination with intravenous 5-fluorouracil-based chemotherapy; first-line treatment of advanced nonsquamous non-small cell lung cancer (NSCLS) in combination with carboplatin/paclitaxel chemotherapy; second-line treatment of glioblastoma; and treatment of metastatic renal cell carcinoma (RCC) in combination with interferon alfa [69].

Tyrosine kinase receptors play key roles in the generation of new blood vessels. SMKIs such as Sunitinib and Sorafenib that target VEGF receptors have shown clinical efficacy and benefit in patients with diverse cancer types including renal cell cancer. Sunitinib has been approved for treatment of GISTs. Sorafenib that inhibits Raf serine kinase has been approved for treatment of hepatocellular carcinoma as well [70]. Besides these, mammalian target of Rapamycin (mTOR) inhibitors and other numerous potent antiangiogenic agents as well as more active treatment strategies are being investigated.

Vascular disrupting agents

VDAs comprise two main classes: ligand directed (biological) VDAs and small molecule VDAs [71]. Ligand-directed VDAs deliver toxins, procoagulant, and pro-apoptotic effectors to disease-associated vessels. The rationale behind ligand-directed VDAs is that endothelial cells in tumor blood vessels express unique receptors on their surface; selectively identifying and targeting these receptors with small molecular drugs, monoclonal antibodies, peptides, growth factors, or gene therapy would cause collapse of tumor vasculature [72]. The localization property of the therapeutic moiety to tumor vessels and its selective destruction effect to tumor vasculature have been shown in preclinical studies [56, 57]. However, the clinical efficacy is limited due to the relatively high cost, lack of specificity, as well as toxicity concerns [57].

Small molecular VDAs comprise two classes: synthetic flavonoids and tubulin-binding agents, both selectively target tumor blood vessels by exploiting differences between normal and tumoral endothelium through either induction of local cytokine production or depolymerization of tubulin [56, 57, 71]. Although through different approaches, the intended results of VDA therapies are the same. Among several VDAs that are actively pursued, small molecules of 5,6-dimethylxanthenone-4-acetic acid (DMXAA) and CA4P are the furthest in clinical trials.

DMXAA is an active analog of flavone acetic acid with a distinct dual mechanism of action that comprises direct effects on cell apoptosis and indirect effects involving the release of tumor necrosis factor-α (TNF) and nitric oxide [58, 71]. DMXAA reorganizes the cytoskeletal network of endothelial cells and disrupts cell-to-cell junctions within minutes of its administration, leaving the cells distorted and basement membrane exposed. Then, platelets begin to aggregate and release serotonin or 5-hydroxytryptamine (5HT) in response to this damage [71]. 5HT is an antivascular agent that is metabolized into 5-hydroxyindoleacetic acid (5HIAA) in the liver. High concentration of 5HIAA in plasma has shown to reflect the intravascular effects of DMXAA in previous studies [73]. Meanwhile, synthesis of TNFa in plasma and tumor tissue is triggered indirectly following DMXAA administration [73]. After 6 h, macrophages release nitric oxide and other cytokines which, when synergize with TNFa, can increase vascular permeability and lead to plasma leakage. These effects raise blood viscosity and restrict the diameter of capillaries, thereby decrease blood flow within the tumor. Approximately 1 h after blood flow has ceased, apoptosis escalates rapidly inside the tumor, and hemorrhagic necrosis develops after few hours of the complete cessation of blood flow. Nonetheless, possibly sustained by absorbing oxygen and nutrients from neighboring unaffected normal vessels, tumor cells at the peripheral rim survive and repopulate quickly after the treatment with DMXAA [58, 73].

Combretastatin A4 Phosphate (CA4P) is a synthetic water-soluble phosphate prodrug of the tubulin-binding agent combretastatin A-4 (CA4) (Fig. 3). Following intracellular uptake, dephosphorylation of CA4P by endogenous phosphatases yields CA4, which binds to either the colchicines or vinblastine sites and causes depolymerization of microtubules in endothelial cytoskeletons [61, 62]. Anticancer activities from this action lead to a change in shape of vascular endothelial cells, i.e., they rapidly change from flat- into balloon-like shape, which causes closure of capillary lumens and blockage of the tumor blood flow, resulting in necrosis of the tumor core within minutes to hours after systemic administration of CA4P [71]. As seen with other VDAs, the tumor edge is less affected due to nutritious supply from the surrounding normal tissues. This selective effect is attributed to the fact that actin as another component of cytoskeleton is absent in tumoral endothelium but present in normal endothelium [74]. Thus, vascular shutdown due to endothelial disruption occurs only in tumors but not normal tissues. Depolymerization of tubulin also affects cancer cells by preventing them from producing

