Recent Advances in the Development of TGF-β Signaling Inhibitors for Anticancer Therapy

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TGF-β is a multifunctional cytokine that plays an important role in both physiologic and pathologic processes, including cancer. Importantly, TGF-β has a dual role in tumorigenesis, acting as a tumor suppressor or a tumor promoter, depending on the stage of tumor development. The aberrantly upregulated production of TGF-β has been strongly implicated in tumor progression, angiogenesis, and metastasis, as well as immune evasion. Therefore, hyperactivated TGF-β signaling is considered a potential therapeutic target for cancer therapy. Numerous inhibitors of overactivated TGF-β signaling have been developed, and some of them are currently in clinical trials. This review focuses on the TGF-β signaling that contributes to tumor progression and immune evasion in the tumor microenvironment and presents recent achievements on TGF-β signaling inhibition as a single or combined therapeutic approach in cancer therapy.

Key Words TGF-β, Tumor microenvironment, Tumor escape, Antineoplastic agents, Immune checkpoint inhibitors

INTRODUCTION

TGF-β was initially identified by its ability to transform and induce anchorage-independent growth of non-transformed fibroblasts [1]. TGF-β is now known to be a multifunctional cytokine that regulates various physiological processes including, cell growth, differentiation, apoptosis, embryonic development, immune responses, and the synthesis of extracellular matrix proteins [2]. Although the effects of the TGF-β signaling depend on the cellular and tissue context, dysregulation of the TGF-β signaling pathway has been involved in the development of human diseases, including cancer [3]. In recent decades, there has been a growing emphasis on the role of the TGF-β signaling pathway in promoting tumor development and progression and inducing an immunosuppressive tumor microenvironment [4,5]. Multiple lines of preclinical evidence suggest that the inhibition of the TGF-β signaling pathway exhibits antitumor activity in various animal models. Notably, high levels of TGF-β expression are correlated with poor prognosis and metastasis and associated with resistance to chemotherapy and shorter survival [6,7].

Based on this rationale, several types of TGF-β signaling inhibitors have been developed, and some of them are currently in clinical trials for cancer treatment. Immunother-apy has recently led to a paradigm shift in cancer therapy, amongst which immune checkpoint inhibitors are the most representative agents approved for treating various advanced solid tumors [8]. Of note, many recent studies have demonstrated that the TGF-β signaling pathway plays a pivotal role in cancer immune escape and resistance to the immune checkpoint inhibitor therapy [9,10]. Therefore, concurrent inhibition of the TGF-β and the immune checkpoint pathway may be a useful strategy to enhance the therapeutic efficacy of immunotherapy.

TGF-β SIGNALING PATHWAY AND CANCER PROGRESSION

TGF-β is synthesized as a latent complex consisting of a mature TGF-β homodimer non-covalently linked to the latency associated protein (LAP) forming the small latent complex (SLC) and the latent TGF-β binding molecule (LTBP), which interacts with extracellular matrix (ECM) [11]. Latent TGF-β can be activated by proteolytic cleavage of LAP by furin, plasmin, and matrix metalloproteinases (MMPs), or by binding to thrombospondin-1 (TSP-1) or αvβ6 integrin [12,13]. Once released, active TGF-β binds to a serine/threonine kinase type II receptor (TGF-β RII), which recruits the type I recep-

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tor (TGF-β RI) and activates it by phosphorylation. Activated TGF-β RI phosphorylates the receptor-regulated Smads (R-Smads), Smad2, and Smad3. Subsequently, phosphorylated R-Smads form a complex with Smad4 and then translocate into the nucleus. In the nucleus, the heteromeric Smad complex regulates transcription through its ability to interact with DNA-binding and recruit transcription cofactors [14]. Besides the Smad-dependent canonical pathway, TGF-β receptors can also activate Smad-independent pathways such as mitogen-activated protein kinases (MAPKs), phosphatidylinositol 3-kinase-AKT (PI-3K/AKT), NF-κB, the small GTPases Rac/Cdc42 and RhoA in a context-dependent manner [15].

TGF-β plays an important but paradoxical role in carcinogenesis and tumor development (Fig. 1). In normal or early stages of tumors, TGF-β acts as a tumor suppressor by inhibiting proliferation and inducing apoptosis of cancer cells. However, during progression, cancer cells become resistant to TGF-β-induced antitumor effects through the acquisition of mutations in the signaling components of the pathway, and then the TGF-β pathway shifts from tumor-suppressive to tumor-promoting functions [16]. In late-stage tumors, TGF-β signaling promotes epithelial-mesenchymal transition (EMT) and invasion, evasion of immune surveillance, and metastatic spread [4]. Also, the tumor-promoting function of TGF-β depends on its effect on the cancer cells and the tumor microenvironment [17].

