Novel therapies emerging in oncology to target the TGF-β pathway

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Abstract

The TGF-β signaling pathway governs key cellular processes under physiologic conditions and is deregulated in many pathologies, including cancer. TGF-β is a multifunctional cytokine that acts in a cell- and context-dependent manner as a tumor promoter or tumor suppressor. As a tumor promoter, the TGF-β pathway enhances cell proliferation, migratory invasion, metastatic spread within the tumor microenvironment and suppresses immunosurveillance. Collectively, the pleiotropic nature of TGF-β signaling contributes to drug resistance, tumor escape and undermines clinical response to therapy. Based upon a wealth of preclinical studies, the TGF-β pathway has been pharmacologically targeted using small molecule inhibitors, TGF-β-directed chimeric monoclonal antibodies, ligand traps, antisense oligonucleotides and vaccines that have been now evaluated in clinical trials. Here, we have assessed the safety and efficacy of TGF-β pathway antagonists from multiple drug classes that have been evaluated in completed and ongoing trials. We highlight Vactosertib, a highly potent small molecule TGF-β type 1 receptor kinase inhibitor that is well-tolerated with an acceptable safety profile that has shown efficacy against multiple types of cancer. The TGF-β ligand traps Bintrafusp alfa (a bifunctional conjugate that binds TGF-β and PD-L1), AVID200 (a computationally designed trap of TGF-β receptor ectodomains fused to an Fc domain) and Luspatercept (a recombinant fusion that links the activin receptor IIb to IgG) offer new ways to fight difficult-to-treat cancers. While TGF-β pathway antagonists are rapidly emerging as highly promising, safe and effective anticancer agents, significant challenges remain. Minimizing the unintentional inhibition of tumor-suppressing activity and inflammatory effects with the desired restraint on tumor-promoting activities has impeded the clinical development of TGF-β pathway antagonists. A better understanding of the mechanistic details of the TGF-β pathway should lead to more effective TGF-β antagonists and uncover biomarkers that better stratify patient selection, improve patient responses and further the clinical development of TGF-β antagonists.

Keywords: Immunosuppression, Ligand traps, Small molecule inhibitors, TGF-β receptor antagonists, Vactosertib

Background

The transforming growth factor-β (TGF-β) signaling pathway regulates a multitude of key processes including cellular growth, differentiation, apoptosis, motility, invasion, extracellular matrix production, angiogenesis, immune responses [1, 2]. Recent advances in understanding the molecular details of the TGF-β signaling cascade, and crosstalk with other pathways, have led to a more coherent roadmap of the programs governed by TGF-β [3–5]. However, difficulty in defining the effect of TGF-β stems from the context-dependent nature of the cytokines effect on different cell types. Cell fate decisions are not determined simply by the available nutrients in the surrounding environment but controlled by a network of communication signals.

TGF-β1, 2 and 3 are evolutionarily conserved, secreted, isoforms that are encoded as large protein precursors with 70–80% homology that govern fundamental aspects
of cellular behavior [6, 7]. TGF-β is synthesized in a latent form that must be activated to allow for engagement of a tetrameric receptor complex composed of TGF-β receptors I and II (TGF-βRI, TGF-βRII). Activated TGF-β complexes, with additional factors, form a serine/threonine kinase complex that binds to TGF-β receptors composed of both type I and type II receptor subunits. After the binding of TGF-β, the type 2 receptor kinase (TGF-βRII) promotes heterotetramerization and phosphorylation that activates the type 1 receptor kinase (TGF-β RI), followed by phosphorylation of Smad2/Smad3 triggering a signaling cascade.

Activation of the TGF-β signaling pathway can elicit either tumor-suppressing or tumor-promoting effects in a cell- and context-dependent manner (Fig. 1). In normal tissues, these effects maintain homeostasis and prevent the early stages of tumor formation [8]. In healthy cells, TGF-β halts the cell cycle at G1 to reduce proliferation, induce differentiation and may promote apoptosis [4, 9, 10]. Activated Smad proteins associate with Smad4 and translocate to the nucleus where they recruit additional transcription factors, DNA-binding transcription factors, co-repressors, co-activators and chromatin remodeling factors. Differential expression of these factors may be responsible for the cell-type and context-dependent response to TGF-β. Nuclear localized SMAD2/3/4 complex induces tumor-promoting effects through cell proliferation (PDGF-β), immune suppression (Foxy3), EMT activation (SNAIL/SLUG, ZEB1/ZEB2, HMGA2), EMT suppression (E-Cadherin, Cytokeratin) and metastasis (HDM2, MMP-9). Tumor-suppressing effects inhibit cell proliferation (p15, p21, p57, 4E-BP1), apoptosis (Bim, DAPK, GADD45β), and autophagy (ATG5, ATG7, Beclin-1), suppress inflammation (Foxy3) and block angiogenesis (Thrombospondin).

In cancer cells, the TGF-β signaling pathway is deregulated or mutated, and TGF-β no longer controls proliferation. As a result of mutations or epigenetic modifications introduced during cancer progression, tumors can become resistant to the suppressive effects with Smad4 and translocate to the nucleus where they recruit additional transcription factors, DNA-binding transcription factors, co-repressors, co-activators and chromatin remodeling factors. Differential expression of these factors may be responsible for the cell-type and context-dependent response to TGF-β. Nuclear localized SMAD2/3/4 complex induces tumor-promoting effects through cell proliferation (PDGF-β), immune suppression (Foxy3), EMT activation (SNAIL/SLUG, ZEB1/ZEB2, HMGA2), EMT suppression (E-Cadherin, Cytokeratin) and metastasis (HDM2, MMP-9). Tumor-suppressing effects inhibit cell proliferation (p15, p21, p57, 4E-BP1), apoptosis (Bim, DAPK, GADD45β), and autophagy (ATG5, ATG7, Beclin-1), suppress inflammation (Foxy3) and block angiogenesis (Thrombospondin).

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of TGF-β signaling [11]. TGF-β1 signaling is hyperactivated in breast cancer, which drives cancer progression and metastasis [12]. Novel regulators of SMAD7, including OTU domain-containing protein 1 (OTUD1) and ubiquitin-specific protease 26 (USP26), attenuate TGF-β1-mediated aggressive phenotypes in breast cancer. SMAD7 is an antagonist of the TGF-β1 signaling pathway forming stable complexes with TGF-βRI to inhibit R-SMAD phosphorylation and downstream signaling. In colon cancer, SMAD7 deletion predisposes to a favorable prognosis compared to patients with two copies of the gene, whereas patients with SMAD7 gene amplification exhibit significantly worse outcomes [13]. TGF-β1 and BMP-7 (bone morphogenetic protein-7), major members of the TGF-β family of receptor ligands, activate downstream signaling pathways and their respective target genes to counter-regulate biological outcomes. BMP7 deficiency accelerates tumorigenesis and negatively correlates with patient survival in prostate cancer [14]. Human prostatic carcinomas generally exhibit significantly lower levels of BMP7 transcripts compared to the normal-appearing gland; daily injections of recombinant BMP-7 protein reduced tumor burden and metastasis in mice transplanted with prostate cancer cell lines directly into the prostate or bone. Loss of BMP7 in glioblastoma, breast and prostate cancers, predisposes to enhanced EMT and acquisition of invasive/metastatic traits suggesting a protective role for BMP7 in cancer progression. Understanding the mechanisms by which the tumor-suppressing or tumor-promoting effects of TGF-β signaling can be regulated may have therapeutic potential for inhibiting the progression of several different types of human cancer.

