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Authors

Akhurst, Rosemary J

Hata, Akiko

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Targeting the TGF β signalling pathway in disease

Rosemary J. Akhurst¹ and Akiko Hata²

¹Helen Diller Family Comprehensive Cancer Center, University of California at San Francisco, San Francisco, California 94158, USA

²Cardiovascular Research Institute, University of California at San Francisco, San Francisco, California 94158, USA

Abstract

Many drugs that target transforming growth factor- β (TGF β) signalling have disease applications. Preclinical and clinical studies indicate the utility of these agents in fibrosis and oncology, particularly in augmentation of existing cancer therapies, such as radiation and chemotherapy, as well as in tumour vaccines. There are also reports of specialized applications, such as the reduction of vascular symptoms of Marfan syndrome. Here, we consider why the TGF β signalling pathway is a drug target, the potential clinical applications of TGF β inhibition, the issues arising with anti-TGF β therapy and how these might be tackled using personalized approaches to dosing, monitoring of biomarkers as well as brief and/or localized drug-dosing regimens.

The transforming growth factor- β (TGF β) superfamily of cytokines, which consists of TGF β s, activins, inhibins, Nodal, bone morphogenetic proteins (BMPs), anti-Müllerian hormone (AMH; also known as Müllerian-inhibiting factor) as well as growth and differentiation factors (GDFs), is conserved through evolution and found in all multicellular organisms¹. The TGF β s *per se* are involved in many cellular processes, including growth inhibition, cell migration, invasion, epithelial-mesenchymal transition (EMT), extracellular matrix (ECM) remodelling and immune-suppression². However, although normally dynamically regulated and involved in maintenance of tissue homeostasis, TGF β s are often chronically over-expressed in disease states, including cancer, fibrosis and inflammation, and this excessive production of TGF β drives disease progression by modulating cell growth, migration or phenotype. The TGF β signalling pathway has therefore become a popular target for drug development.

Knowledge about cellular activities gleaned from studying one disease is often applicable to others. For example, inhibition of TGF β -induced EMT — a process that contributes to cancer progression — is a goal not only of oncologists but also of cardiovascular surgeons to prevent neointimal hyperplasia, and of nephrologists and pneumologists in the treatment of fibrosis³. In addition, the immune-modulatory activities of TGF β have implications in many diseases, including cancer, cardiovascular disease, asthma, rheumatoid arthritis and multiple sclerosis⁴.

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Correspondence to R.J.A.RAkhurst@cc.ucsf.edu.

Competing interests statement

The authors declare no competing financial interests.

Rosemary J. Akhurst's homepage: <http://cancer.ucsf.edu/research/akhurst-lab> Akiko Hata's homepage: <http://www.cvri.ucsf.edu/Scientist/Hata.shtml> ClinicalTrials.gov website: <http://www.clinicaltrials.gov> Flamel Technologies and Digna Biotech Announce Multiple Product Development Agreement (27 June 2011 press release): <http://www.dignabiotech.com/comun/fichero.asp?id=105>

TGF β action is highly context-dependent and influenced by cell type, culture conditions, interaction with other signalling pathways, developmental or disease stage *in vivo* and innate genetic variation among individuals⁵⁻⁹. This makes the pathway a particular challenge for drug development. Nevertheless, over the past decade several drugs targeting the TGF β signalling pathway have been developed by pharmaceutical companies and biotechnology firms alike. Drug design strategies have been numerous and include the development of small-molecule inhibitors (SMIs) and monoclonal antibodies, as well as the inhibition of gene expression; some drugs have reached Phase III clinical trials for a number of disease applications, particularly fibrosis and oncology. There is an increasing number of preclinical examples of TGF β inhibitors that are capable of reducing cancer progression and metastasis, and that augment existing cancer therapies (such as radiation therapy in breast cancer) while simultaneously guarding against radiation-induced fibrosis¹⁰. Additionally, there are novel reports of targeting TGF β signalling in less prevalent indications, such as reduction of vascular symptoms of Marfan syndrome (MFS)^{11,12}. Although there have been many reviews on the pleiotropic action of TGF β during tumorigenesis, which is characterized by tumour-suppressing activity of TGF β at an early stage of cancer and tumour-promoting activity at later stages¹³⁻¹⁶, few focus specifically on drug targets, drug classes and possible therapeutic applications beyond the oncology arena. The translation of anti-TGF β therapies has been pursued most intensively for oncology; however, this Review also discusses the potential of the TGF β signalling pathway as a target for non-neoplastic disease therapies and addresses the associated challenges in the development and application of these strategies.