Role of TGF-β in tumor microenvironment

Solid tumors are not merely composed of cancer cells alone, but they are complex and heterogeneous structures composed of malignant cells that recruit various normal cells, such as fibroblasts, endothelial cells, and immune cells, to promote tumor growth. ECM serves as a structural framework for cell migration and as a reservoir for latent TGF-β, and modulates its activation and turnover [18]. The tumor stroma is a critical component of the tumor microenvironment and is recognized as an essential contributor to tumor growth, metastasis, and immune evasion [19].

TGF-β is normally present at high concentrations in the tumor microenvironment. The increased TGF-β is attributable to the production by both cancer cells and stromal cells, especially cancer-associated fibroblasts (CAFs), immune cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) as well as tumor-associated macrophages (TAMs) [20]. The interplay between these cells within the tumor microenvironment promotes tumor progression by stimulating survival signaling of cancer cells, promoting EMT, angiogenesis, metastasis, and immune evasion [21,22].

While EMT is a physiological process required for normal embryonic development and wound healing in which epithelial cells differentiate into mesenchymal cells, EMT also plays a crucial role in tumor progression and metastasis. TGF-β, which is secreted by cancer cells in an autocrine loop, or a paracrine fashion by stromal and immune cells in the tumor microenvironment, is a potent inducer of EMT [23,24]. TGF-β induces EMT by activating the Smad-dependent and/or Smad-independent transcriptional pathway, which induces the expression of EMT-activating transcription factors, such as Snail (SNAI1), Slug (SNAI2), Zeb1 (TCF8), Zeb2 (SIP1), and Twist [25,26]. The Smad-independent signaling pathways, such as those mediated by the Rho-Rock1 and the AKT, play important roles in promoting cancer cell migration and the manifestation of invasive cellular phenotypes [27]. Cancer cells undergoing EMT acquire invasive properties and enter the surrounding stroma, thereby creating a favorable microenvironment for successful metastasis. EMT is also associated with the acquisition of cancer stem cell-like characteristics and thereby conferring a more aggressive phenotype and resistance to radiotherapy or chemotherapy [28,29].

Angiogenesis in the tumor microenvironment is an essential process for tumor growth and metastasis. New blood vessels can supply the nutrients and oxygen to the cancer
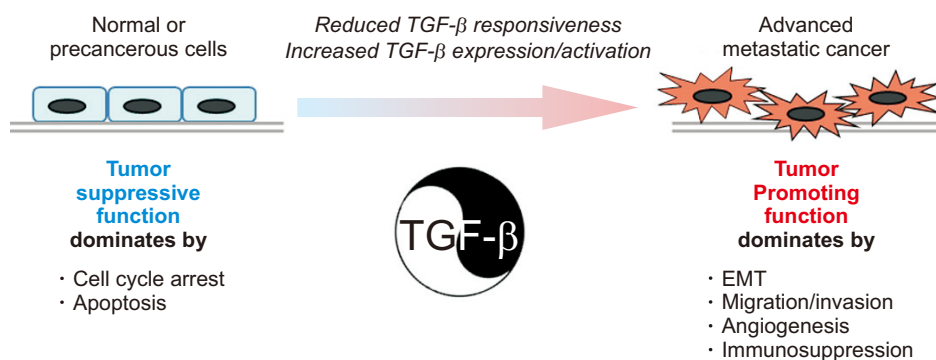
Figure 1. The dual role of TGF-β signaling in tumor progression. TGF-β induces apoptosis and inhibits the proliferation of normal or nontransformed cells but loses its growth inhibitory properties as cells progress to later stages of tumorigenesis. In later stages of tumor progression, TGF-β is actively secreted by cancer cells or stromal cells and acts as a tumor promoter by promoting EMT, cancer cell migration and invasion, and the formation of an immunosuppressive tumor microenvironment. EMT, epithelial-mesenchymal transition.

cells and allow them to have access to the blood system and metastasize to distant sites [30]. TGF-β signaling plays an essential role in normal angiogenesis and vascular integrity, and also neovascularization in the tumor microenvironment [31]. TGF-β directly induces the expression of VEGF, the most potent proangiogenic mediator [32]. TGF-β also recruits endothelial cells, CAFs, and TAMs, which in turn produce proangiogenic factors [33]. TGF-β-mediated MAPK activation promotes cancer cell invasion and tumor angiogenesis through the upregulated expression of MMP-9, which plays an essential role in ECM degradation during tumor angiogenesis and metastasis [34].