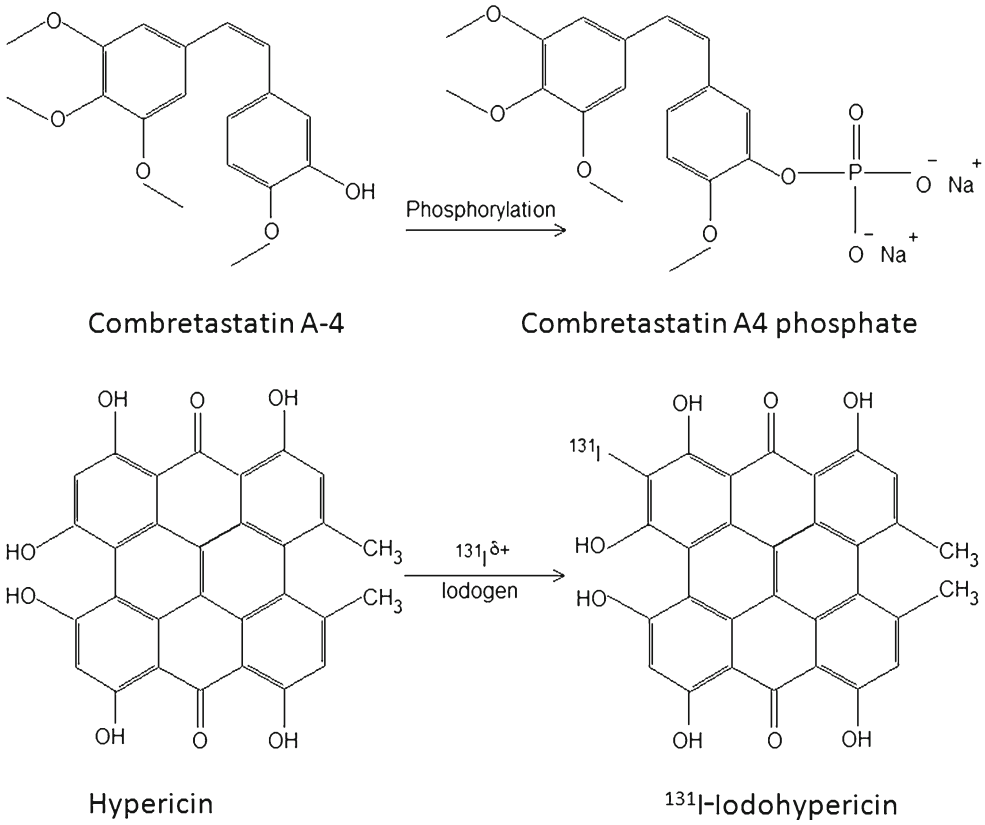


Fig. 3 Molecular structures of CA4P and Hypericin applied with SMSDTTS. With both being small-molecular, naturally existing, and synthetically derivable of proven targetability, Combretastatin A4 disodium Phosphate (CA4P) is a synthetic water-soluble phosphate prodrug of the tubulin-binding agent combretastatin A-4 (CA4). CA4P has a molecular weight of 442 Da and leads the novel class of drug candidates referred to as VDAs. Hypericin, as one of the necrosis avid compounds (NACs), has a structure of polycyclic polyphenolic quinine with molecular weight of 504 Da. It can be labeled with radioiodine using electrophilic substitution method to form ^{131}I-iodohypericin or ^{131}I-Hyp

microtubules. The later is essential to cytoskeleton production, intercellular movement, as well as formation of the mitotic spindle in chromosome segregation and cellular division.

Molecular targeted radiotherapy: combining targeted agents with therapeutic radionuclides

Radiation as cytotoxic surrogate is able to kill tumor tissues without the need for binding or internalizing into individual cancer cells. Combination of therapeutic radionuclides with targeted therapies may result in a more than additive therapeutic effect [75]. Based on the underlying principle that radiation can be delivered in a targeted way by attaching a certain radionuclide to a molecule or antibody, which then attaches itself to receptors specifically on cancerous cells, molecular targeted radiotherapies are designed to emit internal radiation to tumors. To determine the best strategies and to expand them to most tumor types, the selection of radioisotopes and the optimal combinations of targeting therapeutic agents with therapeutic radionuclides are among the most important issues to investigate.