In the tumor microenvironment (TME), TGF-β contributes to favorable tumor growth, invasion and metastatic spread. Advanced tumors produce excessive amounts of TGF-β which, in normal epithelial cells, is a potent growth inhibitor. However, in oncogenically activated cells, the homeostatic action of TGF-β is diverted along alternate pathways and elicits protective or tumor-suppressive effects during the early growth-sensitive stages of tumorigenesis. At late stages of malignancy, tumor progression is driven by TGF-β overload. The TME is a target of TGF-β action that stimulates tumor progression through pro-tumorigenic effects on tumor cells as well as vascular, immune and fibroblastic cells [4, 9, 10].

Epithelial to mesenchymal transition (EMT) is a multi-step, plastic and reversible process during which cells lose epithelial characteristics and gain properties of mesenchymal cells. The transition in cellular phenotype from EMT plays a critical role in tumorigenesis, tumor invasion and metastasis [15, 16], unless the mesenchymal phenotype becomes fixed by subsequent epigenetic changes or additional genetic mutations. The plasticity and reversibility of EMT in response to changing local TGF-β levels have clearly demonstrated in vitro and in vivo. TGF-β-induced EMT is an attractive druggable target since it is thought to drive a more stem cell-like phenotype which is critical for tumor progression, dissemination, homing and colony-initiating activity. Inhibiting TGF-β may reduce the stem cell-like tumor compartment which displays chemotherapeutic resistance.

TGF-β effect on escape from immune surveillance
TGF-β modulates the level and functional activity of numerous immune cells to exert systemic anti-tumor immune suppression and inhibit host immunosurveillance [17–19]. In addition to direct effects on tumor cells, TGF-β regulates infiltration of inflammatory, immune cells and cancer-associated fibroblasts in the TME [20–25]. Neutralizing TGF-β enhances CD8+ T-cell- and NK-cell-mediated antitumor immune responses and increases neutrophil-attracting chemokines resulting in recruitment and activation of neutrophils with an antitumor phenotype. TGF-β also inhibits naïve T cell differentiation into the Th1 phenotype that is the most prominent and best-characterized T cell response against cancer. TGF-β further inhibits T cell proliferation and effector function by silencing expression of IL-2, the cytokine that elicits subsequent CD4+ T cell proliferation. TGF-β signaling directly inhibits the cytotoxic program of CD8+ T cells through mechanisms which stimulate SMADs and transcription factor ATF1 to repress granzyme B and IFN-γ, involved the lytic function of CD8+ T cells [25].

Regulatory T cells (Tregs) suppress the activity of effector T cells to maintain immune homeostasis, and their number is increased in the TME. Tregs facilitate immune evasion and are stimulated to expand and differentiate by TGF-β. TGF-β in combination with IL-2 enforces a suppressor phenotype in ex vivo naïve CD4+ T cells by triggering expression of FOXP3, the master transcription factor of the Treg program [26, 27]. Treg functionality is mediated by TGF-β secretion, and Foxp3 upregulation by TGF-β is mediated by both Smad2 and Smad3 [28]. An environment rich in pro-inflammatory cytokines counteracts TGF-β-driven induction of Tregs as it favors differentiation of CD4+ T cells toward an effector phenotype [29–32].

TGF-β signaling suppresses the generation and function of NK cells by silencing IFN-γ and Th1 transcription factor T-bet expression in NK cells, thus inhibiting Th1 responses [33–37]. Pro-inflammatory signals counteract this mechanism by decreasing TGF-β II levels and
TGF-β signaling inhibits the expression of NKG2D and NKp30, two surface receptors of NK cells that mediate the recognition of stressed and malignant transformed cells [36, 37]. Dendritic cells (DCs) are highly potent antigen-presenting cells and play a key role in tumor immunity and in the regulation of Th1 and Treg-mediated immune responses [38–42]. TGF-β inhibits the antigen presentation capability of DCs in vitro by suppressing MHCII gene expression. Cancer cells direct DCs to secrete TGF-β, which in turn induces conversion of naïve CD4⁺ T cells into Tregs. The TME also polarizes macrophages toward a M2 phenotype with anti-inflammatory, immunosuppressive and pro-angiogenic functions [43–47]. Tumor-associated macrophages (TAMs) produce TGF-β and subsets of macrophages that can mobilize active TGF-β through the activity of integrin αv β8 and MMP1. TGF-β acts as chemoattractant for monocytes to the sites of inflammation and upregulates adhesion molecules that enable monocyte attachment to the ECM. Monocytes differentiate into perivascular macrophages and facilitate tumor cell extravasation by promoting blood vessel leakiness. A TGF-β-rich TME may contribute to immune evasion by dampening the inflammatory functions of macrophages.

TGF-β1-mediated programming of nascent myeloid-derived suppressor cells (MDSCs) leads to a potent antitumor phenotype potentially suitable for adoptive immunotherapy [48, 49]. TGF-β is involved in controlling MDSC differentiation and immunoregulatory function in vivo, and MDSCs regulate T cell immunity. TGF-β increases expansion of the monocytic MDSC (Mo-MDSC) population, expression of immunosuppressive molecules by MDSCs and the ability of MDSCs to suppress CD4⁺ T cell proliferation [50].
TGF-β is a pleiotropic cytokine with a crucial function in mediating immune suppression and evasion of immunosurveillance in the TME. TGF-β produced by T cells has been shown to be an important factor for suppressing antitumor immune responses, but the precise role of tumor-derived TGF-β has been poorly understood. Knockdown of tumor-derived TGF-β using shRNA resulted in dramatically reduced tumor size, slowed tumor formation, prolonged survival of tumor-bearing mice, and inhibited metastatic growth [51]. Mechanistically, reducing the number of MDSCs and CD4+Foxp3+ Treg cells, enhanced IFN-γ production by CTLs. Knockdown of tumor-derived TGF-β also significantly reduced the conversion of naive CD4+ T cells into Treg cells in vitro. Knockdown of TGF-β also suppressed cell migration. TGF-β has also been found to be particularly important for the maintenance of low affinity CD4+ T cells [52]. In the absence of TGF-β, IL-7Rα expression positively correlated with TCR affinity, as TGF-βRII-deficient T cells bearing higher affinity TCRs expressed increased amounts of IL-7Rα and, thus, exhibited better homeostatic survival than their lower affinity counterparts.

Chimeric antigen receptor–modified T cell (CAR T cell) therapy has proven to be a promising approach against eliminating solid tumors, but the immunosuppressive TME remains a significant obstacle. Knocking out the endogenous TGF-β receptor II (TGFBRII) in CAR T cells with CRISPR/Cas9 technology was an approach used to reduce the induced Treg conversion and prevent CAR T exhaustion. TGFBRII-edited CAR T cells had better in vivo tumor elimination efficacy, both in cell line–derived xenograft and patient-derived xenograft solid tumor models, whether administered locally or systemically [53]. Knocking out endogenous TGFBRII greatly improved the in vivo function of CAR T cells in TGF-β–rich TME. The potency of CAR T cells directed to prostate-specific membrane antigen (PSMA) was also enhanced by co-expression of a dominant-negative TGF-βRII (dnTGF-βRII) [54]. Expression of the dnTGF-βRII in CAR T cells increased lymphocyte proliferation, enhanced cytokine secretion, promoted T cell resistance to exhaustion and increased tumor eradication in aggressive human prostate cancer models.

