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## Targeting TGF beta signal transduction for cancer therapy

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### Citation

Liu, S. J., Ren, J., & Dijke, P. ten. (2021). Targeting TGF beta signal transduction for cancer therapy. *Signal Transduction And Targeted Therapy*, 6(1). doi:10.1038/s41392-020-00436-9

Version: Publisher's Version

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Downloaded from: <https://hdl.handle.net/1887/3249673>

**Note:** To cite this publication please use the final published version (if applicable).



## REVIEW ARTICLE OPEN

Targeting TGF $\beta$  signal transduction for cancer therapySijia Liu<sup>1</sup>, Jiang Ren<sup>1</sup> and Peter ten Dijke<sup>1</sup>

Transforming growth factor- $\beta$  (TGF $\beta$ ) family members are structurally and functionally related cytokines that have diverse effects on the regulation of cell fate during embryonic development and in the maintenance of adult tissue homeostasis. Dysregulation of TGF $\beta$  family signaling can lead to a plethora of developmental disorders and diseases, including cancer, immune dysfunction, and fibrosis. In this review, we focus on TGF $\beta$ , a well-characterized family member that has a dichotomous role in cancer progression, acting in early stages as a tumor suppressor and in late stages as a tumor promoter. The functions of TGF $\beta$  are not limited to the regulation of proliferation, differentiation, apoptosis, epithelial–mesenchymal transition, and metastasis of cancer cells. Recent reports have related TGF $\beta$  to effects on cells that are present in the tumor microenvironment through the stimulation of extracellular matrix deposition, promotion of angiogenesis, and suppression of the anti-tumor immune reaction. The pro-oncogenic roles of TGF $\beta$  have attracted considerable attention because their intervention provides a therapeutic approach for cancer patients. However, the critical function of TGF $\beta$  in maintaining tissue homeostasis makes targeting TGF $\beta$  a challenge. Here, we review the pleiotropic functions of TGF $\beta$  in cancer initiation and progression, summarize the recent clinical advancements regarding TGF $\beta$  signaling interventions for cancer treatment, and discuss the remaining challenges and opportunities related to targeting this pathway. We provide a perspective on synergistic therapies that combine anti-TGF $\beta$  therapy with cytotoxic chemotherapy, targeted therapy, radiotherapy, or immunotherapy.

*Signal Transduction and Targeted Therapy* (2021)6:8; <https://doi.org/10.1038/s41392-020-00436-9>

## INTRODUCTION

Transforming growth factor- $\beta$  (TGF $\beta$ ) belongs to a family of multi-functional cytokines that includes TGF $\beta$ s, bone morphogenetic proteins, activins, nodal, growth and differentiation factors, inhibins, lefty, and anti-Müllerian hormone. Members of this family are key regulators of embryonic development, tissue homeostasis, and regeneration, and their malfunction has been implicated in cancer, fibrosis, immune diseases, and many other pathologies.<sup>1–4</sup> In this review, we focus on TGF $\beta$ , which is the prototypical member of the family. There are three highly structurally related mammalian TGF $\beta$  isoforms, that is, TGF $\beta$ 1, TGF $\beta$ 2, and TGF $\beta$ 3.<sup>5,6</sup> In vitro studies showed that all three isoforms elicit similar biological effects, with differences in potency in certain cell types.<sup>7</sup> Mouse studies in which each specific isoform was knocked out revealed strikingly different phenotypes, indicating nonredundant in vivo functions for these three TGF $\beta$  isoforms. TGF $\beta$ 1-deficient mice either die of vascular defects during embryogenesis or autoimmune disease postnatally.<sup>8,9</sup> Mice deficient in TGF $\beta$ 2 have defects in cardiac septation and valve remodeling,<sup>10</sup> and mice lacking TGF $\beta$ 3 have pulmonary defects and cleft palate.<sup>11,12</sup> TGF $\beta$ 1 is the most abundant and studied isoform, and it is particularly highly enriched in platelets and bone.<sup>13,14</sup>

TGF $\beta$  signaling has attracted the interest of cancer biologists because of its numerous roles in regulating cancer cell functions, including cell cycle progression, apoptosis, adhesion, and differentiation.<sup>3,15</sup> The biphasic functions of TGF $\beta$  during cancer progression on tumor cells and other cells in the tumor microenvironment (TME) are summarized in Fig. 1. In different

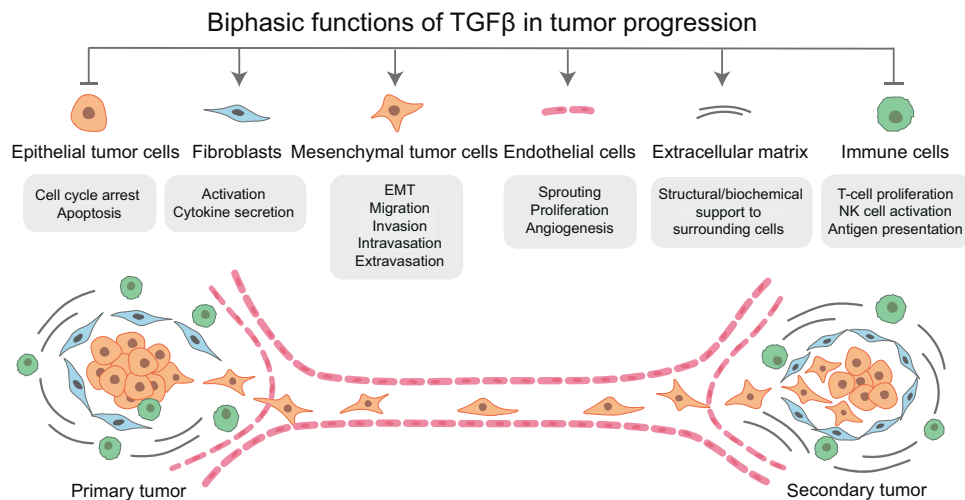
cell types and/or conditions, TGF $\beta$  can have different, even opposite, effects.<sup>16</sup> In normal and premalignant cells, TGF $\beta$  predominantly acts as a tumor suppressor by inhibiting cell proliferation, promoting apoptosis, and maintaining genome stability.<sup>17</sup> However, tumor cells can adapt to or selectively bypass the suppressive functions of TGF $\beta$ ; they utilize TGF $\beta$ 's promotional roles to obtain a growth advantage and undergo processes, such as the epithelial-to-mesenchymal transition (EMT), that enable their migration, invasion, intravasation, and extravasation.<sup>18,19</sup> Moreover, TGF $\beta$  can create a beneficial TME by acting in a paracrine manner to activate cancer-associated fibroblasts (CAFs), promote angiogenesis, produce extracellular matrix (ECM), and suppress anti-tumor immune reaction to trigger cancer progression and promote metastasis.<sup>3,4,20</sup>

Many preclinical results from in vitro cell models and in vivo animal models have shown the great potential of anti-tumor therapeutics with TGF $\beta$ -neutralizing antibodies and ligand traps that block the interaction of TGF $\beta$  with its receptors or selective small-molecule TGF $\beta$  receptor kinase inhibitors.<sup>21,22</sup> However, rendering these pharmacological agents suitable for approved clinical use continues to be a challenge. Because TGF $\beta$  shows highly pleiotropic actions, in addition to the tumor itself, healthy tissues can be affected by the systemic inhibition of TGF $\beta$ , which can lead to unwanted side effects and safety concerns.<sup>23</sup> Therefore, we need to better understand the underlying molecular mechanisms by which TGF $\beta$  signaling controls normal and malignant processes. Moreover, insight into stratifying tumor patients using biomarkers for selecting patients that may benefit from TGF $\beta$  targeting is urgently needed.<sup>24,25</sup> In this review, we

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Received: 23 October 2020 Revised: 4 December 2020 Accepted: 5 December 2020

Published online: 08 January 2021



**Fig. 1** Biphasic functions of TGF $\beta$  during tumor progression. TGF $\beta$  acts as a tumor suppressor in the initial stage of tumor progression by inducing cell cycle arrest and apoptosis of normal and pre-malignant epithelial cells. Upon activation of oncogenes and/or inactivation of tumor suppressor genes, tumor cells become insensitive to the TGF $\beta$ -induced cytostatic effects and undergo uncontrolled proliferation. TGF $\beta$  produced by tumor cells, fibroblasts, immune, and endothelial cells in the tumor microenvironment (TME) can trigger cancer cells to undergo an epithelial-to-mesenchymal transition (EMT). Thereby, late-stage cancer cells acquire the ability to escape from the primary niche, intravasate into the circulation, extravasate and localize to distant sites, and progress to form secondary tumors. Reciprocal TGF $\beta$  signaling between cancer cells and the TME contributes to cancer progression by activating cancer-associated fibroblasts (CAFs), stimulating angiogenesis, promoting protumor cytokine secretion, increasing extracellular matrix deposition, and evading an immune attack. In the metastatic sites, the mesenchymal tumor cells can undergo a mesenchymal-to-epithelial transition. Thus, thereby change back into an epithelial phenotype, which enables rapid outgrowth

discuss recent clinical advancements and bottlenecks in anti-TGF $\beta$  cancer treatment, and provide perspectives of combined treatment to overcome chemo/targeted/radio-therapy resistance and increase the efficiency of immunotherapy responses in cancer patients.

### BIOAVAILABILITY AND ACTIVATION OF TGF $\beta$ LIGANDS

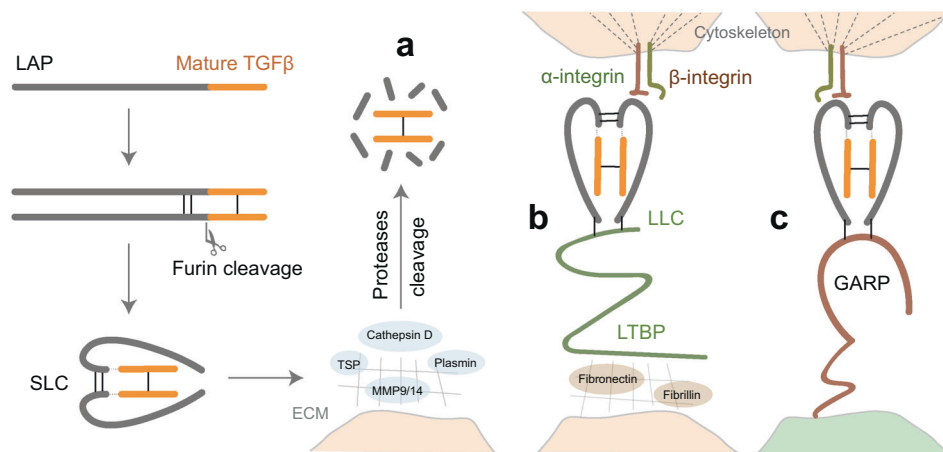
TGF $\beta$  is synthesized as a large precursor protein in the rough endoplasmic reticulum, it consists of a signal peptide, a large N-terminal pro-segment termed latency-associated peptide (LAP), and a short C-terminal mature peptide.<sup>26,27</sup> The pro-peptide assembles into a homodimer in which the LAP portions link via two disulfide bonds and the mature TGF $\beta$  moieties' interaction is stabilized through a disulfide bond.<sup>28</sup> After cleavage of the precursor protein by the convertase furin in the Golgi complex, the LAP portions encircle the mature portions to form the small latent complex (SLC) and shield the mature TGF $\beta$  from binding to its receptors (Fig. 2).<sup>29,30</sup>