CAFs are among the most abundant stromal cell types within the tumor microenvironment and promote tumor progression by supporting cancer cell growth and ECM remodeling, tumor-promoting inflammation, and metastasis [35]. TGF-β promotes the differentiation of fibroblasts to myofibroblasts and stimulates the conversion of normal fibroblasts to CAFs. Activated CAFs enhance angiogenesis by secreting proangiogenic factors, such as VEGF, hypoxia-inducible factor, platelet-derived growth factor, and stromal cell-derived factor-1 [36]. The high density of activated CAFs in the t is closely associated with poor survival, therapy resistance, and recurrence in multiple solid cancers [37,38].

Role of TGF-β in tumor immune evasion

Tumors can escape the host immune system. The malignant tumor cells effectively modulate cellular processes by releasing soluble factors, such as immunosuppressive cytokines (e.g., TGF-β) to protect antitumor immune effector cells. Besides promoting tumor invasion and angiogenesis, TGF-β also plays a critical role in regulating the immune response in the tumor microenvironment [25,39]. TGF-β functions primarily as an immunosuppressive cytokine (Fig. 2). The first in vivo evidence that TGF-β signaling regulates immune response

was demonstrated by knockout studies [40,41]. The lack of TGF-β1 results in severe inflammatory responses by activating the immune cell population and the infiltration of lymphocytes and macrophages in various organs.

Previous studies have demonstrated that TGF-β, secreted by cancer cells and neighboring immune cells, such as Tregs and myeloid suppressor cells, suppress the antitumor activity of effector cells, including CD8$^+$ T cells, natural killer (NK) cells, and macrophages [39]. CD8$^+$ T cells are referred to as cytotoxic T lymphocytes (CTLs), given their ability to kill target cells. TGF-β suppresses the growth and differentiation of CTLs through Smad-dependent downregulation of c-Myc and IL-2, and upregulation of p21 and p27 [42]. NK cells can kill malignant cells without activation process and enhance antitumor effects by secreting cytokines such as IFN-γ and tumor necrosis factor-α (TNF-α). TGF-β signaling inhibits NK cell functions in multiple ways. TGF-β suppresses the expression of IFN-γ in NK cells, thereby blocking Th2 responses [43]. TAMs are also an important source of TGF-β in the tumor microenvironment and are closely associated with poor prognosis and resistance to antitumor agents. TGF-β inhibits the inflammatory response of macrophages mediated by TNF-α/ NF-κB pathway [44]. TGF-β signaling also plays a critical role in inducing Tregs, which suppress the function of effector T cells and participate in cancer immune evasion [45]. TGF-β induces a forkhead box protein 3 (Foxp3), a transcription factor essential for developing Tregs, through Smad3 activation [46].

Recently published data from Tauriello et al. [47] showed that the inhibition of TGF-β signaling with a TGF-β RI kinase inhibitor triggers the infiltration of CTLs, and renders tumors susceptible to immune checkpoint therapies. Likewise, the combination of TGF-β and immune checkpoint pathway inhibitors enhances CTL response and drives potent antitumor immunity and tumor regression [48]. This evidence may imply
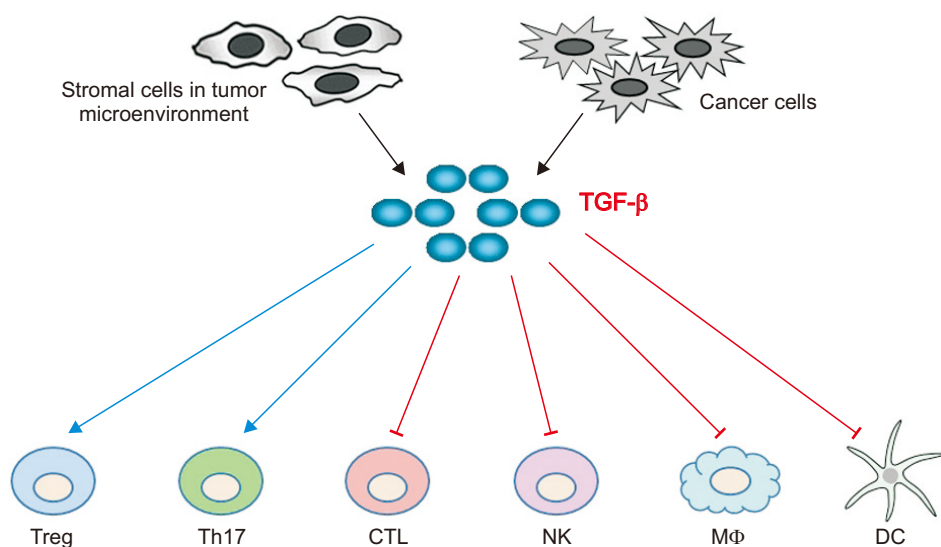
Figure 2. TGF-β secretion in the tumor microenvironment and its effects on immune cells. TGF-β is secreted by cancer cells and stromal cells and exerts immunosuppressive roles for the immune cells involved in the antitumor immune responses. Treg, regulatory T cells; Th17, T helper 17 cells; CTL, cytotoxic T lymphocytes; NK, natural killer cells; MΦ, macrophages; DC, dendritic cells.