**TGF-β pathway biomarkers to predict cancer risk and therapeutic response**

**Blood, serum, and tissue markers**

Measurement of TGF-β pathway components in blood, serum, and tissue represents a rapid, accurate, and inexpensive approach to determine cancer risk, stratify patients into treatment populations, and predict therapeutic response. Early detection of tumors generally improves patient survival, and patients diagnosed at an advanced stage have poorer prognosis. Increased TGF-β levels within the primary tumor as well as high plasma levels of TGF-β correlate with poor prognosis in CRC patients [55, 56]. TGF-β expression is increased in pancreatic cancer and associated with poor prognosis [57, 58]. TGF-β is also highly expressed in HCC, and TGF-β1 levels are associated with disease progression and poor outcome [59]. While during the early phases TGF-β inhibits proliferation of premalignant hepatocytes, later it promotes stromal formation, EMT and invasion [60]. Elevated serum TGF concentration at diagnosis in MM patients may be a favorable predictor of response [61].

A nested case-controlled study using a large real-world population-based dataset exhibited that low serum TGF-β1 concentrations predict death from HCC, with a 160% increase in the risk per one SD decrease in the TGF-β1 concentrations [62]. This association remained statistically significant even after adjusting for confounding factors and in a sensitivity analysis restricted to participants who survived for at least five years after the baseline survey, effectively eliminating the possibility that these patients had undiagnosed HCC at the baseline survey. The data demonstrate that low levels of TGF-β1 can identify patients who are at higher risk of developing HCC. Furthermore, the predictive value was significantly higher among HCV-positive patients. TGF-β1 may be a serum predictor that becomes altered well before the development of clinically detectable malignancy. The findings are in agreement with the general theory that TGF-β1 is frequently present in the tumor microenvironment and prevents premalignant progression during the beginning phases of carcinogenesis.

**Gene expression and transcriptomics**

Signatures identified by gene expression and transcriptomic profiling may serve as diagnostic aids and improve predicting and treatment response compared to single biomarkers. The molecularly guided trials with specific treatment strategies in patients with advanced newly molecular defined subtypes of colorectal cancer (MoTriColor) consortium are performing three two-stage single-arm multi-center open-label phase II studies in molecular- and gene expression-selected mCRC populations. In one treatment cohort, patients with chemotherapy-resistant activated TGF-β signature-like tumors will have received 5FU or capecitabine in the first line of chemotherapy, usually combined with oxaliplatin. Newly identified biomarkers may be included to identify alternative combinations for patients susceptible of developing secondary resistance.
Immune markers

Checkpoint inhibitor-based immunotherapies that target cytotoxic T lymphocyte antigen 4 (CTLA4) or the programmed cell death 1 (PD1) pathway have achieved impressive success in the treatment of different cancer types, but only a subset of patients derive clinical benefit [63]. TGF-β signaling pathway participated in cancer immune escape and ICI resistance. Concurrent TGF-β blockade might be a feasible strategy to enhance the efficacy of immunotherapy and relieve immune checkpoint inhibitor resistance [64]. Selective inhibition of TGF-β1 activation overcomes primary resistance to checkpoint blockade therapy by altering tumor immune landscape [65]. Biomarkers superior to tumor mutational burden would greatly enhance patient selection for immune checkpoint inhibitor therapies.

Agents in clinical development to target the TGF-β pathway in oncology

Despite significant advancements in the diagnosis and treatment of certain cancer types, overall survival (OS) in many cancers has remained relatively constant. Poor initial response rates and the emergence of drug resistance remain significant challenges. Cancer investigators strive to develop more-effective treatments that have fewer side effects than do standard cancer therapies. A number of receptor kinase inhibitors, monoclonal antibodies (mAbs), ligand traps, antisense oligonucleotides and vaccines have been designed to target the TGF-β pathway and have been evaluated clinically (Fig. 1). Many of these agents have been or are being evaluated in clinical trials to treat a number of different cancer types (Tables 1, 2).

Small molecule inhibitors of TGF-β receptor kinase activity

Several TGF-β R kinase inhibitors have been designed to bind the ATP-binding domain of TGF-β R kinase and inhibit ATP kinase activity and block the downstream signaling cascade [66, 67].

Vactosertib (TEW-7197, MedPacto) is an orally available inhibitor of the kinase activity of TGF-β RI/ALK-5 (IC50 = 12.9 nM) [68, 69]. Vactosertib also inhibits ALK-2 and ALK-4 with an IC50 value of 17.3 nM. In murine models, Vactosertib limits the growth and suppresses progression of many solid tumor types and as well as the plasma cell neoplasm MM. Mechanistically, Vactosertib acts pleiotropically on multiple cell types through tumor intrinsic and extrinsic mechanisms (Fig. 3). The safety and efficacy of Vactosertib against a number of different cancer types have been reported in a number of completed trials (Table 1).

A first-in-human phase I dose escalation study investigated the safety, tolerability and pharmacokinetics of Vactosertib in patients with advanced, refractory solid tumors (NCT02160106) [70, 71]. Vactosertib pharmacokinetics were dose proportional when administered once daily for five days. Based upon a relatively short half-life, it was postulated that Vactosertib should be administered twice or thrice daily to maintain a concentration above a minimum effective level over dosing interval. A two-compartment linear model with first-order absorption and absorption lag time adequately described the population pharmacokinetics [70].

An open-label, multi-center study assessed the safety, tolerability, pharmacokinetics and antitumor activity of Vactosertib in combination with Pembrolizumab (Keytruda, Merck) in patients with either metastatic colorectal or gastric cancer or gastroesophageal junction adenocarcinoma (NCT03724851, [72]). Study results indicated objective response rates (ORRs) of 16.7% and 33.3%, respectively, for colon cancer patients who failed entire body anticancer treatment based on RECIST and iRECIST (primary and secondary anticancer evaluation indices). Colon cancer patients who participated in clinical tests demonstrated a microsatellite stable genotype, which previously had a non-existent objective response rate with pembrolizumab alone. A decrease in the colon cancer tumor marker CEA value was observed in half of the patients that received Vactosertib and suggested that tumor was effectively reduced in patients as treatment continued.

Combined inhibition of immune checkpoint and TGF-β signaling is a promising therapeutic strategy since these key pathways have independent but complementary immunosuppressive functions. Safety and efficacy of Vactosertib combined with Durvalumab (Imfinzi, Medimmune, AstraZeneca), a human mAb directed against programmed death-ligand 1 (PD-L1), was evaluated in patients with metastatic non-small cell lung cancer (NSCLC) and urothelial carcinoma (NCT03732274, [73]). The trial was conducted on 15 NSCLC patients and a 16.7% ORR was observed, even though patients demonstrated <25% of PD-L1 expression in tumors. Importantly, imfinzi monotherapy demonstrated an ORR of 2.8% clinical test conducted on the same patient group. A phase II, open-level, non-randomized single-arm study will determine whether the administration of Vactosertib with Durvalumab increased the overall response rate in patients with urothelial cancers that fail to achieve a CR with anti-PD-1/PD-L1-based regimens (NCT04064190). The combination of Vactosertib plus Durvalumab is well-tolerated and without safety concerns.