For the activation of latent TGF $\beta$ , the mature TGF $\beta$  portions need to dissociate from the LAP portions. TGF $\beta$  can be activated in vitro upon heating or treating with a mild acid (pH 4.5) to dissociate the LAP portion from the mature protein.<sup>31,32</sup> In addition, reactive oxygen species can activate TGF $\beta$  in vitro by destroying the ability of LAP to bind to mature TGF $\beta$  via oxidative modification.<sup>33–35</sup> Several ECM serine proteases (plasmin/plasma kallikrein/cathepsin D) and matrix metalloprotease 9/14 (MMP9/14) can release active TGF $\beta$  via the proteolytic cleavage of LAP in vivo (Fig. 2a).<sup>3,31,36</sup> Thrombospondin (TSP) is another activator of latent TGF $\beta$  that is present in the ECM; upon direct binding to LAP, TSP can induce the release of active TGF $\beta$ .<sup>37</sup> In addition, the latent TGF $\beta$  binding protein (LTBP) can covalently bind to LAP, which facilitates the deposition of the SLC in the ECM and forms the large latent complex (LLC).<sup>38,39</sup> LLC can form a covalent interaction with specific ECM proteins such as fibrillin and fibronectin via the N-terminal domain of LTBP (Fig. 2b).<sup>39,40</sup> Moreover, the latent TGF $\beta$  can also interact with the transmembrane glycoprotein A repetition predominant protein (GARP),

which is expressed on the cell surface of regulatory T (T<sub>reg</sub>) cells, platelets, and endothelial cells to facilitate latent TGF $\beta$  activation (Fig. 2c).<sup>41</sup> Finally, integrins, which are cell adhesion receptors that control cell proliferation, survival migration, and invasion, also have a pivotal role in the activation of latent TGF $\beta$ .<sup>42,43</sup> Integrins comprise  $\alpha$  and  $\beta$  heterodimeric subunits that are both type I transmembrane receptors and are expressed in a wide range of cells.<sup>42</sup> Certain integrins have been demonstrated to interact with the Arg-Gly-Asp (RGD) motif of LAP and lead to the mechanical release of LAP by cellular contractions.<sup>44–46</sup>

### FUNCTIONS OF TGF $\beta$ SIGNALING PATHWAYS DURING TUMOR PROGRESSION

Regarding the canonical TGF $\beta$  pathway, active TGF $\beta$  initially binds to the low-affinity accessory TGF $\beta$  type III receptor (T $\beta$ RIII), also named betaglycan, which is abundant on the surface of many cell types (Fig. 3a). Betaglycan can present TGF $\beta$  to the TGF $\beta$  receptor complex, which is present at low levels but high affinity, thereby facilitating signaling.<sup>47</sup> The TGF $\beta$ 2 isoform is, in particular, dependent on T $\beta$ RIII for signaling. Consistent with this notion, endothelial cells that do not express T $\beta$ RIII show weak responsiveness to TGF $\beta$ 2 compared with that of TGF $\beta$ 1 and TGF $\beta$ 3.<sup>7</sup> The TGF $\beta$  receptors complex is a tetramer consisting of two paired transmembrane serine/threonine protein kinases: two T $\beta$ RIIs and two T $\beta$ RIIs.<sup>48–50</sup> TGF $\beta$  binding triggers T $\beta$ RII to transphosphorylate T $\beta$ RI at specific serine/threonine residues that are located in the intracellular juxtamembrane region enriched with glycine and serine residues (the GS domain).<sup>51,52</sup> After the extracellular signal is successfully transduced across the plasma membrane, activated T $\beta$ RI initiates intracellular signaling by phosphorylating SMAD2/3 at their two extreme C-terminal serine residues.<sup>53,54</sup> Thereafter, phosphorylated SMAD2/3 dissociates promptly from T $\beta$ RI and assemble into a heteromeric complex with SMAD4. Subsequently, the SMAD2/3–SMAD4 complex can translocate into the nucleus to activate or repress the expression of target genes.<sup>55,56</sup> SMAD7 is a target gene induced by TGF $\beta$  that encodes for a negative regulator of the TGF $\beta$ /SMAD signaling pathway by associating



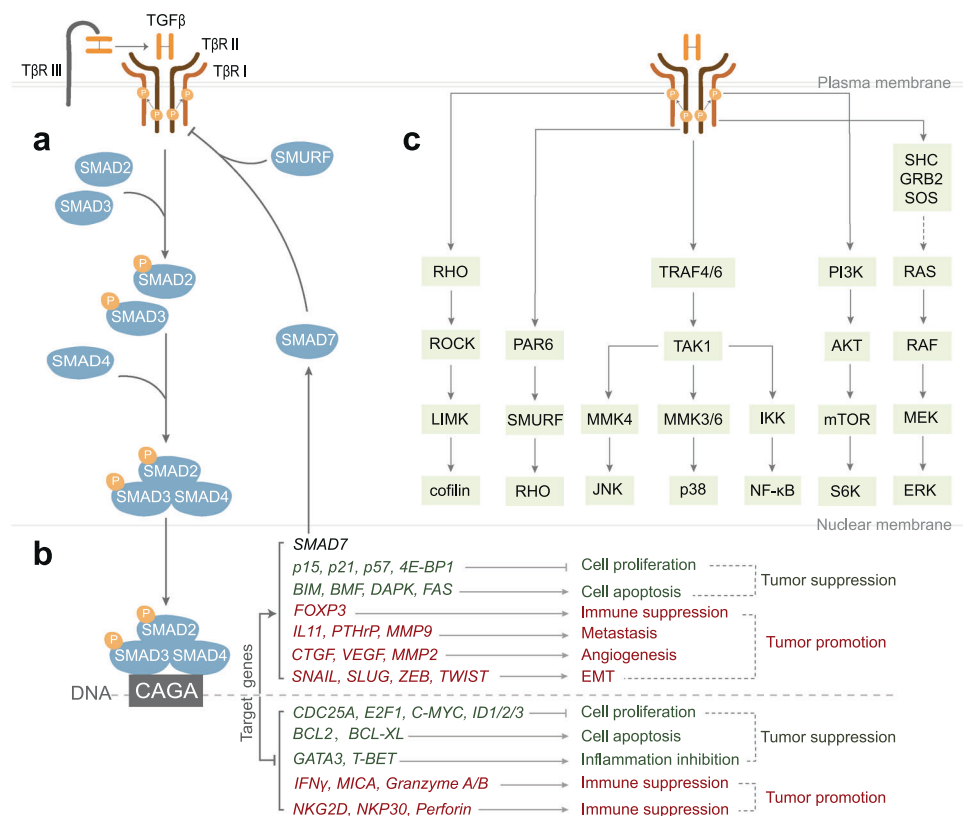
**Fig. 2** A schematic representation of the activation of latent TGF $\beta$ . The pro-TGF $\beta$  precursor is synthesized in the rough endoplasmic reticulum. It consists of an N-terminal signal peptide, latency-associated peptide (LAP), and a mature C-terminal TGF $\beta$  fragment. After cleavage by the convertase furin in the Golgi complex, the LAP dimer binds to mature TGF $\beta$  noncovalently, preventing its binding to cell surface receptors, and forms the small latent complex (SLC). There are three major mechanisms for activation of latent TGF $\beta$ . **a** Proteases (e.g., cathepsin, plasmin, matrix metalloproteinase 9/14 (MMP9/14)) in the extracellular matrix (ECM) cleave LAP and release active TGF $\beta$ . Also, thrombospondin (TSP) can induce activation by direct binding to LAP. **b** SLC is anchored to the ECM proteins (e.g., fibronectin and fibrillin) via latent TGF $\beta$ -binding protein (LTBP) and forms the so-called large latent complex (LLC). Active TGF $\beta$  can be released by cell contraction upon the interaction between LAP and integrins. **c** SLC binds to glycoprotein A repetition predominant protein (GARP) on the cell surface and can also mediate the release of active TGF $\beta$  upon interaction with integrins

with activated T $\beta$ RI, thereby blocking the interaction, phosphorylation, and activation of SMAD2. Subsequent steps in SMAD signaling, including SMAD2–SMAD4 complex formation and its translocation to the nucleus are also inhibited by SMAD7.<sup>57–59</sup> SMAD7 can also antagonize TGF $\beta$  signaling in the nucleus by disrupting the formation of the functional SMAD–DNA complex.<sup>60,61</sup> Moreover, SMAD7 recruits E3 ubiquitin ligases such as SMAD ubiquitination regulatory factor 1/2 (SMURF1/2), WW domain-containing protein 1 (WWP1), and neural precursor cell-expressed developmentally downregulated 4-2 (NEDD4-2) to T $\beta$ RI, and thereby promotes its ubiquitination-mediated proteasomal and/or lysosomal degradation.<sup>62–64</sup> Naturally, the ubiquitination of T $\beta$ RI can be reversed by a set of deubiquitinating enzymes, including ubiquitin-specific protease 4 (USP4), USP11, USP15, and ubiquitin carboxyl-terminal hydrolase L1.<sup>65–68</sup>

The target genes of the canonical TGF $\beta$  pathway that play key roles in regulating tumor progression are summarized in Fig. 3b.<sup>69</sup> In the early stage of tumorigenesis, TGF $\beta$  promotes the expression of cyclin-dependent kinase inhibitors, including *p15*, *p21*, *p57*, and eukaryotic translation initiation factor 4E-binding protein 1 (*4E-BP1*), to induce cell cycle arrest.<sup>70–74</sup> TGF $\beta$  represses the expression of genes encoding several growth-promoting factors, such as cell division cycle 25a (*CDC25A*), E2F transcription factor 1 (*E2F1*) and *C-MYC* proto-oncogene, to induce an anti-mitogenic cellular response.<sup>75–79</sup> Inhibitor of DNA binding 1 (*ID1*) protein functions as a cell differentiation inhibitor and stimulator of cell proliferation, and long-term stimulation of cells by TGF $\beta$  can silence the *ID1* promoter and thereby drive epithelial cells to enter into a cytostatic program.<sup>80</sup> *ID1* was also shown to mediate the escape of pancreatic cancer from TGF $\beta$  tumor suppression.<sup>81</sup> TGF $\beta$  can increase the expression of B-cell lymphoma 2 (*BCL2*)-interacting mediator of cell death (*BIM*), *BCL2*-modifying factor (*BMF*), *FAS* and death-associated protein kinase (*DAPK*) genes<sup>82–85</sup> and decrease the expression of *BCL2* and *BCL-extra large* (*BCL-XL*) genes to induce cell apoptosis.<sup>86,87</sup> In addition, TGF $\beta$  can suppress inflammation in the early stage of tumorigenesis by down-regulating the expression of GATA-binding protein 3 (*GATA3*) and transcription factor T-box expressed in T cells (*T-BET*) genes to inhibit T cell differentiation.<sup>88,89</sup> In the malignant stage, tumor cells can become non-responsive to the TGF $\beta$ -induced cytostatic

and proapoptotic effects when genes that encode key components of the TGF $\beta$  pathway are mutated, and this results in the functional inactivation of their corresponding gene products. For example, *T $\beta$ RII* mutations are frequently found in colorectal cancer (CRC),<sup>90</sup> *SMAD2* mutations have been reported in CRC, hepatocellular carcinoma (HCC), and lung cancer,<sup>91,92</sup> and the deletion or mutation of *SMAD4* is often identified in pancreatic cancer, CRC, and HCC.<sup>93,94</sup> There are several advanced tumors that still retain an intact canonical TGF $\beta$  pathway, such as glioma, melanoma, and breast cancer. These cancer cells can avert TGF $\beta$ -induced cytostatic functions through the acquisition of activating mutations in oncogenes, for example, rat sarcoma (*RAS*) and *MYC*, and inactivating mutations in tumor suppressor genes, for example, *p53* and retinoblastoma protein (*Rb*). TGF $\beta$  can increase forkhead box P3 (*FOXP3*) gene expression,<sup>95</sup> and decrease interferon- $\gamma$  (*IFN $\gamma$* ), MHC class I-chain-related molecules A (*MICA*), *granzyme A/B*, natural killer group 2 member D (*NKG2D*), natural cytotoxicity receptor 3 (*NKP30*), and *Perforin* gene expression to suppress immune function.<sup>96–99</sup> Besides, TGF $\beta$  upregulates interleukin-11 (*IL11*), parathyroid hormone-related peptide (*PTHrP*), and *MMP9* to facilitate metastasis to specific organs.<sup>100–102</sup> Moreover, TGF $\beta$  can promote angiogenesis by upregulating connective tissue growth factor (*CTGF*), vascular endothelial growth factor (*VEGF*), and *MMP2*.<sup>103,104</sup>