Selection of radioisotopes for targeted radiotherapy

Radioisotopes selected for targeted radiotherapy should retain ideal properties for irradiating cancerous cells whilst minimizing damage to surrounding normal tissue. Several factors influence the selection of an appropriate radioisotope, e.g., type of particle(s) it emits, physical half-life, and energy penetration range of the selected radioisotope, etc. Physical half-life directly relates to the delivery rate of an absorbed radiation dose. Rapidly dividing tumor cells are particularly sensitive to a high dose rate. A longer physical half-life and lower dose rate may be more effective for relatively indolent malignancies [76]. The ultimate target to achieve cell death is the nucleus of a cancer cell. Besides the site of cellular radiopharmaceutical concentration, the selected beta- or alpha-emitters must have a suitable path length through tissues to reach the nucleus and appropriate potency to induce cell death. The relatively long particle penetration range (800–10,000 μm) and low linear energy transfer (approximately 0.2 keV/μm) [77, 78] of beta-particles comparing with that of alpha-particles make them suitable for treating bulky tumors. Iodine-131, a long-lived beta-particle emitter has been successfully used for the treatment of hyperthyroidism and differentiated thyroid carcinoma for several decades. Antibody conjugates based on ^{90}Y and ^{131}I for the treatment of NHL have been approved by the FDA in recent years [78]. Alpha-particles have a shorter emitting range (50–80 μm) and a higher linear energy transfer (approximately 100 keV/μm) comparing with that of beta-particles. Radioconjugates emitting alpha particles are the main options for single cell killing or for

treatment of minimal residual tumor cells. Researches on the efficacy of alpha-emitting radioconjugates seem encouraging, and clinical trials for leukemia, cystic glioma, and melanoma are under way. The characteristics of clinically used common radioisotopes are summarized in Table 2.

Targeting tumor with radiolabeled peptides

Peptides have recently showed prominence in targeting malignancies for several reasons. Key properties with peptides include fast clearance, rapid tissue penetration, and low antigenicity, as well as relatively easy and inexpensive production [79]. Radiolabeled peptides appear to be among the most promising vectors for TATs, as they offer an attractive vehicle for clinical use and commercialization [78]. Due to the receptor-mediated internalization and intracellular retention properties of radiopeptides, this approach can deliver adequate radiation doses to the tumor cells for achieving at least volume reduction purpose [80]. Examples of current radiopharmaceuticals include small peptides such as octreotide, neurotensin, α-melanocyte stimulating hormone, vasointestinal peptide, and others [81]. Somatostatin is a peptide hormone that naturally presents in neuroendocrine tumors, i.e., tumors in hypothalamus brain stem, gastrointestinal tract, and pancreas. The best clinically established radiopeptides for in vivo targeting to tumor are based on the somatostatin receptors [78, 80–82]. A particularly large number of excellent radioligands have been developed from somatostatin agonists [82]. ^{111}In-labeled somatostatin has been widely used as a nuclear imaging agent. However, tumor size reduction was seldom achieved with such ^{111}In-labeled somatostatin analogs. Therapeutic radiopeptides with beta-emitting isotopes like ^{90}Y and ^{177}Lu have been most extensively studied [78, 80]. ^{90}Y-DOTA(0)-Tyr^3-octreotide and ^{177}Lu-DOTA(0)-Tyr^3-octreotate have proved to be encouraging and promising in terms of neuroendocrine tumor regression in various studies [78]. Anti-cancer effects of ^{90}Y-DOTA(0)-Tyr^3-octreotide, as reported in literature, vary so much between various studies: complete plus partial regression of 50% or more was achieved in 22±11% of studied patients with neuroendocrine gastroenteropancreatic (GEP) tumors in multi-center phase I studies [83]. With ^{177}Lu-DOTA(0)-Tyr^3-octreotate treatment in patients with neuroendocrine GEP tumors, tumor regression of over 50% in 28% and 25–50% in 19% was achieved with progressive disease in 18% and stable disease in 35% of studied patients [83]. Thus, radiolabeled analogs of somatostatin represented a well-established paradigm of peptide radiopharmaceuticals for targeting tumors [78, 80–84]. To further address the potential of radiopeptide therapy and to establish the optimal treatment scheme, both uniform pathology-oriented trials and randomized clinical trials are required [80].