A phase II study will determine the recommended dose and evaluate the safety of Vactosertib in combination with nanoliposomal irinotecan (Nal-IRI) with 5-FU and leucovorin (FL) in patients with metastatic pancreatic
| Study NCT Registry Number | Agent | Target(s) | Study Population | Number of Patients | Phase | Clinical Efficacy | Most Frequent Adverse Events |
|---------------------------|-------|-----------|------------------|--------------------|-------|------------------|-----------------------------|
| **Small molecule receptor kinase inhibitors** | | | | | | | |
| NCT02160106 (TEW-7197) | Vactosertib | TGF-β RI | Solid tumors | 29 | I | Pharmacokinetics | |
| NCT01682187 | Galunisertib (LY2157299) | TGF-β RI | Advanced solid tumors | 65 | I | Glioma population ORR 14% | Thrombocytopenia, thrombosis, dyspnea |
| NCT01582269 | Galunisertib ± Lomustine | TGF-β RI | Refractory glioma | 180 | II | mOS: Galunisertib 8 m, Lomustine 7 m, Both 6.5 m | Fatigue, nausea, vomiting |
| NCT01220271 | Temozolomide RT ± Galunisertib | CT/RT TGF‑β RI | GBM | 75 | I/II | mOS 18.2 vs. 17.9 m | Fatigue, constipation |
| NCT01373164 | Galunisertib ± Gemcitabine | TGF‑β RI Chemotherapy | Inoperable or metastatic pancreatic | 170 | I/II | mOS 8.9 vs. 7.1 m | Neutropenia, thrombocytopenia |
| NCT02154646 | Galunisertib + Gemcitabine | TGF‑β RI Chemo-therapy | Inoperable or metastatic pancreatic cancer | 9 | I | ORR 0% | Elevated liver enzymes |
| NCT02734160 | Galunisertib + Durvalumab | TGF‑β RI PD-L1 | Metastatic pancreatic cancer | 32 | I | ORR 3% mPFS 1.9 | Elevated liver enzymes neutropenia |
| NCT02240433 | Galunisertib + Sorafenib | TGF‑β RI TKI, Angiogenesis | Metastatic HCC | 14 | I | ORR 9% | Hypophosphatemia Hand-foot syndrome |
| NCT01246986 | Galunisertib | TGF‑β RI | Metastatic HCC | 147 | II | mPFS: 2.7 m (part A) 4.2 m (part B) | Neutropenia |
| **Antibodies** | | | | | | | |
| NCT00356460 | Fresolimumab | TGF-β1, β2, β3 | Advanced melanoma Renal cell cancer | 29 | I | ORR 3 and 5% mPFS 2.75 m | Keratoacanthomas hyperkeratosis |
| NCT01472731 | Fresolimumab RT | TGF-β1, β2, β3 | Glioma | 23 | II | ORR 0% | Fatigue, anemia keratoacanthomas |
| NCT01646203 | LY3022859 | TGF‑β RI | Advanced solid Tumors | 14 | I | Not reported | CRS |
| **Ligand Traps** | | | | | | | |
| NCT04296942 | Bintrafusp alfa, BN-Brachyury, Entinostat, Adotrastuzumab Emtansine | TGF-β RII and PD-L1 | Advanced Stage Breast cancer | 19 | I | ORR 21% | Bullous pemphigoid increased lipase, colitis |
| NCT03427411 | Bintrafusp alfa | TGF-β RII and PD-L1 | HPV-positive advanced solid tumors | 120 (estimated enrollment) | II | ORR 39% | Colitis, hypokalemia gastroparesis |
| NCT02517398 | Bintrafusp alfa | TGF-β RII and PD-L1 | Pre-treated cervical tumors | 25 | I | ORR 28% | Hypokalemia |
| NCT02517398 | Bintrafusp alfa | TGF-β RII and PD-L1 | Refractory head and neck cancer | 32 | I | ORR 22% | Keratoacanthomas hyperglycemia |
| NCT02517398 | Bintrafusp alfa | TGF-β RII and PD-L1 | Pre-treated NSCLC | 80 | II | PD-L1 > 1%, ORR 40% PD-L1 > 80%, ORR 71% | Keratoacanthomas |
| NCT02517398 | Bintrafusp alfa | TGF-β RII and PD-L1 | Pre-treated esophageal adenocarcinoma | 30 | I | ORR 20% | Anemia, pain |
| NCT02517398 | Bintrafusp alfa | TGF-β RII and PD-L1 | Pre-treated gastric cancer | 31 | I | ORR 22% | Anemia, diarrhea, rash |
ductal adenocarcinoma (PDAC) who have failed first-line gemcitabine (GEM) and nab-paclitaxel (NCT04258072). A phase I/II, open-label, non-randomized, multicenter study will evaluate the clinical activity of Vactosertib plus Imatinib in desmoid tumors (NCT03802084). Based upon impressive preclinical and translational studies, Vactosertib has been combined with the immunomodulatory agent Pomalidomide (Pom, Pomalyst, Bristol-Myers Squibb) in a phase Ib/IIa study to treat participants with relapsed and/or refractory MM (NCT03143985) [74, 75]. Results indicated that the combination was safe and that progression-free survival (PFS) was 80%, higher than historical controls seen with Pom alone (PFS = 20%) or that seen with Pom and corticosteroids (PFS = 40%).

A dose escalation, proof-of-concept study of Vactosertib monotherapy in patients with myelodysplastic syndromes (MDS) has also been reported (NCT03074006). A prospective, open-label, multicenter, phase I/II study of Vactosertib in patients with low and intermediate risk of MDS evaluated hematological improvement, maximum tolerated dose, bone marrow response and quality of life. Preclinical and clinical results indicated that TGF-β1 levels were increased in a subset of MDS samples and Vactosertib treatment led to reversal of hematopoietic alteration upon exposure to MDS serum in vitro and increased blood counts in vivo [76, 77].

A two-tiered, single-arm, phase II trial of Vactosertib for anemic myeloproliferative neoplasm (MPN) patients was recently designed (NCT04103645) and will assess how well Vactosertib works in MPN patients to improve anemia and provide new information about how this agent blocks TGF-β signaling in MPN cells. In turn, the study will determine whether Vactosertib can block MPN cells and their effects on bone marrow, e.g., fibrosis.
Table 2  Ongoing clinical trials to evaluate TGF-β pathway antagonists in oncology