The TGF β family

The vertebrate genome contains more than 30 pleiotropic ligands that belong to the TGF β superfamily, including TGF β s, BMPs, GDFs, activins, inhibins, Nodal and AMH¹. TGF β has a conserved motif of nine cysteine residues, eight of which form a tight cysteine knot, with the ninth being crucial for homodimerization². Aberrant expression and activity of many of the ligands of the TGF β superfamily are associated with developmental defects and human diseases¹⁷. Here we focus on TGF β s as there are currently several clinical trials underway involving therapies targeting TGF β signalling, whereas other members of the TGF β superfamily are under-represented in current trials.

Three highly homologous isoforms of TGF β exist in humans: TGF β 1, TGF β 2 and TGF β 3. They share a receptor complex and signal in similar ways but their expression levels vary depending on the tissue¹⁸, and their functions are distinct as demonstrated by the phenotypes of knockout mice¹⁹⁻²³. Each TGF β ligand is synthesized as a precursor, which forms a homodimer that interacts with its latency-associated peptide (LAP) and a latent TGF β -binding protein (LTBP), forming a larger complex called the large latent complex (LLC). The TGF β activation process involves the release of the LLC from the ECM, followed by further proteolysis of LAP to release active TGF β to its receptors². Matrix metalloproteinase 2 (MMP2) and MMP9 are known to cleave latent TGF β . In addition to MMPs, thrombospondin 1 (THBS1) is known to activate latent TGF β ²⁴. Alternatively, upon mechanical stretch, α V β 6 integrin can activate TGF β by binding to the RGD motif present in LAP and inducing the release of mature TGF β from its latent complex^{25,26}.

TGF β signalling

Proteolytic cleavage, interaction with integrins or pH changes in the local environment are known to activate latent TGF β and free active TGF β for binding to its receptors at the cell membrane. TGF β superfamily members signal via heteromeric complexes of two related transmembrane type I and type II serine/threonine kinase receptors. Five type II receptors and seven type I receptors (also termed activin receptor-like kinases (ALKs)) have been

identified. Auxilliary co-receptors (also known as type III receptors) that regulate the access of TGF β superfamily members to signalling receptors also exist. Each subfamily of the TGF β superfamily of ligands binds to type I and type II receptors (Box 1). BMPs can bind to type I receptors alone and, in their absence, can weakly bind to type II receptors, but they show highest affinity when both receptors act together. TGF β and activin display high affinity only for type II receptors and do not normally interact with isolated type I receptors. Binding to the extracellular domains of type I and type II receptors by the dimeric ligand induces close proximity and a productive conformation for the intracellular serine/threonine kinase domains of the receptors, facilitating the phosphorylation and subsequent activation of the type I receptor. The activation of the type I receptor leads to the propagation of signalling by at least two seemingly independent routes: the SMAD-dependent canonical pathway (Box 1; Fig. 1) and the SMAD-independent or non-canonical pathways (Box 2; Fig. 2).

In the SMAD-dependent pathway, activation of TGF β receptor type I (T β RI; also known as TGFBR1 and ALK5) leads to phosphorylation of receptor-specific SMAD (R-SMAD) proteins. SMAD2 and SMAD3 are substrates of T β RI, whereas type I receptors for BMPs utilize SMAD1, SMAD5 and SMAD8 (Fig. 1). Upon phosphorylation by the receptor, R-SMADs together with the common mediator SMAD4 (co-SMAD) translocate to the nucleus, where they interact with other transcription factors (cofactors) to regulate transcriptional responses²⁷ (Fig. 1). In addition to the canonical role of SMADs as transcription factors, a novel role for R-SMADs in the post-transcriptional regulation of microRNA (miRNA) biogenesis has been identified²⁸ (Fig. 1). Therefore, the canonical TGF β -SMAD pathway modulates gene expression both transcriptionally and post-transcriptionally to propagate the physiological and pathological activities of TGF β . In the non-canonical pathway, the activated TGF β receptor complex transmits a signal through other factors, such as tumour necrosis factor (TNF) receptor-associated factor 4 (TRAF4), TRAF6, TGF β -activated kinase 1 (TAK1; also known as MAP3K7), p38 mitogen-activated protein kinase (p38 MAPK), RHO, phosphoinositide 3-kinase (PI3K), AKT (also known as protein kinase B), extracellular signal-regulated kinase (ERK), JUN N-terminal kinase (JNK) or nuclear factor- κ B (NF- κ B) (Box 2; Fig. 2). Thus, cellular responses to TGF β signalling result from the dynamic combination of canonical and non-canonical signalling cascades. In addition to the complexity generated by the canonical and non-canonical TGF β signalling pathway, TGF β signalling can be influenced by different signalling pathways, including the PI3K-AKT, WNT, Hedgehog (HH), Notch, interferon (IFN), TNF and RAS pathways (Box 2; Fig. 2). Interactions with several of these pathways can change the output of TGF β signalling from suppressing growth to inducing cellular plasticity²⁹. Nuclear accumulation and transcriptional activity of R-SMADs can also be negatively regulated through phosphorylation of multiple Ser-Pro and Thr-Pro residues (in the linker region connecting the MH1 and MH2 domains) by ERK, MAPKs, calcium/calmodulin-dependent protein kinase II and cyclin-dependent kinases (CDKs)³⁰. The mode and outcome of the crosstalk between TGF β and other signalling pathways vary considerably but are essential to define the activities of TGF β in propagating spatially and temporally specific outputs^{6,31,32}.