Table 2 Characteristics of several radioisotopes more used for radiotherapeutic purposes

Isotope (symbol)	$T_{1/2}$	Emission	Mean tissue path length (μm)	Decay energy $_{\beta/\alpha}$ (KeV)
Iodine-131 (^{131}I)	8.04 days	Beta/gamma	900	970
Yttrium-90 (^{90}Y)	2.67 days	Beta	3,900	2,280
Lutetium-177 (^{177}Lu)	6.7 days	Beta/gamma	700	497
Samarium-153 (^{153}Sm)	1.95 days	Beta/gamma	1,200	807
Rhenium-188 (^{188}Re)	17 h	Beta/gamma	3,500	2,120
Strontium-89 (^{89}Sr)	50.5 days	Beta	2,400	1,492
Actinium-225 (^{225}AC)	10 days	Alpha	65	5,935
Bismuth-213 (^{213}Bi)	46 min	Beta/alpha	80	1,422/5,982

$T_{1/2}$ half-life, *μm* micrometer, *mean tissue path length* mean range in soft tissue, *decay energy* $_{\beta/\alpha}$ the energy released by a radioactive decay through beta or alpha emission

Radiolabeled MIBG

MIBG or Iobenguane, an iodinated arylalkylguanidine norepinephrine analog, resulted from the combination of the benzyl group of bretylium and the guanidine group of guanethidine [80, 85]. Organ systems with rich adrenergic innervation and/or catecholamine excretion possess a high uptake of MIBG. Thereby, MIBG radiolabeled with iodine-131 has been used for imaging and therapy of neuroectodermally derived tumors such as neuroblastomas, pheochromocytomas, paragangliomas, medullary thyroid carcinomas, carcinoid tumors, and Merkel cell tumors of the skin, etc. [80]. Therapeutic doses of ^{131}I-MIBG have been administered for experimental therapy of malignant pheochromocytoma and other neuroendocrine tumors. The intense uptake and long retention of ^{131}I-MIBG indicate its therapeutic efficacy in metastatic tumors. In a review of 116 patients, partial response in 18–88% of patients was reported with varying doses of ^{131}I-MIBG [86]. A 30% overall objective response (complete and partial tumor response) was reported in a survey of ^{131}I-MIBG practice in over 99% of 537 treated patients with refractory, stage III/IV disease, excluding childhood neuroblastoma [76]. The result was associated with reduction in measurable tumor markers (complete and partial response) in 38% of patients and subjective response in 52% [76]. Currently, large dose of radio-iodinated MIBG has been used to treat relapsed or refractory metastatic neuroblastoma, most studies reported a response rate of 30–40% with ^{131}I-MIBG in these patients. More recent studies mainly focused on the combination of ^{131}I-MIBG with chemotherapy or myeloablative regimens [87].

Monoclonal antibodies mediated targeting radiotherapy

Radioimmunotherapy (RIT) is currently a major research topic for personalized cancer treatment that combines the

cancer killing of radiation therapy with the precise targeting capacity of immunotherapy. Certain target structures of mAbs have been identified for both hematological malignancies and solid tumors. Accordingly, radionuclides have been conjugated to such antibodies to increase specificity of the therapeutic intervention [88]. After intravenous injection of a radiolabeled mAb, the binding ability of the antibody to the tumor-associated antigen ensures that the tumor gets a high dose of radiation, which would be sufficient to kill the targeted cancer cells and the nearby cells. RIT takes advantage of a growing number of mAbs to target tumor cells preferentially while sparing normal and healthy tissues. Recent advances in chemistry have led to increasingly stable conjugation of radionuclide with mAbs [89]. Cancer therapeutic index was potentially increased in comparison with other treatment modalities [77].

Currently the most promising area of RIT is in the treatment of NHL [89]. ^{90}Y-Ibritumomab tiuxetan and ^{131}I-Tositumomab are the two radiolabeled mAbs that have been approved for treatment of follicular and recurred or resisted B cell NHL. High level expression of CD20 on normal and malignant B cells has made it an attractive target for B cell NHL treatment. ^{90}Y-Ibritumomab Tiuxetan and ^{131}I-Tositumomab bind to CD20 antigen on the surface of B cells, therefore deliver its radiation to target cancer cells. ^{90}Y-Ibritumomab Tiuxetan is indicated for the treatment of adults with relapsed or refractory low-grade, follicular, or transformed B cell lymphoma, but its safety has not been determined in children [90]. ^{131}I-Tositumomab is indicated for the treatment of patients with CD20 positive, follicular NHL who are resistant to Rituximab and have relapsed following chemotherapy [89]. Clinical results are very encouraging with a high percentage of patients entering long-term remission with the above-mentioned RIT [89, 90].