that the high abundance of TGF-β in the tumor microenvironment represents the most general and primary mechanism for cancer cells to escape the host immune system.

TGF-β SIGNALING INHIBITION AS ANTICANCER THERAPY

The main strategies for the therapeutic targeting the TGF-β signaling pathway can be exploited by suppressing the production of TGF-β, the activity of TGF-β, the interaction between TGF-β ligand and its receptors, or the kinase activity of TGF-β receptor (Fig. 3). Several types of TGF-β signaling inhibitors are currently under clinical trials in various cancer types, either singly or in combination with other anticancer agents, including immune checkpoint inhibitors (Table 1).

Clinical application of TGF-β signaling inhibitors for cancer therapy

Antisense oligonucleotides can effectively and specifically block the expression and secretion of TGF-β. Trabedersen (AP 12009, OT-101), an antisense oligonucleotide designed to human TGF-β2, has been evaluated in phase I/II clinical trials in patients with various types of advanced solid tumors, including pancreatic and colorectal cancers, glioma, and malignant melanoma [49-51]. Since it is well established that the TGF-β2 isoform plays a pivotal role in the progression of pancreatic cancer and glioma, Trabedersen effectively inhibited proliferation and migration of cancer cells and reversed TGF-β-mediated immune suppression of cancer cells [50]. Although initial clinical trials of Trabedersen demonstrated encouraging results, subsequent trials have been disappointing due to significant side effects and insufficient targeted delivery of antisense oligonucleotide. The TGF-β1-specific anti-

sense oligonucleotide, AP 11014 has been developed for the treatment of non-small cell lung cancer (NSCLC), colorectal cancer, and prostate cancer [52].