Biological actions of TGF β

TGF β is involved in a range of biological processes both during embryogenesis and in adult tissue homeostasis. Although the physiological roles of TGF β have been extensively reviewed elsewhere^{16,33-36}, the major functions of TGF β that are relevant to the topic of this Review are briefly outlined below.

Inhibition of cell proliferation

TGF β strongly inhibits the growth of many cell types, including epithelial, endothelial, haematopoietic and immune cells^{37,38}. TGF β also has pro-apoptotic and differentiation-inducing actions on epithelial cells; together, these actions result in tumour suppression in the context of cancer³⁴. TGF β in epithelial cells activates transcription of cyclin-dependent kinase inhibitor 1A (*CDKN1A*) and *CDKN2A* (which encode p21^{CIP1} and p15^{INK4B}, respectively) to mediate cell cycle arrest at the G1 phase³⁹. Conversely, TGF β represses the transcription of *MYC*, which encodes a potent transcriptional activator of genes that is required for cell proliferation and growth, and inhibitor of DNA binding (ID) family genes, which encode transcription factors that promote cell differentiation and determination⁴⁰. In oncology, many tumours attenuate TGF β growth-inhibitory effects but respond to this ligand in a pro-tumorigenic manner. Thus, depending on the tumour type and the stage of tumour progression, TGF β may provide potent tumour-suppressive or tumour-promoting functions directly on the tumour cell, presumably by mediating differential gene expression programmes (Fig. 3).

Unlike the role of TGF β signalling during tumorigenesis, the contribution of TGF β to vascular disease is more complex and confusing. Studies on clinical samples from vascular disorders, such as atherosclerosis, hypertension and pulmonary hypertension, often find signatures of both upregulation and downregulation of TGF β signalling, as well as complex interactions between this pathway and other ligands of the TGF β family, such as BMPs (Box 3). This has been confirmed by *in vitro* studies, demonstrating the contradictory effects of TGF β in the regulation of vascular cells^{36,41}. Furthermore, the TGF β pathway often exhibits contrasting effects in different vascular cell types, such as endothelial versus vascular smooth muscle cells³⁶. The promiscuous and cell type-specific action of the TGF β pathway on vascular cells makes the application of targeted TGF β signalling therapies for cardiovascular disease a particular challenge.

Induction of epithelial-mesenchymal transition and the myofibroblast phenotype

TGF β can induce an EMT of both epithelial and endothelial cells. This has consequences for disease progression in both cancer and fibrosis³. EMT enhances cellular migration and invasive properties, as cell migration requires loss of cell-cell contacts and acquisition of fibroblastic characteristics. E-cadherin is commonly downregulated in many cancers, and its overexpression can suppress invasion by tumour cells. The TGF β -SMAD pathway mediates the expression of high mobility group AT-hook 2 (HMGA2), which is important for the induction of SNAIL (also known as SNAI1) and SLUG (also known as SNAI2): two zinc-finger transcription factors that are known to repress the E-cadherin gene³³. In breast and skin cancer, tumour cell EMT contributes to cancer progression as cells consequently become more migratory and invasive, and they can ultimately transition to a myofibroblastic phenotype³. The myofibroblast further modulates the basic biology of the tumour by increasing ECM elaboration and eliciting a tissue contraction process, which results in increased interstitial fluid pressure (IFP). This has consequences for the efficiency of drug delivery to the tumour⁴², as drugs cannot penetrate tissue under positive IFP. EMT can also polarize carcinoma cells towards 'stem cell-like' properties, such as increased tumour-initiating capacity and tumour cell drug resistance⁴³. Blocking the TGF β pathway can thus have a threefold benefit: the reduction of tumour invasion and metastasis; the suppression of cancer stem cell-like properties; and the restoration of negative IFP to enhance chemotherapeutic drug delivery⁴⁴.