Tumor necrosis therapy (TNT), an approach stems from RIT, links a radioactive isotope to a targeted monoclonal antibody that is designed to bind to a universal intracellular antigen, i.e., DNA/histone H1 complex, which is exposed only on dead and dying cells [91]. ^{131}I-chTNT-1/B mAb is a genetically engineered, radiolabeled, chimeric mAb specific for the necrotic core of malignant gliomas [91–93]. ^{131}I-chTNT-1/B mAb, which delivers a cytotoxic dose of radiation to the lesion core, has being investigated for the treatment of newly diagnosed and recurrent high grade brain tumors. It remains within the tumor necrosis and bombards the neighboring viable cells with radiation [93]. However, the current use of TNT is severely limited due to low amount of tumor uptake, poor penetration into larger lesions, and heterogeneity of antibody uptake. Clinically, ^{131}I-chTNT-1/B mAb was delivered via convection-enhanced delivery in order to maximize coverage to the tumor and the invasive front of the glial tumor [90–93]. Similar approach has been investigated both clinically and experimentally in extra-cranial tumors [94], which also exposed the same drawbacks of insufficient tumoral tracer distribution as well as unsatisfactory therapeutic outcomes after systemic drug delivery. To compensate insufficient targetability, intratumoral injection was attempted in patients with lung cancers [94].

Small-molecular sequential dual targeting theragnostic strategy (SMSDTTS): a new TAT strategy?

Necrosis avid compounds (NACs) represent a new class of targeting chemicals that show extraordinary affinity to non-viable tissues typically necrosis in the living body [95, 96]. NACs were originally identified after disproving the tumor selectivity of porphyrin derivatives used for photodynamic therapy (PDT) or for tumor-seeking diagnostic imaging [95–97]. Both porphyrin and non-porphyrin species of NACs have been reported [95, 96]. In addition to non-oncological applications such as visualization of myocardial infarction [95, 96, 98], NACs can be exploited for diagnostic and even therapeutic utilities in experimental and clinical oncology, e.g., to assess the presence and extent of spontaneous tumor necrosis, to evaluate the necrotic tumor fraction after necrosis-inducing therapies, and to deliver therapeutic radionuclide to tumor necrosis and kill adjacent living cancer cells by crossfire radiation. The necrotic core of tumors functions as an abundant, insoluble, non-diffusible anchor for NACs. Similar to TNT, NACs can access and bind to the necrotic areas of tumors but with a higher affinity, particularly at the interface between necrotic and viable tumor tissues. Therefore, NACs have the potential to carry therapeutic agents and to preferentially target virtually all solid tumors [99, 100]. By extending the capacity of NACs from diagnostic to therapeutic applications, the radiolabeled derivatives of NACs have shown the property of penetrating and localizing into the tumoral necrotic region and thereafter bombard the adjacent viable tumor cells with ionizing radiation [10].

Hypericin, as a nonporphyrin NAC, is a naturally derived substance isolated from the plant genus *Hypericum*, and it is also synthetically obtainable by binding two molecules of emodin. Hypericin, with molecular weight of 504 Da, has a structure of polycyclic polyphenolic quinine (Fig. 3). A peculiar affinity for necrotic or irreversibly damaged ischemic tissues has been shown with Hypericin [99, 100]. Radiolabeled derivatives of Hypericin mono-[^{123}I]-iodohypericin ([^{123}I]MIH) as diagnostic NACs have been studied in animal models of hepatic and myocardial infarction [100, 101] and found to concentrate in necrotic liver and myocardium of over 20-fold of that in normal surrounding tissues 24 h after systemic injection [100, 101]. Similar affinity was found with native Hypericin in areas of necrosis in tumor

models after ablative therapies [102, 103]. Radiolabeled Hypericin has achieved encouraging results in sensitivity and specificity of necrosis targeting. The virtual tumor-seeking property of hypericin enables it to home to the necrotic core of solid tumor, which prompts our assumption that radioactive "payload" can be delivered to the center of the tumor mass if Hypericin is radiolabeled with therapeutic radionuclide [96, 100]. Experiments using iodine-131 labeled hypericin to form ^{131}I-iodohypericin (^{131}I-Hyp) in different types of tumor models are under investigations with preliminary promising outcomes [10].