| Study NCT Registry Number | Agent Target(s) | Study Population | Number of Patients | Phase | Clinical status |
|---------------------------|----------------|------------------|--------------------|-------|-----------------|
| **Small molecule receptor kinase inhibitors** | | | | | |
| NCT03724851 Vactosertib + Pembrolizumab | TGF-β RI | Metastatic CRC, gastric, or GEJC adenocarcinoma | 67 | Ib/IIa | Active, recruiting |
| NCT03732274 Vactosertib + Durvalumab | TGF-β RI PD-L1 | Advanced NSCLC | 63 | Ib/IIa | Active, recruiting |
| NCT04064190 Vactosertib + Durvalumab | TGF-β RI PD-L1 | Urothelial cancer | 48 | II | Active, recruiting |
| NCT03143985 Vactosertib + Pomalidomide | TGF-β RI IMiD Agent (Cereblon) | Relapsed/Refractory Multiple myeloma | 18 | Ib/IIa | Active, recruiting |
| NCT03698825 Vactosertib + Paclitaxel | TGF-β RI Tubulin/ Mitotic spindle | Second line therapy for metastatic gastric AC | 62 | Ib/IIa | Active, recruiting |
| NCT04103645 Vactosertib | TGF-β RI | Anemic, Ph-neg, MPN | 37 | II | Active, not recruiting |
| NCT04258072 Vactosertib nal-IRI/FL | TGF-β RI Chemotherapy | Metastatic pancreatic Adenocarcinoma | 24 | Ib/IIa | Active, not recruiting |
| NCT03802084 Vactosertib Imatinib | TGF-β RI BCR-abl | Advanced desmoid tumor | 24 | Ib/IIa | Active recruiting |
| NCT02452008 Galunisertib + Enzalutamide | TGF-β RI AR | Castration-resistant prostate cancer | 60 | II | Active, recruiting |
| NCT03206177 Galunisertib + Carboplatin/Paclitaxel | TGF-β RI Chemotherapy | Ovarian carcinoma | 25 | I | Active, recruiting |
| NCT02688712 Galunisertib + Chemo/RT | TGF-β RI | Locally advanced rectal cancer | 37 | I | Active, recruiting |
| NCT04031872 LY3200882 Capecitabine | TGF-β RI Chemotherapy | Advanced Chemo-therapy Resistant Colorectal Cancer and an Activated TGF-beta Signature | 31 | I/II | Active, not recruiting |
| **Antibodies** | | | | | |
| NCT02581787 Fresolimumab + SBRT | TGF-β1, β2, β3 RT | Stage Ia/Ib NSCLC | 60 | I/II | Active, not recruiting |
| NCT03192345 SAR438459 + Cemiplimab | TGF-β1, β2, β3 PD-L1 | Advanced solid tumors | 350 | I | Active, not recruiting |
| NCT04291079 SRK-181 + Anti-PD-L1 | TGF-β1 PD-L1 | Locally advanced or metastatic solid tumors | 183 | I | First-in-human, dose-escalation, dose expansion study to evaluate safety, tolerability, PK, PD |
| **Ligand Traps** | | | | | |
| NCT04349280 Bintrafusp alfa | TGF-β RII and PD-L1 | Metastatic or Locally Advanced/Unresectable Urothelial Cancer With Disease Progression or Recurrence Following Treatment With a Platinum Agent | 40 | Ib | Active, recruiting |
| NCT04501094 Bintrafusp alfa | TGF-β RII and PD-L1 | Checkpoint inhibitor naïve urothelial carcinoma | 75 | II | Active, not recruiting |
| NCT04066491 Cisplatin/Gemcitabine +/- Bintrafusp alfa | Chemotherapy TGF-β RII and PD-L1 | First-line Treatment of Biliary Tract Cancer | 512 | II/III | Active, recruiting |
| NCT04220775 SBRT Bintrafusp alfa | RT TGF-β RII and PD-L1 | Recurrent or Second Primary Head and Neck Squamous Cell Cancer | 21 | I/II | Active, not recruiting |
| Study NCT Registry Number | Agent | Target(s) | Study Population | Number of Patients | Phase | Clinical status |
|---------------------------|-------|-----------|------------------|--------------------|-------|-----------------|
| NCT03833661 Bintrafusp alfa | TGF-β RII and PD-L1 | Locally Advanced or Metastatic Biliary Tract Cancer Who Fail or Are Intolerant to First-line Platinum-Based Chemotherapy | 159 | II | Active, not recruiting |
| NCT03524170 Bintrafusp alfa | TGF-β RII and PD-L1 | Metastatic Hormone Receptor Positive, HER2 Negative Breast Cancer | 20 | I | Active, recruiting |
| NCT04296942 Bintrafusp alfa | TGF-β RII and PD-L1 | Vaccines HER2 HDAC deacetylase | 65 | I | Active, recruiting |
| NCT03840915 Platinum regimen Bintrafusp alfa + and PD-L1 | Chemotherapy TGF-β RII | Stage IV Non-small Cell Lung Cancer | 70 | Ib/II | Active, not recruiting |
| NCT03840902 Chemo-RT Bintrafusp alfa + | Chemo-RT TGF-β RII | Unresectable Stage III Non-small Cell Lung Cancer. Bintrafusp alfa with concurrent chemoradiation followed by bintrafusp vs. concurrent chemoradiation + placebo followed by durvalumab | 350 | III | Active, recruiting |

**Vaccines**

| Study NCT Registry Number | Vaccine | Study Population | Number of Patients | Phase | Clinical status |
|---------------------------|---------|------------------|--------------------|-------|-----------------|
| NCT02346747 Vigil Vaccine | Stage IIIb, IIIc, IV high-grade papillary, serous, clear cell, serous ovarian cancer | 91 | II | Active, not recruiting |
| NCT03073525 Vigil Atezolizumab Vaccine PD-L1 | Stage IIb, IV ovarian cancer. Patients had tumor harvested at surgery and successful manufacturing of Vigil but were ineligible for CL-PTL-119 (the VITAL study) or previously randomized to placebo. | 25 | II | Active, not recruiting |
| NCT02725489 Vigil Durvalumab Vaccine PD-L1 | Advanced Women's cancers. Confirmed diagnosis of women's cancer, inclusive, but not limited to breast, ovarian, fallopian tube, primary peritoneal, uterine, cervical, endometrial, that is locally advanced or metastatic for which the projected response rate to durvalumab is 15% or less. | 13 | II | Active, not recruiting |
### Table 2 (continued)

| Study NCT Registry Number | Agent | Target(s) | Study Population                                                                 | Number of Patients | Phase | Clinical status           |
|---------------------------|-------|-----------|----------------------------------------------------------------------------------|--------------------|-------|--------------------------|
| NCT02511132               | Vigil | Vaccine   | Metastatic Ewing’s sarcoma refractory or intolerant to at least 1 prior line of systemic chemotherapy. | 22                 | IIb   | Active, not recruiting   |
|                           |       | vs. Gem/docetaxel + Temozolomide/Irinotecan | Chemotherapy                                                 |                    |       | (Part A)                 |
| NCT03842865               | Vigil | Vaccine   | Expanded Access Trial of Vigil (Bi-shRNAfurin and GMCSF Augmented Autologous Tumor Cell Immunotherapy) in Advanced Solid Tumors including Ewing’s sarcoma, Ewing’s tumor metastatic, Ewing’s sarcoma metastatic, advanced gynecological cancers, ovarian, cervical, and uterine cancers. | 40                 | Expanded Access | Temporarily not available |

Summary of ongoing clinical trials that are utilizing TGF-β targeting agents in solid tumor as well as hematological malignancies including MM and MPNs. Twelve trials utilized small molecule inhibitors, three utilized antibodies, nine utilized ligand traps, and five utilized vaccination strategies.

![Diagram](image_url)

**Fig. 3** Tumor Intrinsic and Extrinsic Effects of Vactosertib: Vactosertib exerts potent antitumor effects (indicated in red) directly on cancer cells as well as on a number of other cell types.
An open-labeled, multicenter phase I b/Ila, single-arm study was designed to evaluate the safety and tolerability of Vactosertib in combination with weekly paclitaxel as second-line treatment for metastatic gastric adenocarcinoma patients (NCT03698825). A report is that paclitaxel (Taxol; Bristol-Myers Squibb Co.) significantly suppressed the TGF-β /Smad signaling pathway by inhibiting Smad2 phosphorylation in the peritoneum [78]. A multi-center, open-label, phase Ib clinical was designed to evaluate the safety, tolerability and exploratory efficacy of Vactosertib in combination with FOLFOX in patients with PDAC who failed first-line GEM and nab-Paclitaxel (NCT03666832).