In fibrotic conditions, excessive TGF β production induced in the diseased state contributes to EMT elaboration, which can further exacerbate fibrosis, as seen in pulmonary^{45,46}, cardiac⁴⁷ and renal^{48,49} fibrosis, and in arterial restenosis following surgical trauma⁵⁰.

TGF β can also promote a proliferative and/or migratory phenotype on smooth muscle cells that can aggravate some vascular diseases, including neointimal formation following vascular surgery⁵¹⁻⁵³.

Extracellular matrix regulation

The ECM is a complex structure that surrounds mammalian cells. It is the major component of connective tissue and is composed of multiple proteins, such as collagen, elastin, fibrillin, fibronectin, lamin and proteoglycans. Fibrosis is characterized by the accumulation of fibroblasts, which secrete excessive amounts of ECM. As TGF β is widely documented to increase collagen synthesis and deposition by fibroblasts, TGF β has become a central therapeutic target for different types of fibrosis. TGF β activity and the synthesis of ECM proteins are mutually regulated. Several genes encoding ECM proteins that are known to be important in driving fibrosis are directly regulated by TGF β -SMAD signalling pathways. There is a reciprocal regulation of TGF β by the ECM: latent TGF β bound to ECM components, such as fibronectin and fibrillin, is inactive until physiological or pathological processes initiate its release. This is seen in MFS, in which the mutation of a fibrillin-encoding gene results in reduced fibrillin levels and a consequent increase in levels of unbound TGF β ; this, in turn, leads to the activation of TGF β signalling, which is possibly responsible for the aetiology of many Marfanoid features^{11,12}.

Immune-suppression and inflammation

The lethal postnatal inflammatory phenotype of *Tgfb1*-knockout mice^{19,20,54} demonstrates the important immune-suppressor function of this ligand. The widespread expression profile of TGF β receptors on all immune cell types suggests that they have broad activities, including responses in cytotoxic CD8⁺ effector T cells, CD4⁺ effector T helper 1 (T_H1) and T_H2 cells, suppressive regulatory T (T_{Reg}) cells, natural killer (NK) cells, monocytes, macrophages, neutrophils and eosinophils (Fig. 4). Cell type-specific mouse gene knockout studies with *Tgfb2* demonstrate both direct and indirect actions of TGF β on effector T cells⁴.

TGF β has potent growth-suppressing activity on most precursor cells of the immune system, particularly T and B cells of the adaptive arm. TGF β is a potent suppressor of T cell proliferation⁵⁵ and an inducer of B cell apoptosis⁵⁶. Additionally, the ligand can alter the course of immune cell differentiation. Suppressive T_{Reg} cells that are driven by the expression of the transcription factor forkhead box protein P3 (FOXP3) are crucial for maintenance of peripheral immune tolerance as well as regulation of tumour immunity and infection. In CD4⁺ T cells, *Foxp3* expression is positively but indirectly regulated by TGF β 1 through enhanced binding of the SMAD2-induced transcription factor E2A to the *Foxp3* gene promoter, and by relief from GATA3-mediated transcriptional inhibition of the *Foxp3* promoter by competition with TGF β -induced *Id3* (Ref. 57). TGF β suppresses inflammatory T_H1 and T_H2 cell differentiation while stimulating suppressor T_{Reg} cells. Overall, TGF β -mediated suppression of effector CD8⁺ cytolytic cells and T_H cells, together with TGF β dependence for suppressive T_{Reg} cell differentiation, results in the hyper-inflammatory phenotype seen in *Tgfb1*^{-/-} mice.

During tumour progression, excess TGF β suppresses immune surveillance by attenuating the antitumour functions of CD8⁺ T cells, CD4⁺ T cells and dendritic cells. CD4⁺ T cell-specific ablation of TGF β signalling in transgenic mice expressing dominant negative T β RII (DNRII; also known as CD4- Δ T β RII and CD4- Δ TGFB2) led to the development of autoimmunity⁵⁸ and enhanced the differentiation of CD8⁺ cytotoxic T lymphocytes (CTLs). When challenged with tumour cells, these transgenic mice raised a greater tumour-specific CTL response than wild-type littermates⁵⁸. Tumour-derived TGF β also blocks the

differentiation of antigen-presenting dendritic cells⁵⁹ and modifies chemokine receptor expression to blunt dendritic cell chemotaxis⁶⁰, further suppressing immune surveillance.