As can be self-explained by its full terms, SMSDTTS stands for an anticancer strategy using two small molecules to sequentially target tumors for achieving both diagnostic and therapeutic effects. Instead of directly attacking cancer cells as mostly elaborated by others, SMSDTTS primarily targets cancer stromas (soil) and indirectly but more thoroughly destroys parenchymal cancer cells (seeds), which is on the basis of soil-to-seeds hypothesis [10]. As shown in Fig. 4, both chemicals sequentially implemented in SMSDTTS are small-molecular, naturally extractable or synthetically derivable, and clinically injectable. Their respective targeting mechanisms are: (1) CA4P selectively shuts down tumor vasculature and induces ischemic tumor necrosis; and (2) radiolabeled hypericin carries and delivers a therapeutic radionuclide iodine-131 to the prior existing or induced necrotic region in the tumor and kills neighboring residual tumor cells by crossfire radiation. Theoretically, these two components in SMSDTTS represent a perfect match of complementary tumoricidal effects. The VDA CA4P kills the tumor from the inside out and leaves viable tumor cells at the periphery. Since those remaining tumor cells rely on the surrounding normal blood supply for rapid growth, they are sensitive to radiotherapies, which are designed to kill rapidly proliferative and well-oxygenated tumor tissues [9, 104]. Such sequential and complementary treatment may improve the likelihood of complete tumor destruction and, therefore, more satisfactory therapeutic outcomes. With both being small molecules of proven high targetabilities, CA4P and Hypericin possess favorable pharmacokinetics and safety profiles, i.e., high target/non-target ratio, short biological half-lives, and absence of toxic side effects commonly seen with conventional chemotherapies. Relative to currently available anticancer treatments or the newly advocated personalized but sophisticated and costly TAT approaches, this new strategy may prove to be a non-invasive, simple, workable, affordable, and depersonalized anticancer treatment for all solid malignant tumors in visceral organs, whether primary or metastatic in early or late stages [10].

Basic requirements for TATs

To obtain satisfactory therapeutic outcomes with TATs, several basic requirements have to be taken into account.

Dependence on tumor-types

A wide range of tumors have been covered by TATs, e.g., breast cancer treated by trastuzumab and lapatinib, colorectal cancer treated by bevacizumab, lung and pancreatic cancers treated by Gefitinib and erlotinib, lymphoma treated by rituximab and tositumomab, leukemia treated by dasatinib, etc. (Table 1). Although TATs greatly benefit the patients with their respective tumors, tumor cells resistant to TAT drugs are frequently induced in some tumors. TAT that based on the mechanism of SMKI requires the targeting tumor to have identified and overexpressed mutations in kinase domain. Therapies with mAbs rely on sufficient specific tumor-associated antigens expressing on the surface of tumor cells. Due to a lack of particular and generalized TATs that can target all tumor types, many cancers still have not been covered by TATs.

The pathophysiology of solid tumors differs from that of normal tissues and has been explored and utilized for

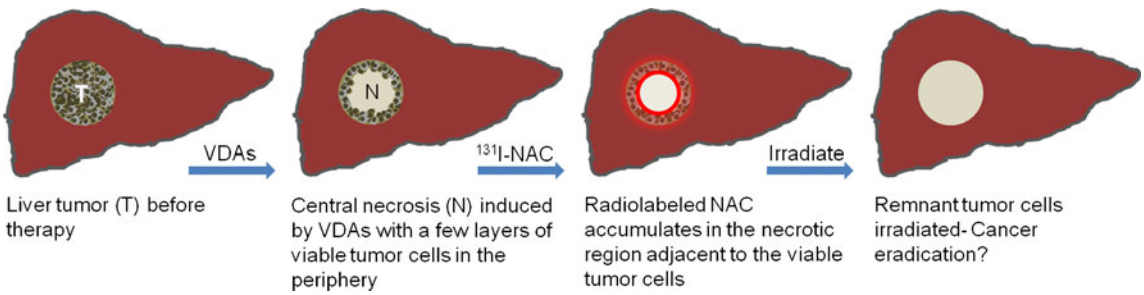


Fig. 4 Schematic hypothesis and components for the novel targeted anticancer therapy of SMSDTTS: predicted sequential dual targeting tumoricidal events: imagine there is an inoperable liver tumor (T), we first treat the tumor using the available VDAs to cause massive tumor necrosis (N) that becomes the reliable target for the second attack launched after 24 h. An IV injected radiolabeled NAC accumulates in the intratumoral necrosis (N) particularly in close vicinity to the peripheral viable rim and constantly irradiates the remnant tumor cells, resulting in complete tumor necrosis (N) or cancer eradication

developing TATs. For example, based on the knowledge that angiogenesis is a continuous process to keep the tumor growth and the fact that tumor vessels are often highly abnormal and are prone to collapse comparing with vasculatures of normal tissues [58], VDAs and angiogenesis inhibitors have been introduced to treat a wider variety of tumor types by targeting these unique features. Another general consensus about solid tumors is that they may contain large necrosis; thereby, TNT has been developed to tentatively target intratumoral necrosis, but the results with TNT are not satisfactory due to the lack of sufficient necrosis avidity and/or uncertain presence of spontaneous tumor necrosis. Along with our recent advances with the SMSDTTS that combines VDAs and radiolabeled necrosis targeting compounds, most solid tumors are expected to be targeted and treated [10].