In addition to the canonical SMAD pathway, TGF $\beta$  can also initiate multiple noncanonical signaling pathways downstream of TGF $\beta$  receptors (Fig. 3c).<sup>105</sup> For example, T $\beta$ RI activates RHO small GTPases and then regulates the activity of RHO-associated protein kinase and LIM kinase to phosphorylate cofilin, leading to actin cytoskeleton reorganization that regulates cell adhesion, motility, and growth.<sup>106</sup> T $\beta$ RII can directly phosphorylate the cell polarity regulator PAR6 that regulates tight junctions and cell migration.<sup>107</sup> TGF $\beta$  also induces the activation of TGF $\beta$ -activated kinase 1 (TAK1) to stimulate c-Jun NH2-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) and nuclear factor  $\kappa$ -light-chain enhancer of activated B cells (NF- $\kappa$ B) pathways. These responses are mediated by tumor necrosis factor-associated factor 4 (TRAF4) and TRAF6 that interact with TGF $\beta$  receptors.<sup>108–110</sup> Serine/threonine kinase protein kinase B (AKT) signaling can also be activated by TGF $\beta$  in a phosphatidylinositol 3-kinase (PI3K)-



**Fig. 3** Schematic of the TGF $\beta$ -induced canonical SMAD and noncanonical signaling pathways. **a** T $\beta$ RII presents TGF $\beta$  to T $\beta$ RI. Thereafter, ligand occupied T $\beta$ RII recruits and phosphorylates T $\beta$ RI to trigger intracellular TGF $\beta$  signaling pathways. In the canonical pathway, activated T $\beta$ RI phosphorylates SMAD2/3 and stimulates the formation of heteromeric complexes with SMAD4. These complexes are translocated into the nucleus and regulate target gene expression. One of the TGF $\beta$ /SMAD-induced target genes is *SMAD7*, of which the gene product participates in a negative feedback loop to regulate the duration and intensity of TGF $\beta$  signaling by recruiting E3 ubiquitin ligase SMURF to T $\beta$ RI. **b** TGF $\beta$  pathway target genes relevant for tumor suppression are listed in green, and the target genes that encode proteins involved in the tumor promotion are listed in red. **c** TGF $\beta$  can also activate many noncanonical pathways, including RHO, JNK, p38, NF- $\kappa$ B, AKT, and ERK signaling components

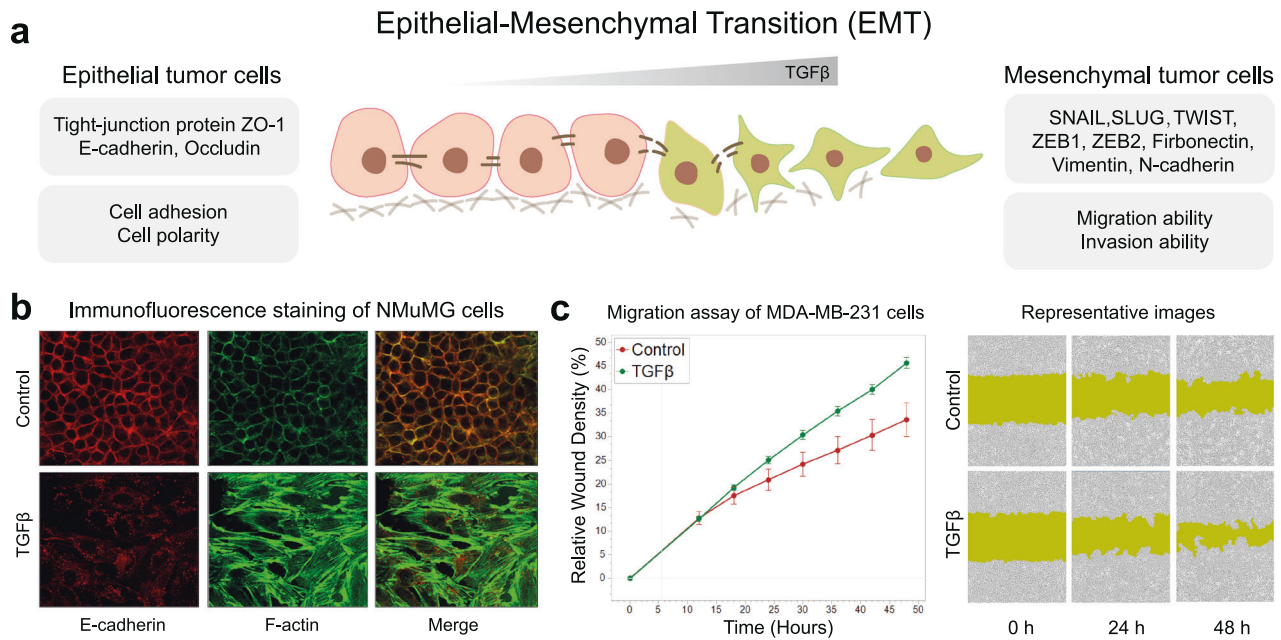
dependent manner.<sup>111</sup> Besides, TGF $\beta$  can induce the phosphorylation of Src homology domain 2-containing protein (SHC) and then recruit growth factor receptor-binding protein 2 (GRB2) and son of sevenless (SOS) to activate the extracellular signal-regulated kinase (ERK) pathway through the RAS, RAF, and mitogen-activated protein kinase (MEK) pathways.<sup>112</sup> A recent study discovered that the RAS-responsive element-binding protein 1 (RREB1) provides a molecular link between RAS and TGF $\beta$  pathways for coordinated induction of a developmental and fibrogenic EMT pathway.<sup>113</sup> These non-SMAD signaling pathways are not unique to TGF $\beta$ ; some are tightly regulated by receptor tyrosine kinases (RTKs) and crosstalk with the canonical SMAD signaling pathway.<sup>114</sup> Moreover, TGF $\beta$  can also indirectly activate these pathways by inducing the expression of secreted growth factors, for example, platelet-derived growth factor (PDGF), which then acts in an autocrine or paracrine manner via specific receptors endowed with intrinsic tyrosine kinase activity.<sup>115–117</sup>

Furthermore, TGF $\beta$  plays an important role in inducing the metastatic capacity of tumor cells by promoting the EMT through the upregulation of transcription factors such as *SNAIL*, *SLUG*, *TWIST*, and *ZEB1/2* through either SMAD- or non-SMAD-dependent pathways.<sup>118–124</sup> Accompanying the increase of TGF $\beta$  levels, EMT leads epithelial tumor cells to lose the capability of adhesion, polarity, and tight junctions by decreasing levels of tight-junction proteins zona occludence-1 (ZO-1), E-cadherin, and occludin, and acquire a highly migratory and invasive mesenchymal phenotype by the increased levels of fibronectin, vimentin, and N-cadherin

(Fig. 4a).<sup>19</sup> EMT has diverse phenotypic manifestations with intermediate epithelial/mesenchymal states and is a reversible process. This has been referred to as epithelial cell plasticity (EMP).<sup>125</sup> TGF $\beta$  can induce different states of EMP and the cellular response to TGF $\beta$  is context-dependent; inflammatory factors, Wnt, Notch, Hippo, and Hedgehog interplay with TGF $\beta$  to orchestrate the EMP response.<sup>126</sup> In Fig. 4b, we show that TGF $\beta$  promotes the EMT in non-transformed mouse mammary gland (NMuMG) epithelial cells.<sup>127</sup> The NMuMG cell line is a frequently used model system to investigate TGF $\beta$ -induced EMT.<sup>128,129</sup> The ability of TGF $\beta$  to promote the migration of the human mesenchymal triple-negative breast cancer (TNBC) MDA-MB-231 cells is shown in Fig. 4c.<sup>130</sup> MDA-MB-231 is highly aggressive and its metastasis to bone occurs in a TGF $\beta$ /SMAD-dependent manner.<sup>101,119,131</sup>

### FUNCTIONS OF TGF $\beta$ IN THE TME

The TME consists of ECM, cytokines, and a large population of different cell types, including resident and infiltrating CAFs, immune-related cells, endothelial cells, and adipocytes that surround the tumor cells. The multipronged effects of TGF $\beta$  on tumor stroma cells built the TME, including its capacity to stimulate ECM production, activate CAFs, suppress the immune system, and promote angiogenesis. Notably, tumor stromal cells are also the main sources of inflammatory factors, including TGF $\beta$ . Reciprocally, the TME exerts profound effects on tumor growth



**Fig. 4** The functions of TGF $\beta$  upon the epithelial-mesenchymal transition (EMT). **a** Schematic of TGF $\beta$  mediation of the EMT process. **b** TGF $\beta$  promotes the EMT in non-transformed NAMRU mouse mammary gland (NMuMG) epithelial cells, as visualized by immunofluorescent staining of cells with anti-E-cadherin antibody (red) and phalloidin (green) to measure filamentous actin expression in the absence and after treatment with 5 ng/ml TGF $\beta$  for 48 h. The typical morphological change from epithelial- to fibroblast-like cells, decreased E-cadherin, and accumulated striated fibers are observed in the NMuMG cells upon TGF $\beta$  stimulation. **c** TGF $\beta$  promotes the migration of MDA-MB-231 human breast cancer cells, as determined via real-time imaging of a wound-healing scratch assay. Left, graph showing the time-lapse relative migration rate; right, images of the cells taken when the initial scratch was made (0 h) and after 24 and 48 h in the absence or presence of 5 ng/ml TGF $\beta$

and progression. In this section, we summarize the functions of TGF $\beta$  in the TME (Fig. 5).