In addition to having a predominant immune-suppressive function, TGF β counterintuitively may have a pro-inflammatory role through its effects on T_H17 cells and cells of the innate immune system. TGF β , together with interleukin-6 (IL-6), was reported to be an essential player in driving pro-inflammatory T_H17 lineage differentiation⁶¹⁻⁶³. However, there is considerable controversy surrounding this topic. First, different laboratories cannot agree on the specific functions of various T_H1, T_H2 and T_H17 cell types in disease progression. T_H17 cells were implicated as major agonists in inflammatory diseases, including inflammatory cancer, asthma and autoimmune disorders⁵⁵. However, recent studies suggest that the active player in disease progression is in fact a T_H17-derived T_H1 cell, or a 'T_H1-T_H17' cell⁶⁴. Second, the role of TGF β in regulating the balance between T_H1 and T_H17 differentiation is in dispute. Despite the widespread acceptance of a role for TGF β in T_H17 differentiation⁶¹⁻⁶³, more recent studies have suggested that TGF β is totally dispensable for the generation of these cells^{65,66}.

With respect to cells of the innate immune system, TGF β directly suppresses NK cell-mediated production of IFN γ (which is required for the tumour killing activity of NK cells) through transcriptional effects of SMAD3 on the IFN γ promoter⁶⁷. It also 'polarizes' macrophages⁶⁸ and neutrophils⁶⁹ from a type I, productive phenotype (that evolved to attack and devour foreign agents such as cancer cells) towards a type II phenotype (that has reduced effector function but produces large quantities of inflammatory molecules, such as IL-6, IL-11 and TGF β). These molecules can exacerbate the local diseased state, resulting in solid tumour progression or inflammation associated with fibrosis or atherosclerosis⁴.

In summary, the regulation of the immune system by TGF β is highly complex and context-dependent. It delicately regulates the tolerogenic versus immunogenic arms of the immune system to balance adequate host defence while limiting collateral inflammatory tissue damage. The molecular details of this regulation have been recently reviewed in depth^{4,55,64}.

Targeting TGF β signalling

Virtually every component of the TGF β pathway has been targeted for drug development (Fig. 5) through numerous design strategies (Fig. 6). Several have been developed through preclinical to clinical trials (Table 1) and many more have been tested only in preclinical systems (Table 2). The drugs that have progressed furthest in clinical development include anti-ligand antisense oligonucleotides (ASOs) from Antisense Pharma⁷⁰⁻⁷⁴, ligand-competitive peptides from Digna Biotech⁷⁵⁻⁷⁸, antibodies that target ligands, receptors or associated proteins spearheaded by Genzyme⁷⁹⁻⁸¹, and SMIs against TGF β receptor kinases developed by many companies, with Eli Lilly having an active clinical programme in Phase II development⁸². The various approaches currently being investigated are discussed in more detail below.

Antisense oligonucleotides and antisense RNA

Antisense Pharma uses the strategy of targeting mRNA translation using ASOs to downregulate ligand synthesis^{70,83}. Its focus has been on targeting TGF β 2, which is produced in excessive quantities by glioblastoma and pancreatic carcinoma cells. Trabedersen (AP12009), a synthetic 18-mer phosphorothioate ASO, binds specifically to human *TGFB2* mRNA, and this drug has progressed to a Phase III clinical trial for oncology applications (Box 4). One of the challenges of this drug is delivering it directly to the tumour to avoid the off-target toxicity associated with systemic delivery of first-generation ASOs. In the case of glioblastoma, this was achieved using intrathecal catheter delivery directly into

the tumour⁷⁴. More recently, the company has started developing intravenous delivery approaches for pancreatic cancer, which appear to be effective in mouse models⁷³ and were recently shown to be safe in humans⁸⁴.

An anti-*TGFB2* antisense strategy has also been used to generate augmented tumour vaccines. Belagenpumatucel-L (Lucanix; NovaRx) is such a drug, in which an ~900-nucleotide *TGFB2* antisense construct is transfected into allogeneic non-small-cell lung cancer (NSCLC) cells, which are then used as a tumour vaccine. Here, drug delivery is not an issue as the 'drug' is in fact genetically engineered NSCLC tumour cell lines. This tumour vaccine has superior activity compared to conventional tumour vaccination approaches^{85,86}. A significant dose-related survival difference was seen in patients who received 2.5×10^7 cells per injection, allowing progression to a Phase III clinical trial⁸⁷.