Monoclonal antibodies (mAbs) targeting ligands are particularly effective in blocking ligand-receptor binding and subsequent ligand-induced signaling. The human mAbs, Metelimumab (CAT-192), Lerdelimumab (CAT-152), and Fresolimumab (GC1008) have been developed and evaluated in clinical trials. Metelimumab and Lerdelimumab are recombinant humanized immunoglobulin (Ig) G4 mAbs that neutralize TGF-β1 and TGF-β2 isoforms, respectively. Although Metelimumab and Lerdelimumab have not entered clinical trials for the treatment of cancers, both mAbs were awarded orphan drug status to treat scleroderma and the prevention of postoperative scarring after glaucoma surgery [53,54]. Fresolimumab (GC-1008) is a human pan TGF-β neutralizing mAb that was evaluated in clinical studies in patients with advanced solid tumors, including melanoma, renal, and squamous cell carcinoma [55,56]. Fresolimumab showed an acceptable safety profile, and there is evidence for its antitumor activity. Recent positron emission tomography (PET) image analysis in patients with glioblastoma and breast cancer using an [89]Zr-labeled Fresolimumab has demonstrated that the inhibition of TGF-β provides survival benefit in the context of radiation treatment [57]. Decoy receptors, such as decorin and bone morphogenetic protein and activin membrane-bound inhibitor (BAMBI) inhibit TGF-β signaling by competing with the TGF-β receptor for ligand binding [14]. AVID200, a protein composed of TGF-β receptor ectodomain fused to the human IgG fragment crystallizable domain, is currently in phase I trials for patients with advanced and metastatic solid tumors (NCT03834662). AVID200 was designed to have the specificity that binds and neutralizes TGF-β1 and
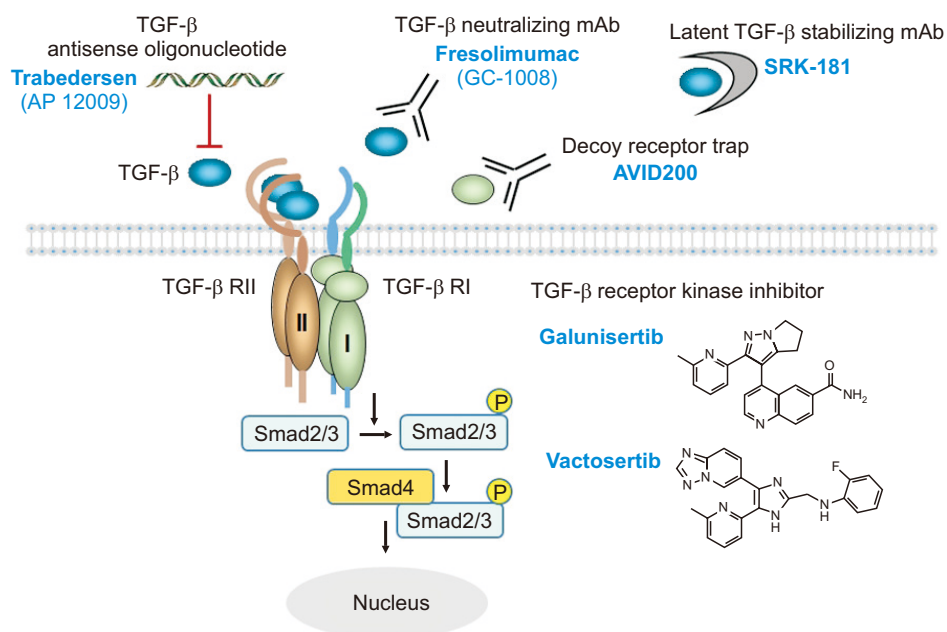
Figure 3. Mechanisms and examples of TGF-β signaling inhibitors being explored in clinical trials. mAb, monoclonal antibody; TGF-β RI, TGF-β receptor type I; TGF-β RII, TGF-β receptor type II.

Table 1. Currently ongoing clinical trials of TGF-β signaling pathway inhibitors for cancer treatment

Drug name	Target type	Treatment	NCT number
Trabedersen (AP 12009)	TGF-β2 AS-ODN	Advanced pancreatic, colorectal cancer, melanoma (phase I)	NCT00844064 (completed)
Fresolimumab (GC1008)	TGF-β1 mAb	NSCLC (phase I/II)	NCT02581787
AVID200	TGF-β1/3 trap	Malignant solid tumor (phase I)	NCT03834662
ABBV-151	GARP/TGF-β1 mAb	Advanced or metastatic solid tumor (phase I)	NCT03821935
SRK-181	TGF-β1mAb	Advanced solid tumor (phase I); alone or in combination with anti-PD-L1	NCT04291079
Bintrafusp alfa (M7824)	TGF-β trap	Advanced NSCLC (phase III)	NCT03631706
		Biliary tract cancer (phase II/III)	NCT04066491
		Thymoma and thymic carcinoma (phase II)	NCT04417660
		Urothelial carcinoma (phase II)	NCT04501094
		HPV associated cancers (phase II)	NCT03427411
		Cervical cancer (phase II)	NCT04246489
		Triple-negative breast cancer (phase II)	NCT04489940
		Esophageal squamous cell carcinoma (phase II)	NCT04595149
		Advanced biliary tract cancer (phase II)	NCT03833661
		Head and neck squamous cell carcinoma (phase II)	NCT04428047
		Cervical cancer (phase II)	NCT04246489
		Advanced colorectal cancers (phase II)	NCT04491955
		Metastatic colorectal cancer (phase I/II)	NCT03436563
		Advanced Kaposi sarcoma (phase I/II)	NCT04303117
		Advanced pancreatic cancer (phase I/II)	NCT04327986
Galunisertib (LY2157299)	TGF-β RI kinase inhibitor	Metastatic prostate cancer (phase II)	NCT02452008
		Advanced hepatocellular carcinoma (phase II)	NCT02178358
		Recurrent glioblastoma (phase II)	NCT01582269
		Advanced hepatocellular carcinoma (phase I)	NCT02906397
		Triple-negative breast cancer (phase I)	NCT02672475
		Carcinosarcoma of uterus or ovary (phase I); in combination with Paclitaxel/Carboplatin	NCT03206177
		Rectal adenocarcinoma (phase I)	NCT02688712
		Recurrent malignant glioma (phase I)	NCT01682187
Vactosertib (TEW-7197)	TGF-β RI kinase inhibitor	Myeloproliferative neoplasms (phase II)	NCT04103645
		Metastatic colorectal or gastric cancer (phase I/II); in combination with Pembrolizumab	NCT03724851
		Advanced NSCLC (phase I/II); in combination with Durvalumab	NCT03732274
		Metastatic gastric cancer (phase I/II); in combination with Paclitaxel	NCT03698825
		Myelodysplastic syndrome (phase I/II)	NCT03074006
		Refractory multiple myeloma (phase I); in combination with Pomalidomide	NCT03143985
		Advanced desmoid tumor (phase I); in combination with Imatinib	NCT03802084
		Urothelial carcinoma (phase I); in combination with Durvalumab	NCT04064190 (not yet recruiting)
		PD-L1 positive NSCLC (phase II); in combination with Pembrolizumab	NCT04515979 (not yet recruiting)
		Pancreatic ductal adenocarcinoma (phase I); in combination with nal-IRI+5FU/LV	NCT04258072 (not yet recruiting)