**Galunisertib** (LY21557299, Eli Lilly & Co.) is a TGF-βRI kinase inhibitor that has been shown to reduce the growth of lung and breast cell lines and was safe in patients with various solid tumors in phase I studies [79–81]. In a phase II study of galunisertib in combination with lomustine, compared to lomustine with placebo, in patients with recurrent glioma failed to demonstrate improved OS relative to placebo plus lomustine (NCT01582269). In a separate phase II study, 40 patients with hepatocellular carcinoma (HCC) that had progressed on or were ineligible to receive sorafenib (Bayer, Onyx) were treated with intermittent dosing of galunisertib (14 days on/14 days off) [82]. HCC patients with normal α-fetoprotein and with TGF-β1 reduction improved in OS compared to patients with non-TGF-β1 reduction (NCT01246986). In a phase II study of patients with unresectable pancreatic cancer, patients treated with galunisertib in combination with GEM improved OS (10.9 vs. 7.2 months) compared to those treated with GEM and placebo (NCT01373164).

The incentive to move galunisertib forward was based largely on the development of an intermittent dosing schedule by predictive pharmacology, pharmacodynamic markers and preclinical toxicology models [83]. The optimal effective dosing schedule that can induce antitumor activity, but lacks cardiac toxicity, consists of iterative cycles (28 days/cycle) comprised of two-week treatment with galunisertib followed by two weeks without drug. The protocol was applied to patients with glioma, and serious cardiac toxicity was not observed [84]. In metastatic PDAC, targeting TGF-β signaling could be a rational approach since it is involved in tumor progression and has been associated with poor prognosis [85]. A randomized phase II study assigned 156 patients to receive galunisertib plus GEM or placebo plus GEM in stage II-IV unresectable PDAC. The combination of galunisertib and GEM resulted in improved OS and PFS and a manageable toxicity profile compared to that of placebo and GEM [85, 86].

**LY3200882** (Eli Lilly) is a next-generation, potent and highly selective ATP competitive inhibitor of the TGF-βRI serine-threonine kinase domain [87]. In a multicenter, non-randomized, open-label study, dose-escalation study of LY3200882 monotherapy, the recommended phase II dose was established at 50 mg bid at two weeks on/two weeks off for patients with advanced or metastatic cancers were treated with (NCT02937272) [88]. Five cohorts of patients with advanced or metastatic cancer following progression on standard therapy received LY3200882 twice daily at increasing doses. Tumor types included were glioma (n=15), GBM (n=5), pancreatic (n=3), cervical (n=3), chondrosarcoma (n=2), appendiceal (n=1) and colorectal adenocarcinoma (n=1). Findings showed that a twice-daily dose of 50 mg at two week-on/two week-off schedule was optimal. Moreover, dose-limiting toxicities were not observed, and all treatment-related adverse events were of grade 1 or 2. Across all cohorts, there was one partial response in the 50 mg cohort—six cases of stable disease, 11 with progressive disease and 12 with non-evaluable disease. The overall response rate (ORR) was 3.3% (n=1), and disease-control rate was 23.3% (n=7). Additionally, an 85.7% tumor reduction, assessed by response assessment in neuro-oncology criteria, was reported in one patient with EGFR-mutant, CDK4-amplified, IDH1/2 wild-type and MGMT-methylated GBM. The patient was treated at 50 mg bid and has remained on treatment for more than 11 months.

**LY573636** (Tasisulam, Eli Lilly) LY573636 is a TGF-βRI inhibitor that exhibited broad spectrum of preclinical antitumor activity [89]. LY573636 was evaluated in a phase I dose-escalation study in patients with advanced or refractory solid tumors. The primary objective was to determine the recommended phase II dose of tasisulam; secondary objectives were to characterize the toxicity and pharmacokinetic profiles of tasisulam and to discern whether there was any antitumor activity [89]. Twenty-six patients were enrolled. No dose-limiting toxicities (DLTs) were observed until cohort 3 (grade 3 hyperbilirubinemia). Interim PK analyses of this and another ongoing phase I study suggested that a lower dose after cycle 1 was necessary for doses ≥ 2,500 mg because of the long half-life of tasisulam (~14 days). When administered as a flat-dose, 24-h infusion, the MTD of drug was a loading dose of 2,500 mg followed by a chronic dose of 1,750 mg, every 28 days. Consistent with the profile of the two-hour infusion in clinical development, bone marrow suppression was the major DLT.

**A83-01** is a small molecule TGF-β RI/ALK-5 kinase inhibitor with an IC50 of 12 nM [90]. Aberrant hepatic growth factor (HGF)/c-MET upregulation and activation
is frequently observed in bladder cancer correlating with cancer progression and invasion. However, the mechanisms underlying HGF/c-MET-mediated invasion in bladder cancer remain poorly defined. As part of a negative feedback loop, SMAD7 binds SMURF2 targeting the TGF-β receptor for degradation. SMAD7 then acts as a SMURF2 agonist by disrupting interactions within SMURF2. Recently, Sim et al. showed that HGF stimulates TGF-β signaling through c-SRC-mediated phosphorylation of SMURF2 resulting in loss of SMAD7 binding and enhanced SMURF2 C2-HECT interaction that inhibits SMURF2 and enhances TGF-βR stabilization [91].

**LY2109761** (Eli Lilly) is an orally active, selective TGF-βRII inhibitor with Ki values of 38 nM and 300 nM, respectively [92]. It is metabolically stable and suitable for in vivo studies. The efficacy of LY2109761 on tumor growth, survival and reduction of metastasis was evaluated in an orthotopic murine model of pancreatic cancer. LY2109761, in combination with GEM, significantly reduced tumor burden, prolonged survival and reduced spontaneous abdominal metastases [93].

**LY364937** (Eli Lilly), **Ki26894** (Kirin Brewery Company), Treatment with the TGF-βRI/ALK-5 inhibitor LY364937 led to a reduction in formation of early bone and lung metastases in a MDA-MB-435-F-L orthotopic xenograft model into nude mice [94]. Ki26894 is another TGF-β type I receptor kinase inhibitor that has shown efficacy against breast and gastric cell lines and xenografts in a mouse cancer model [95, 96].

**LY580276** (Eli Lilly) has been shown to block EMT and tumor cell migration in pancreatic cancer and mouse mammary epithelial cells, respectively [97, 98].

**SB-431542 and SB-505124** (GlaxoSmithKline) are noteworthy TGF-β R kinase inhibitors in pre-clinical development. These TGF-β RI inhibitors block Smad2/3 phosphorylation. The expression of c-myc, which is downregulated by TGF-β in many cell types, was upregulated in MG63 human osteosarcoma cells, suggesting that upregulation of c-myc expression may be the mechanism of blocking growth suppressing signals in of TGF-β in MG63 cells [99]. Inhibitors for a TGF-β type I receptor kinase, SB-431542 and Ki26894, potently enhanced osteoblast differentiation from bone marrow stromal cells as well as MC3T3-E1 cells. The TGF-β inhibition was able to restore osteoblast differentiation suppressed by MM cell conditioned medium as well as bone marrow plasma from MM patients [100]. However, these inhibitors are not specific, are relatively unstable and generate unpredictable outcomes with unwanted, adverse side effects.

**SD-093 and SD-208** are also in pre-clinical development and exhibit antimitastatic effects against gliomas and melanomas [101, 102] and blocked EMT and tumor cell migration in pancreatic cancer [103].

**IN-1130** (In2Gen) that also inhibits TGF-βRI, ALK-4 and ALK-7 signaling decreased tumor growth of prostate cancer xenografts in mice and [104] and inhibited lung metastasis of breast cancer and increased overall survival (OS) of the mice [105].