Monoclonal antibodies

The advantages of monoclonal antibodies are their specificity and extracellular mechanism of action — an advantage when trying to mop up excess extracellular ligand. This is tempered by the less convenient intravenous mode of delivery. However, prolonged pharmacokinetic stability permits infrequent drug administration. Cambridge Antibody Technologies and Genzyme developed humanized (or murinized for preclinical studies) monoclonal antibodies specific to individual ligands, such as lerdelimumab (CAT-152)^{88,89} and metelimumab (CAT-192)⁹⁰, or with pan-ligand specificity, such as fresolimumab (GC-1008)⁹¹⁻⁹³. These antibodies have proceeded through various stages of preclinical and clinical development. Of these three humanized antibodies, fresolimumab has progressed furthest in the clinic for both neoplastic and non-neoplastic applications. This drug was found to be well tolerated and safe at 15 mg per ml in Phase I trials for metastatic melanoma (MetM) plus renal cell carcinoma⁹³ and at 1 mg per ml for the fibrotic disorder focal segmental glomerulosclerosis⁹². Lerdelimumab^{88,89} and metelimumab⁹⁰, despite passing safety tests, failed to show efficacy in fibrotic models of corneal scarring and systemic sclerosis, respectively, and were therefore discontinued⁹⁰. Despite a promising Phase I oncology trial of fresolimumab, after Genzyme was acquired by Sanofi the company made the decision to focus on fibrotic applications of this drug.

Eli Lilly entered the monoclonal antibody arena with a TGFβ1 ligand-selective blocking antibody, LY2382770, which has progressed to Phase II trials for kidney fibrosis (Table 1). Since merging with ImClone, Eli Lilly has also developed a TβRII-blocking antibody, IMC-TR1 (Ref. 94), which has just entered clinical trials for breast and colon cancer (ClinicalTrials.gov identifier: NCT01646203). In addition, Biogen Idec and Stromedix have developed an anti-integrin β6 antibody that prevents the activation of TGFβ and has been used efficaciously in preclinical studies of fibrosis and cancer⁹⁵; it is in a Phase II trial for fibrosis (ClinicalTrials.gov identifier: NCT01371305).

Ligand traps and peptides

Genzyme developed a ligand trap by fusing Fcγ to the extracellular domain of TβRII, but this construct never reached clinical trials⁹⁶. However, an alternative ligand trap approach, pursued by Digna Biotech, using peptide mimetics of TβRIII (also known as betaglycan and TGFBR3), completed a Phase IIa clinical trial for scleroderma and skin fibrosis, showing safety and efficacy when topically applied to skin (Table 1; Box 4). This company has plans to extend to Phase IIb/III trials in 2013 (J. Dotor, personal communication). A peptide antagonist of TGFβ activation, LSKL (Leu-Ser-Lys-Leu), binds to a conserved sequence in the LAP region of the latent complex and has demonstrated efficacy in reducing TGFβ signalling *in vitro*⁹⁷. This antagonist is based on thrombospondin and specifically blocks TGFβ activation. The issue of peptide drug delivery is not a problem for topical application;

however, to progress to systemic delivery, Digna Biotech has partnered with Flamel Technologies to investigate proprietary peptide delivery systems.

Small-molecule inhibitors

There are a plethora of SMIs that specifically target the type I receptor of TGF β to inhibit the phosphorylation of SMAD2 and SMAD3 while keeping at least some non-canonical responses, such as TAK1 activation, intact. These drugs are generally ATP mimetics that bind competitively within the hydrophobic ATP binding pocket of the receptor kinase. The chemistry of these compounds has been extensively reviewed^{98,99} and some molecular structures are shown in Fig. 6. The obvious advantages of these molecules over most others are their economical production, stability and ease of oral administration, set against a possible disadvantage of cross-inhibition of other kinases. The short half-life of these drugs provides the possibility of rapid drug withdrawal should adverse events arise. Many successful preclinical studies for metastatic cancer have been undertaken with these SMIs, as reviewed previously^{100,101}. However, the only company to continue pursuit of a T β RI-targeted SMI into clinical trials for oncology is Eli Lilly with LY2157299 (Ref. 82) ([ClinicalTrials.gov](https://clinicaltrials.gov) identifier: NCT01373164).