AS-ODN, antisense oligonucleotides; NCT, National Clinical Trial; NSCLC, non-small cell lung cancer; mAb, monoclonal antibody; GARP, glycoprotein A repetitions predominant; anti-PD-L1, anti-programmed death-ligand 1; HPV, human papillomavirus; TGF-β-RI, TGF-β receptor type I; nal-IRI+5FU/LV, nanoliposomal irinotecan plus 5-fluorouracil/leucovorin. Data from ClinicalTrial.gov (https://www.clinicaltrials.gov/).

TGF-β3 isoforms, which can reverse immunosuppressive effects of TGF-β in the tumor microenvironment as well as resistance to anticancer therapies [58].

Specific inhibition of the activity of TGF-β RI, activin-like kinase 5 (ALK5) by small molecules is an attractive therapeutic strategy for developing anticancer drugs. Although several small molecule inhibitors such as SB-431542, SB-505124 (GlaxoSmithKline; Brentford, UK), and LY36493/LY580276

(Eli Lilly and Company; Indianapolis, IN, USA) have been developed and evaluated in preclinical and clinical trials, many of them exhibit low selectivity and undesired side effects, especially cardiac toxicity [59]. However, ALK5 inhibitors, such as Galunisertib and Vactosertib, are currently undergoing clinical trials in cancer treatment through clinical pharmacokinetic and pharmacodynamic studies to establish a safe and effective therapeutic window.

Galunisertib (LY2157299) is an oral ALK5 inhibitor with an IC_{50} of 50 nM that specifically inhibits the activation of the downstream molecule, Smad, thereby blocking the TGF-β signaling pathway [60]. The antitumor activity of Galunisertib has been shown in various animal models for breast and colorectal cancers and hepatocellular carcinoma either alone or in combination with other standard anticancer agents [21,61]. Galunisertib is one of the most extensively studied small molecule ALK5 inhibitors in clinical development and shows a safety profile from various clinical trials [62,63]. Two phase Ib/IIa clinical trials were conducted with Galunisertib combined with gemcitabine or with temozolomide-based radiochemotherapy in patients with advanced pancreatic cancer or with malignant glioma, respectively [64,65]. When given together, both treatments had an acceptable safety and tolerability profile, although the clinical benefits are not statistically significant. A recent phase II study has demonstrated that Galunisertib combined with a multikinase inhibitor Sorafenib prolonged the overall survival of over 14 months in patients with advanced hepatocellular carcinoma [66]. Galunisertib is currently being evaluated in a phase I clinical trial in patients with carcinosarcoma of the uterus or ovary combined with Paclitaxel or Carboplatin (NCT03206177). A phase II clinical trial is ongoing with Galunisertib combined with Enzalutamide, a nonsteroidal antiandrogen, in patients with metastatic castration-resistant prostate cancer (NCT02452008).