Aspects on targeting agents

Specificity and affinity of TATs

Agents for TATs are designed to interact specifically with particular receptors to avoid or reduce side effects. On the other hand, how tightly a TAT agent binds to its receptor is also a substantial concern. Tumor specificity and affinity are the most essential requirements for TATs. The exquisite target specificity as well as the high affinity to targeting antigens enables mAbs to selectively bind to antigen and therefore reduce off-target effects. However, most antigens are not tumor specific because they are expressed not only by certain cancer cells but also by normal cells. Meanwhile, the expression of antigens in tumors recognized by mAbs is often heterogeneous, and loss of expression may be observed in anaplastic transformation and may result in immune escape [105]. SMKIs show great promise as a new class of TAT because they target the ATP binding site in protein kinases domain, whereas ATP site is present in all of the more than 500 protein kinases identified in the human genome, which makes cross-reactivity inescapable. Molecular specificity and off-target interactions of SMKIs against kinases must be assessed and identified before its clinical use. Yet for most SMKIs, specificity and affinity have been determined against only relatively small sets of kinases [106].

Cytotoxicity concerns

TATs should have the potential to induce selective tumor cytotoxicity while sparing normal tissues. Despite certain abilities to localize into the tumor and to bind to tumor cells, spectacular tumor regressions are not always seen with unconjugated mAbs and SMKIs due to the following reasons. First, tumors express the target but are not dependent thoroughly on the target for proliferation and/or survival. Secondly, the development of resistance occurs regardless of the size and type of the tumor. Moreover, generally insufficient cytotoxicity of mAbs and SMKIs to cancer cells may hinder the therapeutic efficacy. Furthermore, most cancers are not sensitive to single-agent targeted therapies. Even when sensitive to single-agent therapies, cancers develop resistance. Thus, novel cytotoxic agents with unique mechanisms of actions are continuously being pursued [107] and TATs are required to combine with multiple agents or different mechanisms to achieve sufficient cytotoxicity and to gain synergistic anticancer effects [108]. Efforts to improve the cytotoxicity of mAbs have been focused on conjugates with cytotoxic agent, radioisotopes, and immunotoxins [109]. Our endeavors in developing SMSDTTS represent another example [10].

Highlighted by ^{90}Y-Ibritumomab Tiuxetan and ^{131}I-Tositumomab, which have been clinically approved as radioimmunoconjugates used for the treatment of lymphomas, RIT could be heading for the mainstream in TAT development. Radioimmunoconjugates are produced either by covalently binding the radioisotope directly to the mAb or by crosslinking them through a chemical linker or a chelator [110]. Besides the targeting specificity of the antibody to the cancer cells, the stability of the antibody-radionuclide conjugate, the cytotoxic potentiality of the selected radionuclide with regard to the targeted cells, are the key components for the optimization of RIT.

Toxicity

Although TAT agents were deliberately chosen or designed to act on specific molecular targets, which may lead to fewer and less toxic side effects than conventional chemotherapy, toxic effects associated with TATs such as hypertension, fatigue, bone marrow toxicity, skin toxicity, gastrointestinal side-effects as well as immunosuppression, metabolic alterations, interstitial pneumonitis, and hypothyroidism do commonly present [111]. Patients treated with TATs need to be closely monitored for the development of drug-related toxicities. Meanwhile, supportive measures to prevent interruptions of treatment, dose reductions, and eventual development of life-threatening complications should be vigorously taken to manage with drug-related toxicities even at mild and moderate levels [112].

Considerations for TATs

Cancer biology: inherent hurdles for cancer cure

Oncogenesis is a multi-step process with various genes and pathways, different mechanisms, multifarious carcinogens,

viruses, cytokines, hormones, bacteria, etc., as well as a whole bunch of possible gene mutations or disruptions involved, which together allow the cells to undergo uncontrolled division, thus forming a malignant mass [113]. Besides, other nonexclusive detailed mechanisms that trigger resistance can be envisaged: target mutation, target amplification, activation of a complementary pathway that bypasses the target requirement, upregulation of mechanisms that lower the intracellular concentrations of the target, etc [14]. Such an increasingly known complexity makes cancer cure biologically almost impossible.

What to target and how to target in cancer

All targeted cancer therapies aim to maximize tumor destruction while minimize side-effects, which makes tumor affinity or target binding an essential demand. High target tissue binding is the most important goal, whereas blood pool residence and nonspecific binding of a TAT agent are also important considerations. Equally important is the biodistribution of metabolized components and their excretion routes particularly for those radioactively labeled compounds. Although the discussed TATs by inhibiting a single molecular target, antigen, or neoangiogenesis may prevent tumor cell proliferation or kill targeted malignant cells effectively, tumor progression is unlikely to depend on a sole signal transduction pathway. Furthermore, for any genetically unstable diseases including cancer, resistance is an inevitable consequence of the treatment with a single molecular targeted agent or antibody [114]. Apparently, the newly introduced SMSDTTS which chooses noncancerous, less mutant, and more stable stromal targets may confront less drug resistance and more therapeutic response [10].