Other approaches

A novel approach to the suppression of ligand production has been the preclinical development of pyrrole-imidazole polyamides that bind with sequence specificity to the *TGFB1* gene promoter to attenuate gene expression^{50,102,103}. These large ~17 kDa polymeric molecules (Figs 5,6) bind within the minor groove of DNA to prevent transcription factor binding. Challenges associated with these drugs include the specificity of promoter binding, along with drug delivery issues owing to their large molecular size and the high local concentration required for activity. However, preclinical studies suggest that these molecules might be used in drug-eluting stents for the purpose of reducing restenosis after coronary or carotid artery surgery⁵⁰.

An alternative approach to suppress TGF β signalling is gene transfer of antagonizing signalling molecules, such as the inhibitory SMAD7. This approach has been applied in model systems to treat or prevent various pathological conditions, including colonic and hepatic fibrosis, vascular remodelling and diabetic kidney disease^{104,105}. Such an application has the potential to be applied to many other systemic diseases to attenuate the activity of the TGF β pathway, with the caveat that gene therapy is still far from being widely accepted as a therapeutic approach¹⁰⁶.

As an approach to stimulate immune destruction of cancer cells by tumour-infiltrating T cells, human tumour antigen-specific CTLs have been engineered to express DNRII using a clinical grade retrovirus vector. TGF β -resistant CTLs were found to have a functional advantage over unmodified CTLs in clearing TGF β -secreting Epstein-Barr virus (EBV)-positive lymphoma *in vitro* and *in vivo*¹⁰⁷, and this approach to therapy has progressed to a Phase I clinical trial for EBV-positive lymphoma. A further modification of the CTLs, by engineering in an HER2 (also known as ERBB2) chimeric receptor as well as a DNRII, allows the CTLs to target HER2-positive tumour cells¹⁰⁸⁻¹¹¹. This approach is in a Phase I clinical trial for advanced HER2-positive lung malignancy, labelled the HERCREEM trial ([ClinicalTrials.gov](https://clinicaltrials.gov) identifier: NCT00889954).

Finally, Renova has developed a recombinant TGF β 3 ligand as an anti-scarring agent on the basis of the hypothesis that this ligand has activity that is independent of and antagonistic to TGF β 1 (Ref. 112). The drug, administered by injection around a surgical wound site,

progressed to a Phase III clinical trial, but unfortunately it did not reach its primary or secondary efficacy end points.

Pre-existing drugs that inhibit TGF β

Pre-existing drugs that have been extensively used for other applications may act, in part, by inhibiting TGF β . Examples are losartan and candesartan, which are angiotensin type II receptor inhibitors that were originally developed for the treatment of hypertension. Both appear to reduce TGF β signalling, although the precise molecular mechanisms of this action are still unclear^{12,113-115}. Pirfenidone acts in part by reducing the fibrotic effects of TGF β ¹¹⁶ via unknown targets. It is the first approved drug in Europe for idiopathic pulmonary fibrosis (IPF), and is in a Phase III trial in the United States^{117,118}. On the other side of the coin, some common drugs, including aspirin, elevate circulating TGF β levels, which — in certain cases such as arteriosclerosis — correlates with disease suppression¹¹⁹.

Therapeutic uses of TGF β signalling inhibition

Cancer

TGF β has a biphasic action during tumorigenesis, suppressing tumorigenesis at early stages but promoting tumour progression later on (Fig. 3). This is a paradigm for the action of TGF β during disease progression in general, including that of fibrosis, inflammation and cardiovascular disease, and it is rooted in the fact that the normal function of this ligand is in the regulation of homeostasis. During disease progression, TGF β signalling can go into override and, once unharnessed, results in more damage than good. The main goal in cancer therapy is therefore to downmodulate excessive levels of TGF β ligands.

A major challenge in developing TGF β inhibitors for cancer therapy has been the fact that these compounds are not cytotoxic or cytostatic to most tumour cells *in vitro*. They were developed to target properties of the tumour that are required for cancer progression, including migration, invasion and metastasis, as well as effects on the tumour microenvironment (Figs 3,4). Standard cytotoxic screens used by the pharmaceutical industry to identify anticancer drugs were therefore not relevant, and therapeutic utility could only be determined by *in vivo* efficacy in animal models and ultimately in the clinic.