#### TGF $\beta$ and CAFs in the TME

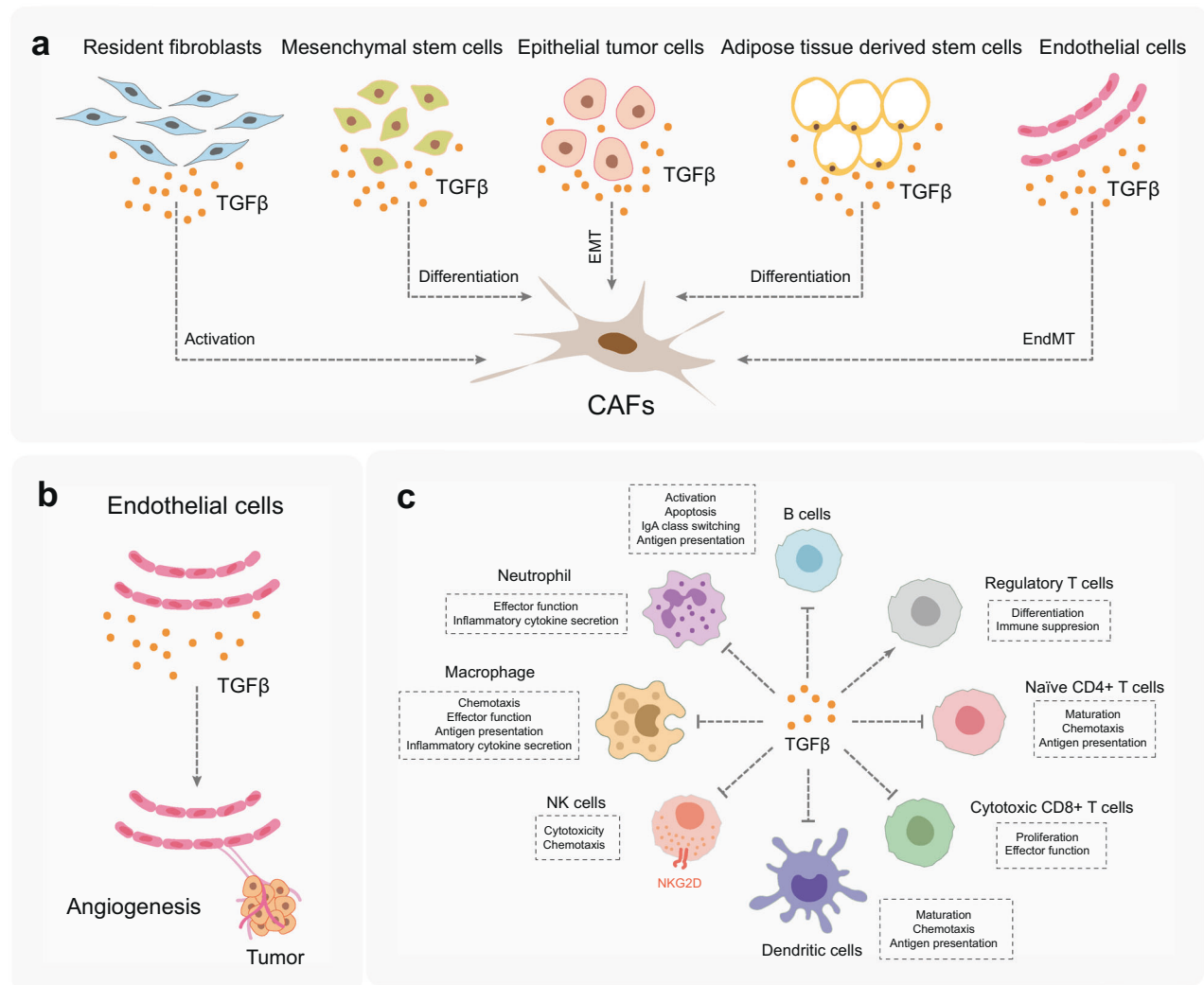
CAFs are key components of the TME and play roles in providing a favorable environment to support tumor progression by producing ECM and cytokines, stimulating immune evasion, and promoting angiogenesis.<sup>132–134</sup> Next to tumor-promoting CAFs, also tumor-suppressive CAFs have been identified.<sup>135</sup> Within a tumor, the CAF population can be highly heterogeneous. For example in breast tumors, at least four distinct CAF subpopulations, that is, vascular CAFs, matrix CAFs, cycling CAFs, and developmental CAFs (dCAFs), were defined.<sup>136</sup> In pancreatic tumors, CAFs have been classified into inflammatory CAFs, which are found distant from tumor foci with high IL6 and low  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) expression, and myofibroblastic CAFs that are located adjacent to tumor foci with high  $\alpha$ -SMA expression and that demonstrate a strong TGF $\beta$  responsiveness.<sup>137–139</sup> The characterization of molecular markers and mechanistic insights of different CAF subtypes may provide opportunities for their precise targeting in cancer therapy.<sup>140</sup>

During the formation of CAFs, TGF $\beta$  is a major driver in activating resident quiescent fibroblasts, and differentiating bone marrow-derived mesenchymal stem cells and adipose tissue-derived stem cells into CAFs (Fig. 5a).<sup>141–144</sup> The activation of the TGF $\beta$  signaling pathway can also induce epithelial cancer cells into myofibroblasts via EMT, and differentiate endothelial cells into fibroblast-like cells by endothelial-mesenchymal transition (EndMT) (Fig. 5a).<sup>145,146</sup> In turn, CAFs autocrine TGF $\beta$  can serve as an attractant to recruit more fibroblasts into the TME around the tumor invasion front and promote metastasis.<sup>147,148</sup> CAFs surround and throughout the tumor often restrict the accessibility of anti-cancer drugs to tumor cells since the dense CAFs with stiff surrounding matrix can reduce the density of blood vessels and form a physical barrier surrounding the tumor cells.<sup>149</sup> In hypoxic

TME, CAF-secreted TGF $\beta$ 2 can cooperate with hypoxia-inducible factor to increase stemness phenotype and induce robust chemotherapy of cancer cells.<sup>150</sup> Radiation-induced TGF $\beta$  signaling can activate CAFs in the TME, which enhances the invasiveness of the associated tumor cells via promoting EMT.<sup>151</sup> One recent study in which *TBR1*-mutant pancreatic cancer was investigated, found that the anti-tumor efficacy of TBR1 blockade is due to the inhibition of the TGF $\beta$  signaling in CAFs.<sup>152</sup> Furthermore, an increasing number of studies reported that the overactive TGF $\beta$  signaling in CAFs is a major reason of immunotherapy failure.<sup>153</sup> Analysis of the single-cell landscape of CAFs in pancreatic cancer identified a TGF $\beta$ -driven CAF population expressing leucine-rich repeat-containing 15 (LRRC15).<sup>154</sup> This transmembrane protein is associated with poor outcome in immunotherapy trial data.<sup>154</sup> It will be interesting to explore if the targeting of LRRC15<sup>+</sup> CAF in the TME will boost the response of cancer patients towards immune checkpoint blockade therapy.<sup>154</sup>

#### TGF $\beta$ and endothelial cells in the TME

Endothelial cells are located at the surface of blood and lymphatic vessels. Blood vessels nourish tumors by delivering blood/oxygen/nutrients, removing waste products, and mediating the entry and exit of immune cells and other substances.<sup>155–158</sup> A positive correlation between the TGF $\beta$  level and microvessel density was observed in various types of tumors.<sup>159</sup> Endothelial cells express two TBRIs (activin receptor-like kinase 1 (ALK1) and TBR1/ALK5). The TGF $\beta$ -ALK5 signaling axis can directly mediate inhibition of endothelial cell proliferation and migration (Fig. 5b).<sup>160,161</sup> Indirectly, the expression of some key angiogenic factors, such as VEGF, CTGF, and fibroblast growth factor 2 (FGF2), can be induced by the TGF $\beta$ -ALK5 signaling axis.<sup>162–165</sup> In the presence of endoglin, the TGF $\beta$ -ALK1 signaling axis can directly promote endothelial cell proliferation and migration.<sup>160,166</sup> Moreover, the JNK pathway also mediates the proangiogenic response of TGF $\beta$  in endothelial cells.<sup>167</sup> A recent study showed that TGF $\beta$  can



**Fig. 5** Functions of TGF $\beta$  in the tumor microenvironment (TME). **a** TGF $\beta$  can activate/differentiate resident fibroblasts, mesenchymal stem cells, epithelial tumor cells, adipose tissue-derived stem cells, and endothelial cells into cancer-associated fibroblasts (CAFs) in the TME. **b** TGF $\beta$  promotes angiogenesis in the TME by acting directly and indirectly on endothelial cells stimulating their proliferation, migration, and sprouting. **c** TGF $\beta$  crucially suppresses the immune system by regulating the functions of immune cell populations in the TME. The specific actions of TGF $\beta$  are indicated in the boxes next to the different immune cells that are depicted

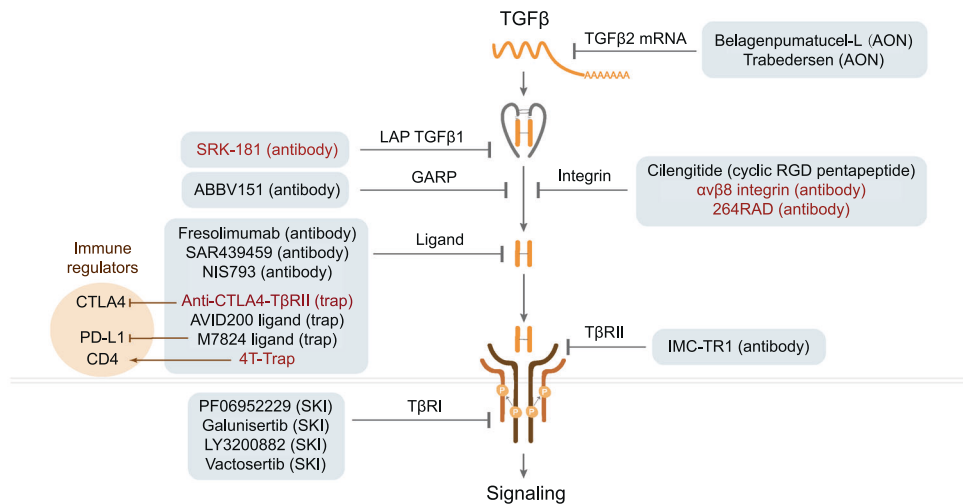
promote VEGF-C production in tubular epithelial cells, macrophages, and mesothelial cells to induce lymphangiogenesis in renal and peritoneal fibrosis. However, the function of TGF $\beta$  in the formation of the cancer-associated lymphatic system is not well understood.<sup>168</sup>

#### TGF $\beta$ and immune-related cells in the TME

There are a variety of innate and adaptive immune cells dispersed throughout the TME (Fig. 5c). During tumorigenesis, myeloid cells (including myeloid-derived suppressor cells (MDSCs), macrophages, neutrophils) typically accumulate in the early stage of tumor outgrowth to suppress the T cell response and sustain an immunosuppressive environment.<sup>169</sup> Dendritic cells (DCs) deliver tumor antigens to T cells and natural killer (NK) cells that exert antitumor cytotoxic effects.<sup>170</sup> However, antitumor immune reactions often become suppressed during tumor development; TGF $\beta$  can exhibit pivotal immunosuppressive effects on the intrinsic antitumor potential of DCs and NK cells in the TME (Fig. 5c). At an early stage of cancer, TGF $\beta$  mitigates myeloid proliferation and differentiation by repressing the expression of

cytokine IFN $\gamma$ .<sup>171,172</sup> Progressively, myeloid cells in advanced-stage cancer produce TGF $\beta$  and MMPs that further inhibit antitumor immune reactions and promote tumor metastasis.<sup>173–175</sup> Indeed, experimentally induced inactivation of TGF $\beta$  signaling in myeloid cells can lead to an increase in antitumor activity.<sup>175,176</sup> TGF $\beta$  blocks the activation of NK cells and suppresses their cytotoxic potential by inhibiting C-type lectin receptor NKG2D expression directly and indirectly.<sup>177</sup> IL1 receptor-associated kinase M (IRAK-M) is a potent negative regulator of Toll-like receptor (TLR) signaling and is predominantly expressed in macrophages.<sup>178</sup> By inducing IRAK-M expression and antagonizing TLR signaling, TGF $\beta$  protects tumors from the potential TLR-mediated antitumor activities of macrophages.<sup>179</sup>

TGF $\beta$  suppresses adaptive immunity during cancer progression mainly by inhibiting the activation, proliferation, differentiation, and migration of T cells. TGF $\beta$  can suppress the differentiation of naïve CD4 $^{+}$  helper T cells into distinct effector subtypes. However, it induces the conversion of naïve T cells into T<sub>reg</sub> cells (previously known as suppressor T cells) that suppress the immune response.<sup>180</sup> A recent study showed that depletion of T<sub>reg</sub> in



**Fig. 6** Schematic of strategies utilized in (pre)clinical trials targeting TGF $\beta$  signal transduction for cancer therapy. Various pharmacological interventions are grouped into the targeting of different TGF $\beta$  signaling components, that is, TGF $\beta$  mRNA, GARP/integrins that are involved in activation of latent TGF $\beta$ , and ligands that interact with TGF $\beta$  receptors and T $\beta$ RI kinase activity. The promising new targeting molecules that have been studied in pre-clinical models are highlighted in red color. Different strategies for targeting TGF $\beta$  signaling, including antisense oligonucleotide (AON), neutralizing antibody (antibody), cyclic RGD pentapeptide, TGF $\beta$  ligand trap (trap), and small-molecule kinase inhibitor (SKI) are indicated. The immune regulatory targets (CTLA4/PD-L1/CD4) of the bispecific molecules that sequester TGF $\beta$  with a T $\beta$ RII extracellular domain containing trap are highlighted in the orange circle