**TGF-β-directed antibodies to block ligand activation or prevent ligand receptor binding**

Antibodies are used to interfere with TGF-β ligand binding to its cognate receptor as well as to block activation of latent TGF-β. Both steps are critical for TGF-β to elicit its pro-tumorigenic and immune suppressive responses.

**SRK181-mlgG1** (Scholar Rock). Cancer immuno-therapy, including immune checkpoint blockade, has achieved increased prominence in the recent years [65]. Despite breakthroughs achieved with cancer checkpoint therapy, only a fraction of patients respond to anti-PD-1 therapy because of primary or secondary (acquired) resistance. Studies have suggested that inhibition of TGF-β may help overcome resistance to immune checkpoint blockade (NCT04291079). Conventional TGF-β antagonists do not discriminate among the three isoforms of TGF-β. It was reasoned that a lack of isoform selectivity may underlie the toxicities observed in both preclinical and human studies. To achieve improved safety and a therapeutic window enabling higher dosing, antibodies were designed and aimed at selectively targeting the latent TGF-β1 complex, without meaningfully affecting other isoforms. Retrospective analyses of clinical tumor samples identified TGF-β1 as the most prevalent TGF-β isoform in solid cancers. Preclinical results demonstrated that the highly selective inhibition of TGF-β1 activation with SRK 181-mlG1 overcame primary resistance to checkpoint inhibitors. SRK181-mlG1 is a potent and highly selective inhibitor of the TGF-β1 isoform and is an investigational product candidate being developed to overcome primary resistance to checkpoint inhibitor therapy, such as anti-PD-(L)1 Abs. Results demonstrated the effectiveness and safety of the agent with immune checkpoint blockade in mouse cancer models.

**Fresolimumab** (GC1008, Genzyme) is a human mAb that neutralizes TGF-β1 and TGF-β2 that was tested in a phase I trial of 28 patients with malignant melanoma and one patient with RCC. Seven patients showed partial response, four developed reversible cutaneous keratoacanthomas and SCCs, and one patient acquired hyperkeratosis as unwanted side effects [106].

**LY3022859** (Eli Lilly), an anti-TGF-βRII mAb, inhibits receptor-mediated TGF-β signaling activation and was tested in a phase I trial composed of 14 patients with advanced solid tumors. The maximum dose tolerance
was not determined. Dose escalation beyond 25 mg dose was considered unsafe due to negative symptoms, e.g., uncontrolled cytokine release, despite prophylaxis. Unfortunately, a safe dose at which the drug was active was not determined [107].

264RAD (AstraZeneca) is a human mAb that targets αvβ6 integrin that has a key role in activation of latent TGF-β, has been shown to reduce tumor growth and metastasis in vivo [108, 109]. Targeting αvβ6 with 264RAD antibody alone or combined with trastuzumab (Herceptin, Genentech), a human epidermal growth factor receptor 2 (EGFR) neutralizing Ab, can benefit patients with high-risk, trastuzumab-resistant disease [110].

1D11 (Genzyme Corp., Sanofi) binds TGF-β1, 2 and 3, which suppresses lung metastasis in a breast cancer mouse model, by increasing in the antitumor response of CD8+ T cells [111].

2G7 (Genentech) is a second mAb that has shown efficacy in preclinical trials to inhibit breast cancer metastasis by increasing NK cell activity [112, 113].

TGF-β ligand traps

AVID200 (BMS) is an engineered TGF-β ligand trap comprised of TGF-βR ectodomains fused to a human Fc domain. AVID200 has antibody-like properties and is 1,000 times more selective for neutralizing TGF-β1 and TGF-β3, compared to TGF-β2 [114]. AVID200 does not target TGF-β2, which is a positive regulator of hematopoiesis and normal cardiac function, and blockade of TGF-β2 is undesirable. Since TGF-β1 and TGF-β3 are negative regulators of hematopoiesis, while TGF-β2 is a positive regulator of hematopoiesis, the unique isoform selectivity profile of AVID200 makes it an attractive agent for the treatment of MDS-associated anemia. The ability of AVID200 to selectively target TGF-β1 and TGF-β3 positions it to be an effective and well-tolerated therapeutics in oncology.

Bintrafusp alfa (GSK-4045154, M7824, MSB0011359C, EMD Serono/GlaxoSmithKline/ Merck KGaA) is an innovative first-in-class bifunctional fusion protein composed of a mAb against PD-L1 fused to the extracellular domain of the TGF-β receptor II [115]. In the 3+3 dose-escalation component of phase I study (NCT02517398), eligible patients with advanced solid tumors received Bintrafusp alfa once every 2 weeks until confirmed progression, unacceptable toxicity or trial withdrawal. Bintrafusp alfa demonstrated manageable safety profile in patients with heavily pre-treated advanced solid tumors. A phase 1, open-label trial included 80 patients with advanced NSCLC that progressed after platinum doublet therapy or platinum-based adjuvant or neoadjuvant treatment and those who also had not received previous immunotherapy. Patients were randomized at a one-to-one ratio to receive Bintrafusp alfa 500 mg or the recommended phase 2 dosage of 1200 mg every 2 weeks [116]. Bintrafusp alfa had encouraging efficacy and manageable tolerability in platinum-treated NSCLC patients. While pembrolizumab received accelerated FDA approval based upon ORR and duration of response (DOR) for patients with recurrent/metastatic cervical cancer with tumors that express PD-L1, the ORR of 14.3% suggests that there remains a significant unmet need for patients with previously treated advanced cervical cancer unselected for PD-L1. A multi-center, global single-arm phase II study (NCT04246489) will assess the clinical activity and safety profile of Bintrafusp alfa in platinum-experienced cervical cancer [117].

Luspatercept (Luspatercept-aamt, Acceleron) is a first-in-class erythroid maturation agent being developed to treat patients who have serious blood disorders associated with ineffective erythropoiesis. It is a recombinant fusion protein derived from human activin receptor type IIb (ActRIIib) linked to a protein derived from IgG. Luspatercept binds TGF-β ligands to reduce SMAD2 and SMAD3 signaling. Luspatercept was shown to reduce the severity of anemia in patients with lower-risk MDS with ring sideroblasts who had been receiving regular red-cell transfusions and who had disease that was refractory to erythropoiesis-stimulating agents or who had discontinued such agents [118].

Soluble TGFβRII (sTGF-βRII)-Fc and soluble betaglycan (sBetaglycan)-Fc were constructed as Fc fusion proteins, in which immunoglobulin fragment crystallizable (Fc) was fused to the extracellular domain of TGF-βRII and betaglycan, respectively [119]. In a manner similar to that of neutralizing antibodies, targeting TGF-β with sTGF-βRII-Fc and sBetaglycan-Fc also reduced tumor growth and metastasis in preclinical models. TβRII-Fc was shown to induce apoptosis in primary tumors and reduced tumor cell motility, invasion and lung metastases from transplanted 4T1 and EMT-6 mammary tumors in syngeneic mice [120].

ASOs targeting the TGF-β pathway

Antisense oligonucleotides (ASOs) offer a novel approach to specifically target and inactivate genes involved in cancer progression, especially those that are difficult to inhibit by small molecules or mAbs [121]. Due to their instability, nanoparticles and chemically modified ASOs have been developed to improve delivery and stability of oligonucleotides to target cells or tissues [122, 123].