Vactosertib (TEW-7197) is a novel orally bioavailable inhibitor of ALK5 with an IC_{50} of 11 nM that was designed to have more potent and specificity in comparison to other small molecule inhibitors [67]. The antitumor activity of Vactosertib has been demonstrated in various types of cancers, including breast and lung cancer, melanoma, and hepatocellular carcinoma [68-70]. Vactosertib prolonged the survival of breast tumor-bearing mice by inhibiting invasion and metastasis and improved the ability to elicit CTL activity [69]. A recent study showed that combination treatment of Vactosertib with nal-IRI + 5-FU/LV, a chemotherapy regimen for advanced pancreatic cancer, significantly improved the overall survival of mice by suppressing the invasion of pancreatic cancer cells [71]. Using RNA-sequencing analysis, this study has demonstrated that Vactosertib significantly induces the CCDC80 gene, which regulates E-cadherin expression, and the ectopic expression of CCDC80 suppresses migration and expression of EMT markers in pancreatic cancer cells. Based on the promising results of antitumor effects in various preclinical models, Vactosertib has been tested first in a human phase I study in

patients with advanced solid tumors (NCT02160106) [72,73]. Vactosertib is currently evaluated in phase I/II clinical trials for various cancer types combined with conventional chemotherapeutic agents. Two phase I clinical trials are currently evaluating the safety and efficacy of Vactosertib in combination with Paclitaxel in patients with metastatic gastric cancer (NCT03698825) and in combination with Pomalidomide, a thalidomide analog in patients with relapsed and refractory multiple myeloma (NCT03143985). Vactosertib combined with Imatinib is currently investigated in clinical trials for patients with advanced desmoid tumor (NCT03802084).

Combination therapy of immune checkpoint and TGF-β signaling pathway inhibition

Cancer immunotherapy is currently more focused on targeting immune inhibitory checkpoints, such as programmed death-1 (PD-1) or its main ligand PD-L1, or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) [74]. Several mAbs that block the immune checkpoint pathway are currently approved in specific clinical indications for immunotherapy. Although a subset of patients achieves durable clinical responses after immunotherapy, the majority of patients show little response or resistance. As described above, the aberrant TGF-β signaling contributes to tumor immune escape and immune checkpoint inhibitor resistance. Therefore, concurrent inhibition of TGF-β and immune checkpoint pathway might be a promising strategy for increasing therapeutic efficacy.

One approach targeting TGF-β signaling is to block the release of active TGF-β from the latent complex or the TGF-β ligand activation. Glycoprotein A repetitions predominant (GARP) is a transmembrane protein that serves as the cell surface docking receptor for latent TGF-β [75]. GARP is abundantly expressed on the surface of activated Tregs and platelets and plays an important role in the bioavailability and activation of TGF-β [76]. ABBV-151 is a mAb that binds to the GARP-TGF-β complex and blocks the release of TGF-β. Preclinical data have shown that targeting both GARP-TGF-β and PD-1 improves antitumor effects compared with anti-PD-1 alone. ABBV-151 was evaluated to determine the safety, efficacy, pharmacokinetics, and pharmacodynamics in patients with locally advanced or metastatic solid tumors as a single agent or combined with an anti-PD-1 mAb (NCT03821935). SRK-181 is a humanized mAb that selectively binds to latent TGF-β1 and inhibits its activation [77]. The combined treatment of SRK-181 with an anti-PD-1 antibody demonstrated significant antitumor response and survival benefit in bladder tumor-bearing mice by increasing intratumoral CD8^{+} T cells and decreasing immunosuppressive myeloid cells. SRK-181 is currently undergoing clinical trials in patients with locally advanced or metastatic solid tumors as an independent agent and combination with anti-PD-L1 antibody (NCT04291079).

Bintrafusp alfa (M7824) is a novel first-in-class bifunctional fusion protein composed of the extracellular domain of TGF-β