CD4<sup>+</sup> T cells suppresses cancer progression as a result of tissue healing and remodeling of the blood vasculature, causing cancer hypoxia and death in distant avascular regions.<sup>181</sup> TGF $\beta$  can block the activation and maturation of cytotoxic CD8<sup>+</sup> T cells by repressing the tumor antigen processing and presentation of DCs and inhibit CD8<sup>+</sup> T cell proliferation through suppressing the expression of IFN $\gamma$  and IL2.<sup>172,182–184</sup> TGF $\beta$  promotes antigen-induced programmed cell death protein-1 (PD-1) expression in CD8<sup>+</sup> T cells, which causes T cell exhaustion.<sup>185</sup> Recently, researchers found that the TGF $\beta$  signal maintains the immune-suppressive identity of CD8<sup>+</sup> T<sub>reg</sub> cells. Transcription factor Eomesodermin (Eomes) controls the follicular location of CD8<sup>+</sup> T<sub>reg</sub> cells.<sup>186</sup> Both TGF $\beta$  and Eomes coordinate to promote the homeostasis of CD8<sup>+</sup> T<sub>reg</sub> cells.<sup>186</sup> TGF $\beta$  can regulate the activation, proliferation, apoptosis of B cells, and stimulate the antibody switching in B cells. However, its function on B cell-mediated antitumor immunity is not well investigated.<sup>187–189</sup>

## PHARMACOLOGICAL INTERVENTIONS OF TGF $\beta$ IN CANCER THERAPY

There are numerous anti-cancer pharmacological interventions that target specific mediators of TGF $\beta$  signaling pathway or TGF $\beta$  activators, which have been tested in human clinical trials or that displayed very promising results in pre-clinical animal models (Fig. 6). In this section, we introduce the recent advancements and bottlenecks of the main anti-TGF $\beta$  strategies, including neutralizing antibodies, ligand traps, small-molecule kinase inhibitors, and antisense oligonucleotides (AONs), and summarize the pharmacological interventions that have been or are currently being studied in clinical trials (Table 1).

### Neutralizing antibodies

Neutralizing antibodies can be selectively directed against critical domains of native ligands and extracellular domains of receptors to block their biological activity. Fresolimumab (GC1008), a human IgG4k monoclonal antibody that neutralizes TGF $\beta$ 1, 2, and 3, demonstrated acceptable safety and antitumor activity in a phase 1 clinical trial for patients with malignant melanoma or renal carcinoma.<sup>190</sup> Phase 2 clinical trials of this antibody have been

conducted for patients with glioma, metastatic breast cancer, or relapsed malignant pleural mesothelioma, which showed good toleration.<sup>191–193</sup> The phase 2 clinical evaluation of this antibody for the treatment of early-stage non-small cell lung cancer (NSCLC) is still ongoing (NCT02581787). LY3022859 is an anti-T $\beta$ RII IgG1 monoclonal antibody blocking TGF $\beta$  binding to the ectodomain of T $\beta$ RII that showed significant antitumor function against primary tumor growth and metastasis in several mice tumor models.<sup>194</sup> When tested for use in the treatment of advanced solid tumors in a phase 1 clinical trial, the maximum-tolerated dose for this antibody was not determined since the patients suffered from uncontrolled cytokine release despite prophylaxis.<sup>195</sup>

Integrins are major activators of TGF $\beta$  ligand, including  $\alpha$ v $\beta$ 1,  $\alpha$ v $\beta$ 3,  $\alpha$ v $\beta$ 5,  $\alpha$ v $\beta$ 6, and  $\alpha$ v $\beta$ 8.<sup>46,196–199</sup> An increase number of studies showed that integrin-induced TGF $\beta$  signaling pathway modulates the tumor stroma and in particular the immune microenvironment.<sup>200</sup> Thus, besides their role to promote cell proliferation, survival, and migration, the ability of  $\alpha$ v integrins to stimulate TGF $\beta$  activation contributes to cancer progression.<sup>201,202</sup> Thus, integrins can be pharmacological targets for cancer treatment, and their targeting may enhance the efficacy of immune therapies.<sup>203</sup> For example, previous research found that  $\alpha$ v $\beta$ 8 integrin-expressing tumor cells can evade host immunity by upregulating TGF $\beta$  signaling in immune cells.<sup>204</sup> The engineered antibody against  $\alpha$ v $\beta$ 8 integrin can block the release of active TGF $\beta$  by cancer cells and promote the anti-tumor immune reaction in pre-clinical mice models of colon carcinoma and lung cancer.<sup>205</sup> A recent study showed that  $\alpha$ v $\beta$ 6-neutralizing antibody 264RAD can suppress TGF $\beta$  signaling and significantly reduce tumor growth in  $\alpha$ v $\beta$ 6-positive human pancreatic ductal adenocarcinoma (PDAC) xenografts mice model and immunocompetent transgenic mice bearing  $\alpha$ v $\beta$ 6-positive PDAC tumors.<sup>206</sup> Although most studies showed that anti- $\alpha$ v $\beta$  integrin therapy has promise in cancer treatment, an opposite finding was reported for antibody-mediated inhibition of  $\alpha$ v $\beta$ 6 that while leading to a strong decreased TGF $\beta$  signaling, it promoted pancreatic cancer progression.<sup>207</sup>

### Ligand traps

TGF $\beta$  ligand traps are TGF $\beta$  receptor ectodomain-based chimeric fusion proteins that are rationally designed to prevent TGF $\beta$ s from

**Table 1.** Summary of pharmacological strategies targeting TGF $\beta$  for cancer therapy in clinical trials (information was obtained from <https://www.clinicaltrials.gov/>)

| Strategy              | Drug         | Target              | Cancer                                                        | Identifier  | Phase | Treatment                                                                        | Status     |
|-----------------------|--------------|---------------------|---------------------------------------------------------------|-------------|-------|----------------------------------------------------------------------------------|------------|
| Neutralizing antibody | Fresolimumab | TGF $\beta$ 1/2/3   | Glioma                                                        | NCT01472731 | 2     | Monotherapy                                                                      | Completed  |
|                       |              |                     | Metastatic breast cancer                                      | NCT01401062 | 2     | Combination with radiotherapy                                                    | Completed  |
|                       |              |                     | Relapsed malignant pleural mesothelioma                       | NCT0112293  | 2     | Monotherapy                                                                      | Completed  |
|                       |              |                     | Renal cell carcinoma or malignant melanoma                    | NCT00356460 | 1     | Monotherapy                                                                      | Completed  |
|                       |              |                     | Early stage non-small cell lung cancer                        | NCT02581787 | 2     | Combination with radiotherapy                                                    | Recruiting |
|                       | SAR439459    | TGF $\beta$ 1/2/3   | Advanced solid tumors                                         | NCT03192345 | 1     | Monotherapy/combination with cemiplimab                                          | Recruiting |
|                       | NIS793       | TGF $\beta$ 1/2/3   | Metastatic pancreatic ductal adenocarcinoma                   | NCT04390763 | 2     | Monotherapy/combination with spartalizumab/gemcitabine and paclitaxel            | Active     |
|                       |              |                     | Breast/lung/hepatocellular/colorectal/pancreatic/renal cancer | NCT02947165 | 1     | Monotherapy/combination with spartalizumab                                       | Recruiting |
|                       | IMC-TR1      | T $\beta$ RII       | Advanced solid tumor                                          | NCT01646203 | 1     | Monotherapy                                                                      | Completed  |
|                       | ABBV151      | GARP: TGF $\beta$ 1 | Advanced/metastatic solid tumors                              | NCT03821935 | 1     | Monotherapy/combination with ABBV-181                                            | Recruiting |
| Ligand trap           | AVID200      | TGF $\beta$ 1/3     | Advanced/metastatic solid tumors                              | NCT03834662 | 1     | Monotherapy                                                                      | Active     |
|                       | M7824        | TGF $\beta$ /PD-L1  | Advanced non-small cell lung cancer                           | NCT03631706 | 3     | Monotherapy                                                                      | Recruiting |
|                       |              |                     | HPV-associated cancers                                        | NCT04432597 | 2     | Combination with HPV vaccine PRGN-2009                                           | Recruiting |
|                       |              |                     | Cholangiocarcinoma/gallbladder cancer                         | NCT03833661 | 2     | Monotherapy                                                                      | Active     |
|                       |              |                     | Recurrent respiratory papillomatosis                          | NCT03707587 | 2     | Monotherapy                                                                      | Active     |
|                       |              |                     | Biliary tract cancer                                          | NCT04066491 | 3     | Combination with gemcitabine and cisplatin                                       | Recruiting |
|                       |              |                     | Relapsed small cell lung cancers                              | NCT03554473 | 2     | Combination with topotecan/temozolomide                                          | Recruiting |
|                       |              |                     | Metastatic triple-negative breast cancer                      | NCT03579472 | 1     | Combination with eribulin mesylate                                               | Recruiting |
|                       |              |                     | Thymoma/thymic carcinoma                                      | NCT04417660 | 2     | Monotherapy                                                                      | Active     |
|                       |              |                     | Stage II–III HER2-positive breast cancer                      | NCT03620201 | 1     | Monotherapy                                                                      | Recruiting |
|                       |              |                     | Advanced Kaposi sarcoma                                       | NCT04303117 | 2     | Combination with M9241                                                           | Recruiting |
|                       |              |                     | Advanced adenocarcinoma of the pancreas                       | NCT03451773 | 2     | Combination with gemcitabine                                                     | Completed  |
|                       |              |                     | Metastatic colorectal cancer/advanced solid tumors            | NCT03436563 | 2     | Monotherapy                                                                      | Recruiting |
|                       |              |                     | Advanced pancreas cancer                                      | NCT04327986 | 2     | Combination with M9241 and radiotherapy                                          | Active     |
|                       |              |                     | Metastatic non-prostate genitourinary malignancies            | NCT04235777 | 1     | Combination with M9241/radiotherapy                                              | Recruiting |
|                       |              |                     | Resectable head and neck squamous cell carcinoma              | NCT04247282 | 2     | Monotherapy/combination with N-803 and TriAd Vaccine                             | Recruiting |
|                       |              |                     | Metastatic/locally advanced urothelial cancer                 | NCT04349280 | 1     | Monotherapy                                                                      | Active     |
|                       |              |                     | HER2-negative breast cancer                                   | NCT03524170 | 1     | Combination with radiotherapy                                                    | Recruiting |
|                       |              |                     | Advanced-stage breast cancer                                  | NCT04296942 | 1     | Combination with ado-trastuzumab emtansine, entinostat, and BN-brachyury vaccine | Recruiting |
|                       |              |                     | AT-hook 2 (HMG2) expressing triple-negative breast cancer     | NCT04489940 | 2     | Monotherapy                                                                      | Active     |
|                       |              |                     | Platinum-experienced cervical cancer                          | NCT04246489 | 2     | Monotherapy                                                                      | Recruiting |
|                       |              |                     | Recurrent/second primary head and neck squamous cell cancer   | NCT04220775 | 2     | Combination with radiotherapy                                                    | Recruiting |