Possible reasons for unsuccessful TATs

SMKIs are generally designed with intention to target one specific kinase. However, as a result of the evolutionarily conserved nature of the ATP binding pocket, a SMKI may potently inhibit lots of other kinase members while targeting their specific kinase. Such off-target kinases may be a potential safety liability of SMKIs therapies and may hinder drug development [115, 116]. For both mAb and its radiolabeled derivatives, only a handful of studies have shown a significant number of complete remissions up to now. Several reasons account for the failure or unsatisfactory results from mAbs. Firstly, the specificity of antigen expression on tumor cells is poor, and tumor antigens often express to some degree on normal cells. Secondly, intracellular compartments, in particular the cytoplasm or nucleus, have generally been poorly accessible or inaccessible to monoclonal antibodies [75, 117]. Moreover, the activity dose in RITs is limited by myelotoxicity as a result of the continuous radiation exposure of the red bone marrow to the slow-clearing antibody. Thus, the success of mAb therapy and RIT for treatment of solid tumors has been limited so far [118]. As well, radiolabeled peptides and MIBG only show effects in limited types of malignant tumors.

Limitations and obstacles of molecular targeted radiotherapies

Molecular targeted radiotherapy, being an evolving and promising modality of cancer treatment, is required to be efficacious with minimal normal tissue toxicity [119]. Limitations concerning research on molecular targeted radiotherapies mainly include non-uniqueness of antibodies for tumor cell antigens (antibodies may bind to non-target antigens on normal cells), heterogeneous antigen expression on tumor cells, formidable myelotoxicity, slow blood clearance, and sub-optimal distribution of the relatively large (molecule weight 150 kDa) radiolabeled antibodies in the tumor [120]. Besides, other obstacles include inadequate understanding of the molecular mechanism and pharmacology of the agent, physical characteristics of selected cytotoxic radionuclides, intrinsic inferior cellular radiosensitivity, cancer cell resistance factors, normal cell toxicity, and criteria of clinical trial designs, etc. However, the major clinical limitation of targeted radiotherapy, particularly for treatment of solid tumors, lies in immunogenicity, cell specificity, and cell permeability of the targeting molecular ligands.

Despite the collective and enriching knowledge on molecular targeted radiotherapy thus far, many obstacles are still in suspense and require further exploration. Basic requirements for future molecular targeted radiotherapies should involve cytotoxic radioligands with high target specificity, uniqueness for tumor cell or tumor stroma, rapid blood clearance, suitable physical characteristics for systemic administration, appropriate potency for cancer cells, and a wide coverage of tumors. In addition, the conjugation of the targeting molecule to the radionuclide should be reliable. The final radioligand must be practical, affordable for clinical use, as well as stable in vivo and effective at targeting the tumoral binding site [78]. Small molecular necrosis avid Hypericin with a high target specificity as we proposed is likely to play a crucial role in such a context [10].

Potential challenges and new opportunities for TATs

Resulting from immature tumor blood vessels, discrepancy between nutrition supply and tumor growth, immune response and incomplete treatment, one unique characteristic of most solid tumors is that they encompass a proportion of dead tissue in addition to numerous proliferating viable cancer cells. The accumulation of dead cells results in the

formation of a necrotic core presented in virtually all solid tumors beyond a certain size. On the other hand, clinically, the most critical problem for all cancer therapies is incomplete treatment, which sooner or later leads to tumor relapse. For instance, VDAs can cause tumor vessel shutdown and lead to tumor core necrosis. However, tumor relapses quickly due to peripheral remaining viable cells. Radiofrequency ablation (RFA) may destroy the tumor, but the remaining viable cells frequently cause tumor recurrence. Thereby, sequential combination of targeted therapies by non-overlapping complementary mechanisms is imperative and should be designed to achieve synergetic outcomes [58]. SMSDTTS literally destroy the tumor "from the inside out" with minimal radiation exposure to healthy tissues due to the high target-to-nontarget ratio. Sequential use of CA4P and Iodine-131 labeled Hypericin may provide an ingenious approach and simplify the cancer problems. Encouraging results have been achieved with SMSDTTS in recent preliminary experiments in rodent tumor models [10]. Further optimizations are warranted before the implementation of this new strategy in clinical oncology.

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