RII that functions as a TGF-β sequestering or trap fused to a human IgG1 mAb against PD-L1 [78]. Bintrafusp alfa not only inhibits PD-L1 signaling but also blocks the TGF-β-mediated immune evasion in the tumor microenvironment. Preclinical data have demonstrated that Bintrafusp alfa induces immune cell infiltration and antibody-dependent cell-mediated cytotoxicity for a wide range of human carcinoma cells, including those of lung, urothelial bladder, and breast origin [79-81]. According to this study, TGF-β reduces NK cell activation and NK cell-mediated cytotoxicity of tumor cells. The immunosuppressive activities of TGF-β were reduced by Bintrafusp alfa but not by anti-PD-L1 [78]. Bintrafusp alfa prolonged survival of mice bearing EMT-6 breast tumors and suppressed spontaneous lung metastasis more efficiently than did treatment with either anti-PD-L1 or TGF-β trap alone [79]. Bintrafusp alfa treatment reduced expression of α-smooth muscle actin (α-SMA), a maker of CAFs, which serve as a significant tumor microenvironment component and contribute to drug resistance. Bintrafusp alfa also promoted the antitumor immune activity of tumor-infiltrating lymphocytes, CD8$^+$ T cells, and NK cells by enhancing their cytotoxic activity. Bintrafusp alfa has undergone phase I clinical trials in patients with various types of advanced solid tumors, including NSCLC, biliary tract cancer, and hepatocellular carcinoma [82-84]. Results from these clinical trials showed that the treatment with Bintrafusp alfa had a tolerable safety profile and preliminary encouraging evidence for antitumor activity in patients with advanced NSCLC and hepatocellular carcinoma. Bintrafusp alfa is currently investigated in multiple phase II clinical trials in patients with advanced solid tumors including colorectal, head and neck squamous cell, cervical, triple-negative breast cancer, and NSCLC as a single therapy and also in combination with other standard chemotherapeutic agents. Interestingly, a phase III clinical study has been undertaken to evaluate the efficacy and safety of Bintrafusp alfa compared with Pembrolizumab, an anti-PD-1 mAb, as the first-line treatment for patients with advanced NSCLC that has high PD-L1 expression (NCT03631706).

Ravi et al. [85] reported a similar bifunctional antibody-ligand trap comprising an antibody targeting CTLA-4 or PD-L1 fused to an ectodomain of TGF-β RII. This fusion antibody inhibits tumor growth more efficiently than either anti-CTLA-4 or anti-PD-1 alone and the combination of anti-CTLA-4 and anti-PD-1 in human melanoma and triple-negative breast cancer models, respectively. The anti-CTLA-4-TGF-β RII fusion protein significantly reduced and counteracted tumor-infiltrating Tregs and activating tumor-reactive IFN-γ-expressing CD8$^+$ T cells and memory cells.

Besides mAbs and TGF-β traps, the small molecule inhibitors of TGF-β receptor kinase are also currently tested in clinical trials combined with immune checkpoint inhibitors. The phase I/II study of Galunisertib combined with Nivolumab, an anti-PD-1 antibody, was investigated in patients with refractory NSCLC and hepatocellular carcinoma (NCT02423343),

and with Durvalumab, an anti-PD-L1 antibody with metastatic pancreatic cancer (NCT02734160). A phase Ib/IIa trial of the combination of Vactosertib with Durvalumab, an anti-PD-L1 antibody, is being conducted in patients with advanced NSCLC (NCT03732274). A phase II clinical trial of Vactosertib combined with Pembrolizumab to treat PD-L1 positive NSCLC (NCT04515979) and a phase I trial of Vactosertib combined with Durvalumab to treat advanced urothelial carcinoma (NCT04064190) are scheduled to begin shortly to evaluate their therapeutic benefits.

CONCLUSION

TGF-β is a pleiotropic cytokine that can display either tumor-suppressive or tumor-promoting effects in cancer, depending on the stages of tumor and cellular context. Cancer cells also frequently exhibit aberrant regulation of TGF-β signaling either by upregulated expression of TGF-β or by activation of downstream pathways mediated by crosstalk with other signaling pathways. Increasing evidence demonstrates that the TGF-β pathway primarily contributes to generating a favorable microenvironment for tumor growth, angiogenesis, metastasis, and evasion from immune surveillance throughout various tumor progression steps. Therefore, targeting the tumor microenvironment, including immune checkpoint molecules, has led to a paradigm shift in cancer therapy. Although cancer immunotherapies such as immune checkpoint inhibition have shown remarkable promise in the management of various types of malignancies, their efficacy is still limited by various immune-resistant mechanisms. Recent clinical studies of Bintrafusp alfa and small molecule inhibitor of ALK5 combined with an immune checkpoint inhibitor have demonstrated enhanced antitumor efficacy in advanced solid tumors. These results suggest that TGF-β signaling inhibition overcome therapeutic resistance and ultimately enhances the antitumor immune responses. In conclusion, use of TGF-β signaling pathway inhibitors as a single treatment or combined with chemotherapeutic or immunotherapeutic agents is a promising cancer treatment strategy. The challenge going forward is to identify reliable biomarkers for predicting the therapeutic response and determining the patient selection and optimal timing of treatment.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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