**Table 1.** continued

| Strategy                  | Drug         | Target                 | Cancer                                                              | Identifier  | Phase | Treatment                                                  | Status     |
|---------------------------|--------------|------------------------|---------------------------------------------------------------------|-------------|-------|------------------------------------------------------------|------------|
| Small-molecule inhibitors | Galunisertib | T $\beta$ RI           | Advanced small bowel/colorectal cancers                             | NCT04491955 | 2     | Combination with N-803, M9241, and CV301 vaccine           | Active     |
|                           |              |                        | Advanced hepatocellular carcinoma                                   | NCT02906397 | 1     | Combination with radiotherapy                              | Active     |
|                           |              |                        | Metastatic castration-resistant prostate cancer                     | NCT02452008 | 2     | Combination enzalutamide                                   | Recruiting |
|                           |              |                        | Rectal cancer                                                       | NCT02688712 | 2     | Combination fluorouracil/capecitabine and radiotherapy     | Recruiting |
|                           |              |                        | Metastatic breast cancer                                            | NCT02538471 | 2     | Combination with radiotherapy                              | Terminated |
|                           | Vactosertib  | T $\beta$ RI           | Metastatic pancreatic cancer                                        | NCT02734160 | 1     | Combination with durvalumab                                | Terminated |
|                           |              |                        | Advanced refractory solid tumors                                    | NCT02423343 | 1     | Combination with nivolumab                                 | Active     |
|                           |              |                        | Recurrent/refractory non-small cell lung cancer                     | NCT02423343 | 2     | Combination with nivolumab                                 | Active     |
|                           |              |                        | Hepatocellular carcinoma                                            | NCT01246986 | 2     | Combination with sorafenib/ramucirumab                     | Active     |
|                           |              |                        | Metastatic androgen receptor-negative triple-negative breast cancer | NCT02672475 | 1     | Combination with Paclitaxel                                | Recruiting |
|                           | LY3200882    | T $\beta$ RI           | Newly diagnosed malignant glioma                                    | NCT01220271 | 2     | Combination with temozolomide and radiotherapy             | Completed  |
|                           |              |                        | Carcinoma of the uterus or ovary                                    | NCT03206177 | 1     | Combination with paclitaxel/carboplatin                    | Recruiting |
|                           |              |                        | Advanced/metastatic unresectable pancreatic cancer                  | NCT01373164 | 2     | Combination gemcitabine/placebo                            | Completed  |
|                           |              |                        | Advanced solid tumors                                               | NCT02160106 | 1     | Monotherapy                                                | Completed  |
|                           |              |                        | Refractory multiple myeloma                                         | NCT03143985 | 1     | Combination with pomalidomide                              | Recruiting |
|                           | PF06952229   | T $\beta$ RI           | Urothelial carcinoma                                                | NCT04064190 | 2     | Combination with durvalumab                                | Active     |
|                           |              |                        | Metastatic colorectal cancer                                        | NCT03724851 | 1     | Combination with pembrolizumab                             | Recruiting |
|                           |              |                        | Advanced non-small cell lung cancer                                 | NCT03732274 | 2     | Combination with durvalumab                                | Recruiting |
|                           |              |                        | Myeloproliferative neoplasm                                         | NCT04103645 | 2     | Monotherapy                                                | Active     |
|                           |              |                        | Advanced desmoid tumor                                              | NCT03802084 | 2     | Combination with imatinib                                  | Recruiting |
|                           | Cilengitide  | $\alpha$ v $\beta$ 3/5 | Metastatic gastric cancer                                           | NCT03698825 | 2     | Combination with paclitaxel                                | Recruiting |
|                           |              |                        | Metastatic pancreatic ductal adenocarcinoma                         | NCT04258072 | 1     | Combination with irinotecan, fluorouracil, and leucovorin  | Active     |
|                           |              |                        | Solid tumors                                                        | NCT02937272 | 1     | Monotherapy                                                | Active     |
|                           |              |                        | Activated colorectal cancer                                         | NCT04031872 | 2     | Combination with capecitabine                              | Active     |
|                           |              |                        | Advanced solid tumors                                               | NCT03685591 | 1     | Monotherapy                                                | Recruiting |
|                           |              |                        | Diffuse intrinsic pontine glioma                                    | NCT01165333 | 1     | Combination with radiotherapy                              | Completed  |
|                           |              |                        | Advanced non-small cell lung cancer                                 | NCT00842712 | 2     | Combination with cetuximab and platinum-based chemotherapy | Completed  |
|                           |              |                        | Brain metastases from lung cancer                                   | NCT00884598 | 1     | Combination with radiotherapy                              | Completed  |
|                           |              |                        | Advanced solid tumors/glioblastoma multiforme                       | NCT01122888 | 1     | Combination with sunitinib                                 | Terminated |
|                           |              |                        | Metastatic prostate cancer                                          | NCT00103337 | 2     | Monotherapy                                                | Completed  |
|                           |              |                        | Recurrent glioblastoma multiforme                                   | NCT00093964 | 2     | Monotherapy                                                | Completed  |
|                           |              |                        | Diagnosed inoperable glioblastoma                                   | NCT01558687 | 1     | Monotherapy                                                | Terminated |
|                           |              |                        | Advanced solid tumors/lymphoma                                      | NCT00077155 | 1     | Monotherapy                                                | Completed  |
|                           |              |                        | Childhood recurrent/progressive high-grade glioma                   | NCT00679354 | 2     | Monotherapy                                                | Completed  |
|                           |              |                        | Newly diagnosed glioblastoma                                        | NCT00689221 | 3     | Combination temozolomide and radiotherapy                  | Completed  |

**Table 1.** continued

| Strategy                  | Drug        | Target        | Cancer                                                                                | Identifier  | Phase | Treatment                                               | Status     |
|---------------------------|-------------|---------------|---------------------------------------------------------------------------------------|-------------|-------|---------------------------------------------------------|------------|
| Antisense oligonucleotide | Trabedersen | TGF $\beta$ 2 | Childhood refractory primary brain tumors                                             | NCT00063973 | 1     | Monotherapy                                             | Completed  |
|                           |             |               | Prostate cancer                                                                       | NCT00121238 | 2     | Monotherapy                                             | Completed  |
|                           |             |               | Unresectable/metastatic melanoma                                                      | NCT00082875 | 2     | Monotherapy                                             | Terminated |
|                           |             |               | Acute myeloid leukemia                                                                | NCT00089388 | 2     | Monotherapy                                             | Terminated |
|                           |             |               | Advanced solid tumors                                                                 | NCT00022113 | 1     | Monotherapy                                             | Completed  |
|                           |             |               | Undergoing surgery for recurrent/progressive glioblastoma multiforme                  | NCT00112866 | 2     | Monotherapy                                             | Terminated |
|                           |             |               | HIV-related Kaposi's sarcoma                                                          | NCT00006222 | 1     | Monotherapy                                             | Terminated |
|                           |             |               | Locally advanced/metastatic cancer                                                    | NCT00004258 | 1     | Monotherapy                                             | Completed  |
|                           |             |               | Progressive/recurrent glioma                                                          | NCT00006093 | 2     | Monotherapy                                             | Completed  |
|                           |             |               | Relapsed/refractory high-grade gliomas/diffuse intrinsic pontine gliomas              | NCT01517776 | 2     | Combination with temozolomide and radiotherapy          | Terminated |
|                           | Lucanix     | TGF $\beta$ 2 | Recurrent/metastatic squamous cell carcinoma of the head and neck                     | NCT00705016 | 2     | Combination with cetuximab, cisplatin, and fluorouracil | Completed  |
|                           |             |               | Triple-negative breast cancer/advanced solid tumors that cannot be removed by surgery | NCT01276496 | 1     | Combination with paclitaxel                             | Completed  |
|                           |             |               | Newly diagnosed MGMT-promoter unmethylated glioblastoma                               | NCT01044225 | 3     | Combination with radiotherapy and temozolomide          | Terminated |
|                           |             |               | Advanced tumors known to overproduce TGF $\beta$ 2                                    | NCT00844064 | 1     | Monotherapy                                             | Completed  |
|                           |             |               | Glioblastoma and anaplastic astrocytoma                                               | NCT00761280 | 3     | Monotherapy                                             | Terminated |
|                           |             |               | Recurrent/refractory high-grade glioma                                                | NCT00431561 | 2     | Monotherapy                                             | Completed  |
|                           |             |               | Non-small cell lung cancer                                                            | NCT01058785 | 2     | Monotherapy                                             | Completed  |
|                           |             |               | Non-small cell lung cancer                                                            | NCT00676507 | 3     | Monotherapy                                             | Completed  |

binding to their receptors. AVID200 is a selective trap of TGF $\beta$ 1 and TGF $\beta$ 3 that showed the capacity to enhance the anti-tumor efficacy by inhibiting the growth of syngeneic 4T1 TNBC homograft in immunocompetent host mice.<sup>187</sup> A phase I clinical trial of AVID200 revealed that it was safe and well tolerated for patients with advanced solid tumors.<sup>208,209</sup>

#### Small-molecule inhibitors

Small-molecule kinase inhibitors of T $\beta$ R kinases are undergoing intensively clinical trials for anti-TGF $\beta$  signaling in cancer treatment. Galunisertib (LY2157299) is an orally available small-molecule inhibitor that selectively binds to T $\beta$ RI (and weakly to T $\beta$ RII) and inhibits its kinase activity. It showed favorable preclinical results for antitumor growth, for example, in Calu6 and EMT6-LM2 mouse tumor models.<sup>210</sup> In phase 1 clinical trials, this inhibitor exhibited acceptable safety and dose tolerability for patients with HCC/metastatic pancreatic cancer/malignant glioma/advanced solid tumors.<sup>211–214</sup> Phase 1/2 clinical trials of this inhibitor for patients with metastatic prostate cancer/TNBC/advanced HCC/rectal cancer/recurrent glioblastoma are ongoing.<sup>213,215,216</sup> A phase 2 study of this inhibitor for patients with advanced HCC showed acceptable safety and prolonged overall survival.<sup>215</sup> However, in a phase 2 study of patients with recurrent glioblastoma, this inhibitor failed to improve the overall survival.<sup>217</sup> Vactosertib (TEW-7197) is another orally bioavailable kinase inhibitor of T $\beta$ RI that revealed promising antitumor function in a mouse model of myeloma.<sup>218</sup> This inhibitor displayed a favorable safety profile and antitumor efficacy in phase 1 clinical trials for patients with advanced solid tumors or desmoid tumors.<sup>219,220</sup> Phase 1 clinical trial investigation of this inhibitor for patients with refractory multiple myeloma/metastatic CRC/metastatic PDAC and phase 2 study for patients with metastatic gastric cancer/CRC/urothelial carcinoma/ NSCLC/myeloproliferative neoplasm/advanced desmoid tumors are ongoing.<sup>221–224</sup> LY3200882 is a next-generation highly selective potent ATP-competitive T $\beta$ RI inhibitor that showed antitumor efficacy in a preclinical mouse model of TNBC cancer.<sup>225</sup> The phase 1 clinical trial results showed that LY3200882 had a tolerable safety profile and early signs of antitumor efficacy for patients with advanced or metastatic cancers (NCT04031872). PF06952229 is a selective and orally available T $\beta$ RI inhibitor that is being studied in phase 1 clinical trials for patients with advanced/metastatic breast cancer/castration-resistant prostate cancer (NCT03685591).