AP12009 (Trabedersen, Antisense Pharma GmbH/Isarna) targets TGF-β2 expression and is being studied to treat malignant glioma, pancreatic carcinoma and...
malignant melanoma with an immunotherapy approach. After providing preclinical proof-of-concept results, the safety and efficacy of AP12009 were assessed in an open-label phase I/II dose-escalation study for recurrent and/or refractory high-grade glioma patients [124, 125]. Median survival time after recurrence exceeded the current literature data for chemotherapy. AP12009 has undergone or is currently used in phase III trials against astrocytoma (NCT00761280).

AP11014 and AP15012 are antisense molecules used in pre-clinical trials for treatment of non-small cell lung cancer, prostate carcinoma, CRC and MM, respectively [56, 126].

Vaccine-based approaches to modulate TGF-β signaling

**Vigil** (Vital, Gradalis) is composed of autologous tumor cells harvested from the patient at the time of initial de-bulking surgery which are then transfected extracorporeally, with a plasmid encoding the gene for the immune-stimulatory cytokine GM-CSF, and a bifunctional short hairpin RNA (shRNA) which specifically knocks down expression of furin, a critical convertase responsible for production of TGF-β1 and TGF-β2. GM-CSF enhances surface antigen expression, making cancer cells more visible to the patient's immune system and further stimulates the immune system by recruiting and maturing antigen-presenting cells, e.g., DCs. Vigil (Vital) is being investigated as a maintenance therapy in patients with high-risk stage 3b-4 ovarian cancer in a multicenter, randomized, double-blind, placebo-controlled, phase II/III trial (NCT02346747). The study involves more than 80 patients diagnosed with stage 3B, 3C or 4 high-grade papillary serous/clear cell/endometrioid ovarian, fallopian tube or primary peritoneal cancer. These patients were randomized to receive either vigil or placebo, and the primary goal is to determine patients’ recurrence-free survival (RFS) and overall survival (OS). Phase II of Vital in 42 cancer patients that demonstrated that Vigil is safe, leads to T-cell activation and improves RFS [127].

Another phase II crossover study (NCT03073525) is evaluating the safety and antitumor activity of vigil in women diagnosed with advanced gynecological cancers, e.g., ovarian, cervical and uterine. Vigil’s safety and antitumor activity, in combination with the immune checkpoint inhibitor atezolizumab (Tecentriq, Genentech), are being evaluated. Biomarkers are being used to measure antitumor systemic immune response generated either by Vigil alone, atezolizumab alone or the combination of the agents.

A pilot study (NCT02725489) is evaluating the safety, tolerability and efficacy of the combination of Vigil immunotherapy, and durvalumab (Imfinzi), a PD-L1 inhibitor, in women diagnosed with different types of advanced breast, ovarian, cervical, uterine, fallopian, primary peritoneal and endometrial cancer is recruiting patients. The main purpose of the study is to investigate the effects of the combination treatment may have on patients, and whether or not the combination helps in slowing down the progression of the disease. An open-label pilot study (NCT02574533) examined the combination of vigil and pembrolizumab in inducing cancer-specific T-cell immunity in patients with incurable locally advanced or metastatic melanoma. Pembrolizumab is a humanized mAb which activates T-cell-mediated immune responses against tumor cells. The safety of vigil immunotherapy in combination with other drugs is also being evaluated in phase Ib trial (NCT02511132) involving patients with metastatic Ewing’s sarcoma that could not be treated with at least one prior line of systemic chemotherapy. Finally, a multicenter, expanded access protocol of intra-dermal autologous vigil in solid tumor patients is ongoing (NCT03842865).

**Belagenpumatucel-L** (Lucanix, NovaRx Corp.) was prepared by transfecting allogeneic non-small cell lung cancer (NSCLC) cells with a plasmid containing a TGF-β2 antisense transgene [128]. Patients that received belagenpumatucel-L had a dose-related survival advantage (NCT00676507) [129]. Tumor cells promote immunosuppressive Treg cell proliferation directly through TGF-β production or by DC conversion into regulatory cells that secrete TGF-β. TGF-β2 antagonizes natural killer (NK) cells, lymphokine-activated killer cells, and DC function and belagenpumatucel-L reduced TGF-β2-mediated immunosuppression [130–134].

**Shortcomings of TGF-β antagonists**

The dual role of TGF-β in cancer highlights the need to better understand the contextual effects of this cytokine to better guide patient selection for the use of anti-TGF-β therapies in oncology. Furthermore, TGF-β is secreted by nearly every cell type, is therefore ubiquitous and regulates numerous critical physiologic processes in healthy cells. Limited mechanistic understanding of the dual, opposing actions of TGF-β as a tumor suppressor and tumor promoter remains a challenge in the development of TGF-β antagonists as cancer therapy. Combined with the pleiotropic activities of TGF-β and the lack of biomarkers, patient selection criteria and optimal dosing regimens are yet to be determined. However, many of these agents have limited activity when given as monotherapy, effective combinations with other anticancer strategies, e.g., immunotherapy, checkpoint inhibitors. Moreover, molecular biomarkers to stratify patient selection and treatment decisions are only beginning to emerge. Future clinical trials that incorporate bioinformatic tools and define biomarkers that identify patients
who would benefit from TGF-β therapy are needed to advance the incorporation of TGF-β receptor antagonists into frontline cancer treatment.

Conclusions
A number of TGF-β pathway antagonists have advanced to clinical trials and demonstrated acceptable safety profiles and significant therapeutic efficacy in cancer patients. Vadapt, in combination with chemotherapy and immunotherapeutics, has been well tolerated and shown promising activity against a number of cancer types. AVID200 was well tolerated with peripheral target engagement across the entire dosing period and led to TGF-β target modulation and immune activation. Bintrafusp alfa, the first-in-class bifunctional fusion protein TGF-β trap fused to a human immunoglobulin G1 antibody blocking PD-L1, had encouraging efficacy and manageable tolerability in patients with NSCLC previously treated with platinum. We conclude that TGF-β pathway antagonists, in combination with other modalities, are highly relevant since they provide a rapidly acting, cost-effective and feasible approach to improve cancer treatment. In addition, TGF-β pathway antagonists serve as a reasonable option for patients resistant to conventional therapies. While challenges remain, perspectives are enhanced by recently completed and ongoing clinical trials.

Abbreviations
ALK: activin receptor-like kinase; ASO: antisense oligonucleotides; BMP: bone morphogenic protein; CDK: cyclin-dependent kinase; CSC: cancer stem cells; CTLA-4: cytotoxic T lymphocyte antigen-4; DC: dendritic cell; DLT: dose-limiting toxicity; EMT: epithelial to mesenchymal transition; EGFR: epithelial growth factor receptor; FC: fragment crystallizable; GM-CSF: granulocyte macrophage colony-stimulating factor; GIC: glioma-initiating cells; NSCLC: non-small cell lung cancer; ORR: overall response rate; MDSC: myeloid-derived suppressor cells; MM: multiple myeloma; MDS: myelodysplastic syndrome; NK: natural killer; OS: overall survival; PDAC: pancreatic ductal adenocarcinoma; PFS: progression-free survival; PD-L1: programmed death ligand 1; RECIST: Response Evaluation Criteria in Solid Tumors; RFS: recurrence-free survival; Smad: Caenorhabditis elegans Sm genes and the Drosophila mothers against decapentaplegic proteins; TGF-β: transforming growth factor beta; Th: T helper; Tki: tyrosine kinase inhibitor; TME: tumor microenvironment.

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