Previous studies showed that genetically and pharmacologically inhibition of  $\alpha$ v $\beta$  integrin can inhibit TGF $\beta$  signaling and suppress metastasis.<sup>201,203</sup> For example, the cyclic RGD pentapeptide cilengitide (EMD121974) is a potent and selective inhibitor of integrin  $\alpha$ v $\beta$ 3/5 that showed a reduction of TGF $\beta$ 1 and TGF $\beta$ 2 messenger RNA (mRNA) and protein expression, SMAD2 phosphorylation, and TGF $\beta$ -mediated reporter gene activity in most glioma cells.<sup>226</sup> The intracranial LN-308 glioma xenograft mice model also displayed decreased SMAD2 phosphorylation in response to cilengitide.<sup>226</sup> This inhibitor has been evaluated in phase 1 clinical trials for patients with brain tumors/lung cancer/advanced solid tumor/lymphoma/HIV-related Kaposi's sarcoma,<sup>227–230</sup> in phase 2 clinical trials for patients with NSCLC/prostate cancer/metastatic melanoma/acute myeloid leukemia/head neck squamous cell carcinoma,<sup>231–237</sup> and in phase 3 for patients with glioblastoma.<sup>238</sup> The clinical trials of cilengitide were terminated for the treatment of patients with metastatic melanoma, acute myeloid leukemia, or HIV-related Kaposi's sarcoma since it neither exhibited antitumor efficacy nor improved overall survival. However, the phase 3 clinical study of cilengitide in glioblastoma patients showed well toleration and single-agent activity.<sup>237,238</sup>

#### Antisense oligonucleotides

AONs are short oligonucleotides designed to suppress the expression of specific genes by blocking their translation.<sup>239</sup> Trabedersen

(AP12009) is a phosphonothioate antisense oligodeoxynucleotide that specifically targets TGF $\beta$ 2 mRNA.<sup>240</sup> Trabedersen was evaluated in the phase 1 clinical studies in patients with melanoma/pancreatic cancer/CRC, and showed good safety and encouraging survival results.<sup>241</sup> The phase 2 clinical trials' evaluation of this AON for patients with glioblastoma or anaplastic astrocytoma enabled the determination of the optimal dose for further clinical development.<sup>242</sup> However, the phase 3 clinical trial of trabedersen for patients with glioblastoma was terminated because of the lack of patient recruitment.<sup>243–245</sup> Lucanix (belagenpumatucel-L) is a vaccine expressing AON TGF $\beta$ 2 RNA that was evaluated in clinical trial studies for NSCLC patients.<sup>246,247</sup> This vaccine did not meet its survival endpoint in a phase 3 clinical trial for NSCLC patients; however, it improved the overall survival of patients who had completed chemotherapy within the previous 12 weeks.<sup>248</sup>

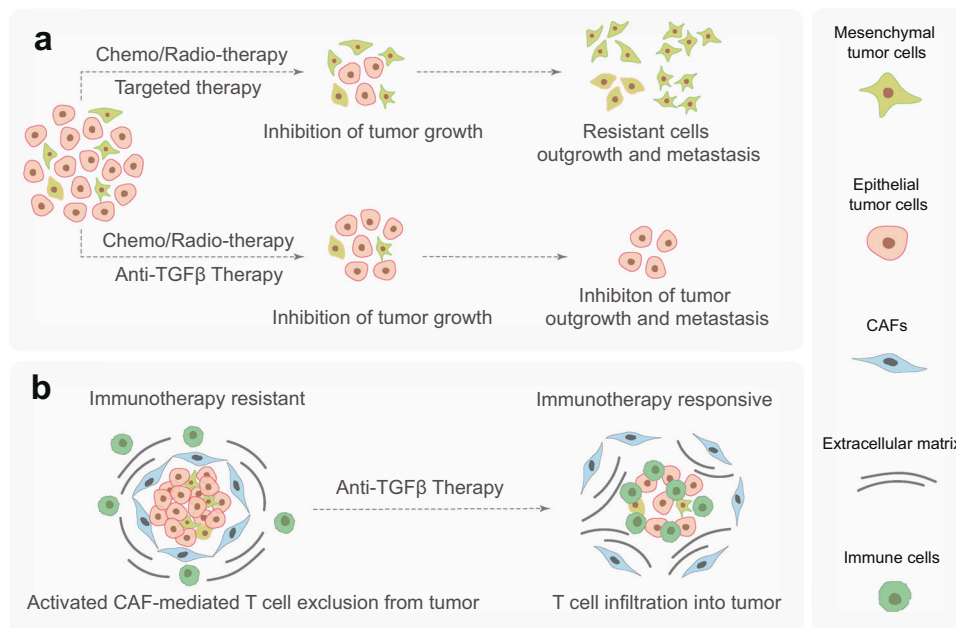
### TARGETING TGF $\beta$ IN CANCER THERAPY: CHALLENGES AND OPPORTUNITIES

Overcoming the adverse effects of anti-TGF $\beta$  therapies  
Cancer patients who were treated with blockers of TGF $\beta$  signaling can experience side effects if the function of TGF $\beta$  in physiological processes is compromised. For example, some T $\beta$ RI kinase inhibitors showed therapeutic effects in cancer patients, but their cardiac toxicity at high doses (hemorrhagic, degenerative, and inflammatory lesions in heart valves) and skin toxicity (eruptive keratoacanthomas, hyperkeratosis, cutaneous squamous-cell carcinomas, and basal cell carcinoma) limits their safe therapy window.<sup>22</sup> These adverse effects have (and continue to) challenge the clinical application of many other anti-TGF $\beta$  therapies. Careful dosing of TGF $\beta$  inhibitors to cancer patients may attenuate toxicity. Moreover, the adverse effects may be mitigated by pulsatile therapy, in which the patient has so-called "drug holidays" during the dosing period. Galunisertib (LY2157299) has been applied as a therapy regimen of 2 weeks on and 2 weeks off drug treatment to reduce the (cardiac) side effects.<sup>249</sup> In addition, predictive biomarkers may aid in selecting patients who benefit most from treatment with anti-TGF $\beta$  agents. Transcriptional profiling of samples from patients with many different cancer types, including glioblastoma, pancreatic cancer, breast cancer, ovarian cancer, CRC, and NSCLC, revealed that cancer patients with mesenchymal subtypes have high expression of TGF $\beta$  target genes and that this correlates with poor prognosis. Therefore, subgroups of patients with cancers that carry a mesenchymal phenotype may particularly benefit from anti-TGF $\beta$  therapies.<sup>250–255</sup>

#### Perspectives on the synergy of combination therapies

Increased TGF $\beta$  activity has been implicated in the resistance to various anticancer therapies, including cytotoxic chemotherapy, targeted therapy, radiotherapy, and immunotherapy.<sup>4</sup> Therefore, combining anti-TGF $\beta$  therapy with these established strategies may dampen therapy resistance (Fig. 7). A future opportunity and challenge are to identify the optimal combination of a synergistic therapy regimen for each individual patient.

*Anti-TGF $\beta$  therapy with chemotherapy, targeted therapy, or radiotherapy.* Chemotherapy is a frequent standard first-line cancer therapy regimen consisting of one or more anticancer chemicals designed to stop and kill rapidly proliferating cancer cells. However, chemotherapy has strong side effects due to its toxicity to normal cells. Targeted therapy is performed by using small molecules that interfere with specific signaling pathways that promote cancer cell proliferation and survival.<sup>256</sup> Examples of such small-molecule compounds are selective tyrosine kinase inhibitors.<sup>257</sup> Selective small-molecule inhibitors have less severe side effects than standard chemotherapy, but both types of therapy can lead to therapy resistance and relapse, as some cells within the heterogeneous tumor population survive and proliferate after



**Fig. 7** Sketch of synergistic combination therapies. **a** Chemo/radio/targeted therapy alone inhibits the growth of epithelial-like tumor cells, and in combination with anti-TGF $\beta$  therapy, invasive escape and resistance to these therapies are attenuated, and metastasis of mesenchymal tumor cells is restrained. **b** Activated CAFs mediated by high TGF $\beta$  activity suppresses immunotherapy efficacy by blocking T cell infiltration into tumors and inducing T cell dysfunction. In combination with anti-TGF $\beta$  therapy; however, T cell exclusion is inhibited, and the antitumor efficacy of the immunotherapy is improved

an initial beneficial anticancer response.<sup>258</sup> TGF $\beta$ -induced cancer cell acquisition of a mesenchymal phenotype has emerged as a key mechanism that confers the therapy-resistant and stemness phenotype to cancer cells.<sup>22</sup> Blocking TGF $\beta$  may kill two birds with one stone by impairing metastasis and preventing resistance against anticancer treatments.

T $\beta$ RI kinase inhibitors, such as galunisertib (LY2157299), have been combined with multiple chemotherapies, including paclitaxel, sorafenib, or gemcitabine in patients with TNBC, glioblastoma, HCC, or PDAC to improve antitumor efficacy.<sup>215,216,259–261</sup> LY3200882 is combined with gemcitabine/paclitaxel/cisplatin in an ongoing phase 1 trial for the treatment of patients with solid tumors (NCT02937272), or is tested in combination with capecitabine for patients with resistant metastatic CRC (NCT04031872). The efficacy of a combination of vactosertib (TEW-7197) and paclitaxel is explored in an ongoing phase 1/2 clinical trial for the treatment of metastatic gastric cancer (NCT03698825). NIS793 is a pan anti-TGF $\beta$ -neutralizing antibody that is currently being tested in a phase 2 clinical trial for patients with metastatic PDAC in combination with gemcitabine/nab-paclitaxel chemotherapy (NCT04390763).<sup>262</sup> T $\beta$ RI kinase inhibitors have also been combined with targeted therapies to overcome drug resistance in pre-clinical cancer models. For example, downregulation of mediator complex subunit 12 (MED12) leads to resistance to multiple tyrosine kinase inhibitors in multiple cancer cells. Mechanistically, MED12 depletion was found to increase T $\beta$ RII protein levels, mediate MEK/ERK activation, and induce the expression of mesenchymal markers. Treatment with the T $\beta$ RI kinase inhibitor galunisertib (LY2157299) sensitized the therapeutic response of metastatic non-small cell lung cancers with downregulated MED12 to RTK inhibitors.<sup>263</sup>

In radiotherapy, ionizing radiation is used to kill or control the growth of malignant cells. Some types of cancer are notably radioresistant, such as glioblastoma, which produces abundant TGF $\beta$  in the microenvironment that leads to the radioresistance of glioma-initiating cells.<sup>264</sup> Radiation can also cause normal tissues to become more fibrotic by inducing the expression and release of

active TGF $\beta$ .<sup>265</sup> Therefore, the combination of anti-TGF $\beta$  therapy with radiotherapy may improve treatment by enhancing tumor cell radiosensitivity and protecting normal tissues.<sup>266,267</sup> In a phase 2 clinical trial, the TGF $\beta$ -blocking antibody fresolimumab (GC1008) was combined with focal irradiation for the treatment of metastatic breast cancer, and it was found to prolong median overall survival and demonstrated a favorable systemic immune response.<sup>268,269</sup> In addition, fresolimumab (GC1008) combined with stereotactic ablative radiotherapy is in a phase 2 clinical trial for early-stage NSCLC treatment (NCT02581787). The combination of the T $\beta$ RI kinase inhibitor LY3200882 with intensity-modulated radiotherapy is currently being tested in a phase 1 clinical trial of patients with solid tumors (NCT02937272).

**Anti-TGF $\beta$  therapy with immunotherapy.** Immune checkpoints are crucial regulators in the process of the immune response. Two types of immune checkpoint signals have been described. Costimulatory signals are mediated by CD27, CD28, CD40, CD134, CD137, and so on, for cytotoxic effector T cell proliferation and their migration toward the tumor.<sup>270,271</sup> Coinhibitory signals increase T cell exhaustion and dysfunction, and this process engages coinhibitory molecules, such as PD-1, cytotoxic T-lymphocyte-associated protein 4 (CTLA4), lymphocyte activation gene 3 (LAG3), T cell immunoglobulin domain and mucin domain 3 (TIM3), and sialic acid-binding immunoglobulin-type lectin 7 (SIGLEC7).<sup>270</sup> Normal physiological context balance costimulatory and coinhibitory signals for maintaining self-tolerance and preventing autoimmunity. However, this balance is perturbed in the TME; coinhibitory signals are used by tumor cells to evade an immune attack. Therefore, costimulatory signal agonists and coinhibitory signal antagonists have been applied in cancer immunotherapy, of which CTLA4, PD-1, and its partner programmed death-ligand 1 (PD-L1) are the most studied.<sup>271</sup> PD-L1 is a transmembrane ligand expressed on the surface of cancer cells, tumor-infiltrating DCs and macrophages, and binds to PD-1 receptor located in the plasma membrane of cytotoxic CD8<sup>+</sup> T cells to suppress effector T cell antitumor function.<sup>272</sup> CTLA4 is a

receptor expressed on T<sub>reg</sub> cells and downregulates immune responses.<sup>273</sup> Immune checkpoint inhibitors (ICIs) are currently at the forefront of cancer therapy, as they display successful long-lasting antitumor efficacy, even for certain metastatic cancers. However, only 15% of patients respond to immunotherapy.<sup>274</sup> Anti-PD-1 therapy not only initiates a tumor rejection program, but also induces a competing TGF $\beta$ -driven immunosuppressive program.<sup>262</sup> TGF $\beta$  has also been found to drive immune response evasion and attenuate the tumor response to anti-PD-L1 therapy by contributing to the exclusion of CD8<sup>+</sup> effector T cells.<sup>275,276</sup> In addition, the unsuccessful antitumor activity of chimeric antigen receptor (CAR) T cell therapy has been attributed in part to TGF $\beta$ -induced immunosuppression.<sup>277</sup> Because of these insights into TGF $\beta$ 's immunosuppressive function, the combination of anti-TGF $\beta$  therapy and immunotherapy is under intensive investigation.

Many preclinical studies have shown a great success in the combination of immunotherapy with anti-TGF $\beta$  therapy (Fig. 6). For example, the T $\beta$ RI kinase inhibitor galunisertib (LY2157299) has been combined with anti-PD-1/L1 immunotherapy in the treatment of breast cancer in a mouse model. The combined treatment showed an efficient antitumor immunity, leading to more persistent and complete responses.<sup>278</sup> The anti-CTLA4-T $\beta$ RII chimera has been studied in preclinical melanoma and TNBC tumor xenograft mouse models and demonstrated superior results with respect to antitumor efficacy than treatment with anti-CTLA4 antibody alone.<sup>279</sup> The TGF $\beta$  ligand trap AVID200 combined with ICIs showed enhanced antitumor immunity in TNBC homograft models.<sup>280</sup> Recently, researchers engineered a bispecific receptor decoy named CD4 TGF $\beta$  Trap that selectively blocks TGF- $\beta$  signaling in CD4<sup>+</sup> T cells and promotes reorganization of tumor vasculature and cancer cell death in a mouse mammary tumor virus promoter-driven Polyoma middle T-antigen transgenic mouse model of breast cancer.<sup>281</sup> SRK-181-mIgG1 is a fully human antibody that selectively binds to latent TGF $\beta$ 1 and inhibits its activation, which avoided toxicities observed with pan-TGF $\beta$  inhibitors.<sup>282</sup> The coadministration of SRK-181-mIgG1 together with anti-PD-1 inhibitors has been applied in the treatment of checkpoint blockade-resistant syngeneic mouse tumors that displayed an increased influx of intratumoral CD8<sup>+</sup> T cells and a lower level of immunosuppressive myeloid cells.<sup>282</sup> Researchers designed pH-responsive clustered nanoparticles that can deliver T $\beta$ RI kinase inhibitor galunisertib (LY2157299) and small interfering RNA targeting PD-L1 to the PDAC stroma microenvironment, and this regimen showed significant antitumor efficiency by both provoking antitumor immunity and suppressing tumor growth in PDAC mouse models.<sup>283</sup> However, despite these highly encouraging results mentioned above, we like to provide a note of caution as different tumor models with different immunogenicity can exhibit totally different, sometimes even opposite, results.<sup>284</sup>

Some therapies in which anti-TGF $\beta$  and immunotherapy are combined have entered clinical evaluations with patients (Table 1). For example, the T $\beta$ RI kinase inhibitor vactosertib (TEW-7197) was combined with the anti-PD-L1/PD-1 monoclonal antibody durvalumab in a phase 2 clinical trial for patients with advanced urothelial carcinoma or NSCLC (NCT03732274), and with the anti-PD-1 antibody pembrolizumab in a phase 1/2 clinical evaluation of patients with metastatic colorectal or gastric cancer (NCT03724851). The combination therapies showed a good safety profile and antitumor activity in both trials.<sup>285,286</sup> SAR439459 is a pan-TGF $\beta$  ligand-neutralizing antibody, and the combination of SAR439459 and anti-PD1 antibody lead to tumor regression in the MC38 tumor-bearing mice model.<sup>287</sup> SAR439459 in combination with the anti-PD1 antibody cemiplimab is now being tested in a phase 1 clinical trials for patients with advanced solid tumors (NCT03192345). NIS793 is a monoclonal antibody blocking TGF $\beta$ 1 and TGF $\beta$ 2 and is being tested in combination with the anti-PD1

antibody PDR001 in a phase 1 clinical trial of patients with advanced malignancies (NCT02947165).<sup>262</sup> M7824 is a bifunctional fusion protein consisting of the ectodomain of T $\beta$ RII, which serves as a TGF $\beta$  ligand trap, and a human monoclonal antibody against PD-L1.<sup>288</sup> The fusion protein can target both TGF $\beta$ - and PD-L1-mediated signaling pathways, and it displayed promising results by eliciting antitumor activity in multiple mouse cancer models.<sup>289</sup> M7824 showed a manageable safety profile and encouraging clinical efficacy in phase 1 clinical trials, including patients with advanced solid tumors, NSCLCs, recurrent glioblastoma, cervical cancer, metastatic TNBC, heavily pretreated CRC, or human papillomavirus (HPV)-associated cancers.<sup>290–296</sup> M7824 is now being evaluated in a phase 2 clinical trial for patients with advanced/metastatic biliary tract cancer, gallbladder cancer, recurrent respiratory papillomatosis, thymoma, CRC, head and neck squamous cell cancer, advanced pancreas cancer, or recurrent prostate cancer.<sup>297</sup> Moreover, the evaluation of M7824 for patients with advanced NSCLC or biliary tract cancer is now under investigation in phase 3 clinical trials.<sup>278,298</sup> A recent study discovered that an anti-GARP:TGF $\beta$ 1 mAb (ABBV151), which selectively blocks TGF $\beta$ 1 production by T<sub>reg</sub> cells, can induce the regression of anti-PD-1 immunotherapy-resistant tumors in a mouse cancer model.<sup>299,300</sup> The phase 1 clinical trial of an anti-GARP:TGF $\beta$ 1 mAb (ABBV151) as monotherapy and in combination with the anti-PD1 mAb budigalimab (ABBV-181) were recently initiated for the evaluation of their safety and tolerability for patients with advanced solid tumors (NCT03821935).

CAR T cell therapy has demonstrated remarkable success by utilizing engineered T cells with tumor antigens that lead to the recognition and attack of tumor cells in blood cancers; however, this method still remains a big challenge for treating solid tumors partially due to TGF $\beta$ -mediated immunosuppression.<sup>301</sup> Inhibiting TGF $\beta$  signaling in CART cells can boost their antitumor efficacy in solid tumors.<sup>302,303</sup> Other strategies also reported overcoming CART cell repression by interfering with TGF $\beta$  signaling, such as coexpression of the dominant-negative T $\beta$ RII,<sup>304</sup> coexpression of T $\beta$ RII-41BB to switch the TGF $\beta$ -suppressive signal to a 41BB stimulatory signal,<sup>305</sup> coexpression of TGF $\beta$  binding single-chain variable fragment,<sup>306</sup> and constitutively active AKT.<sup>307</sup>

Anti-TGF $\beta$  therapy can also be combined with cancer vaccines to enhance treatment efficacy.<sup>308</sup> Vaccination against the EMT transcription factor TWIST1 can induce effector T cell responses and suppress murine tumor growth and spontaneous metastasis.<sup>309</sup> The combination of an adenovirus cancer vaccine encoding tumor-associated antigen TWIST1 (Ad-TWIST) and bifunctional fusion protein M7824 that targets both TGF $\beta$  and PD-L1 displayed superior improvement of the antitumor efficacy as compared to Ad-TWIST monotherapy in multiple murine models of human solid tumors.<sup>310</sup> In addition, a phase 2 clinical trial study of combination therapies with M7824 and therapeutic Tri-Ad (ETBX-011, ETBX-051, and ETBX-061) vaccine for patients with head and neck neoplasms is ongoing (NCT04247282). M7824 has also been combined with the HPV vaccine PRGN-2009 in phase 1/2 clinical trials for subjects with HPV-associated cancers (NCT04432597) and combined with cancer vaccines that target the EMT driver transcription factor brachyury in phase 1/2 clinical trials for patients with advanced breast cancer (NCT04296942) or metastatic castration-resistant prostate cancer (NCT03493945).<sup>291,311,312</sup>

## CONCLUDING REMARKS

TGF $\beta$  was discovered in the late 1970s/early 1980s,<sup>313,314</sup> and its role as a multifunctional regulator of normal and cancer cell growth became apparent shortly thereafter.<sup>315</sup> The purification of TGF $\beta$  and cloning of its complementary DNA, as well as the identification of TGF $\beta$  receptors, paved the way for in vitro and in vivo studies on its mechanism of action and revealed the pleiotropic roles of TGF $\beta$  in controlling pathophysiological processes.<sup>1–4,27,55,316–318</sup> Moreover,

these advances allowed the pharmacological interference of key pathways with neutralizing antibodies against TGF $\beta$  or TGF $\beta$  receptor kinase inhibitors.<sup>319,320</sup> While preclinical studies in mouse models have shown great promise for TGF $\beta$  pharmacological agents, the role of TGF $\beta$  as a tumor suppressor and critical role in maintaining tissue homeostasis have made its clinical translation demanding and prevented TGF $\beta$  targeting strategies from reaching clinical approval for the treatment of cancer patients. Significant progress has been made to (potentially) make treatment with TGF $\beta$ -targeting agents more safer and effective. For example, by performing intermittent dosing strategies bypassing cardiovascular toxicity,<sup>321,322</sup> by specifically inhibiting only the TGF $\beta$ 1 isoform that has the strongest link with cancer progression,<sup>282</sup> or by selecting cancer patients for treatment with a mesenchymal phenotype and high TGF $\beta$  activity that are likely to benefit most from the TGF $\beta$  targeting.<sup>22,250</sup>

We now know that cancer cells frequently escape from the TGF $\beta$ -induced cytostatic response and that TGF $\beta$  drives the EMT of cancer cells. Mesenchymal cancer cells have been linked to metastasis and chemotherapy, targeted therapy, and/or radiotherapy resistance. As most cancer patients die of metastasis and demonstrate relapse after chemotherapy, targeted therapy, and/or radiotherapy, the combination of these therapies with TGF $\beta$  inhibitors are being tested. Moreover, TGF $\beta$  strongly promotes cancer progression by acting on the TME, activating CAFs, stimulating angiogenesis, and eluding the immune system. How TGF $\beta$  inhibitors elicit their anticancer effect in patients is frequently unclear, but its reversal of immunosuppressive activity in the TME might be of key importance. Moreover, as the clinical failure of immune checkpoint inhibitors for cancer treatment has been linked to overactive TGF $\beta$  signaling activity (at least under some circumstances), we are witnessing a profound renewed interest in TGF $\beta$  as a target for cancer therapy. Thus, by combining chemotherapy, targeted therapy, radiotherapy, and immunotherapy with TGF $\beta$ -targeting drugs, treatments can be made more efficient by improving antitumor efficacy and reducing therapy resistance. One aspect remains of key importance, finding reliable biomarkers that enable clinicians to select the best (combinatorial) treatment for each individual cancer patient.

## ACKNOWLEDGEMENTS

We apologize to authors whose papers could not be cited due to space limitations. We are grateful to Dieuwke Marvin and Adilson Fonseca Teixeira for a critical reading and comments. We thank Gerard van der Zon for the immunofluorescent staining images of NMuMG cells. Research on targeting TGF $\beta$  for cancer therapy in our laboratory is supported by the Cancer Genomics Centre Netherlands.

## AUTHOR CONTRIBUTIONS

P.t.D. and S.L. conceived of the paper; S.L. wrote the initial draft of the paper and generated the figures and tables; P.t.D. and J.R. edited and revised the paper. All authors approved the final paper.

## ADDITIONAL INFORMATION

**Competing interests:** The authors declare no competing interests.

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