Two major concerns in TGF β drug development have been the inadvertent inhibition of the tumour-suppressing arm of TGF β signalling in cancer¹²⁰⁻¹²² and the development of adverse side effects unrelated to cancer, such as widespread inflammation, autoimmunity or cardiovascular defects that have been revealed by mouse gene knockout studies^{19-21,123}. Preclinical studies suggested that attenuation of TGF β -mediated growth inhibition would not be a major issue^{96,124,125}. However, clinical trials to date⁸² have not revealed the cardiac valvulopathy¹²⁶ or hyperostosis and chondrocyte hypertrophy and hyperplasia¹²⁷ observed in rat preclinical toxicology studies. Moreover, there has been no widespread evidence of inflammatory complications in clinical trials reported to date^{54,82}. These reassuring safety findings are supported by evidence from patients with the rare disease multiple self-healing squamous epithelioma (MSSE), who have germline-null mutations in the gene encoding T β RI but develop only self-limiting and mostly non-malignant skin lesions¹²⁸. Intriguingly, in a Phase I clinical trial of GC-1008 for the treatment of MetM, patients developed skin lesions, keratoacanthoma or squamous cell carcinoma (SCC) that were similar to the skin abnormalities reported in MSSE, with the appearance of keratoacanthoma and SCC seemingly influenced by the extent of exposure to GC-1008. These lesions, which appeared on sun-damaged skin, were manifested in approximately 25% of patients who received higher dose levels of GC-1008 and/or longer exposure to the drug, and the lesions resolved on drug withdrawal^{91,93}. To put this toxicity into context, non-melanoma skin cancers, such as SCC and keratoacanthoma, develop in approximately 15-30% of patients with MetM who

are treated with BRAF inhibitors such as vemurafenib and dabrafenib¹²⁹, and therapy with sorafenib and TNF antagonists produced similar findings^{130,131}. Recent data from studies with vemurafenib for MetM therapy suggest that these lesions arise from pre-existent mutant RAS-containing cells within sun-damaged skin¹³². Intriguingly, one study of keratoacanthoma that appeared in sorafenib-treated patients showed somatic *TGFBR1* missense mutations¹³³, one of which was also identified as a causative germline mutation for MSSE¹²⁸.

Cancer ‘stem cells’, or tumour-initiating cells (TICs), are defined by their capacity to self-renew and to initiate and persistently propagate the entire tumour. Targeting the cancer stem cell for destruction or irreversible quiescence is therefore the Holy Grail of oncology, especially as these cells are exceedingly resistant to both chemotherapy and radiotherapy, and are responsible for tumour metastasis and recurrence after therapy¹³⁴. Several groups have now reported the phenomenon that TGFβ-induced EMT can drive tumour cells towards a more ‘stem cell-like’ phenotype characterized by increased expression of stem cell markers and enhanced tumour-initiating activity *in vitro* and *in vivo*^{43,135}. In breast cancer¹³⁵, the TGFβ and WNT signalling pathways were shown to be the most commonly activated signalling pathways in cancer stem cells that had been fractionated from the bulk tumour on the basis of expression of stem cell markers such as CD44^{hi} and CD24^{low}. In preclinical studies, TGFβ inhibitors have been shown to deplete the stem cell compartment in various cancers — including breast cancer¹³⁵, glioblastoma¹³⁶⁻¹³⁸ and chronic myeloid leukaemia¹³⁹ — which leads to increased lifespan in several mouse models of metastatic cancer. Anido *et al.*¹³⁷ showed that glioblastoma-initiating cells (GICs, which express the stem cell markers CD44, ID1, ID3, SOX2 and SOX4) responded to LY2109761 by downregulating the expression of ‘stem cell’ genes. Moreover, patient-derived glioblastoma neurospheres transplanted orthotopically into non-obese diabetic/severe combined immunodeficient mice (NOD/SCID mice, which do not have T cells or B cells) responded to LY2109761 by decreasing in size and reducing their expression of stem cell markers¹³⁷. The same research team is currently undertaking a Phase I/II clinical trial for glioblastoma using the closely related drug LY2157299 (Ref. 137). Importantly, they showed a reduction of CD44 and ID1 RNA levels after 2 months of LY2157299 treatment in tumour biopsy material from one patient with glioblastoma for whom a salvage surgical resection was performed both before and after 2 months on the trial¹³⁷. The ability to reduce the number of stem cells in an aggressive tumour such as glioblastoma is a major coup.

It has been argued that TGFβ inhibitors might, however, release isolated and disseminated tumour (stem) cells from dormancy by initiating proliferation and/or disrupting the stem cell niche. A couple of recent studies may give credence to this notion, as systemic TGFβ inhibition resulted in increased numbers of circulating tumours as well as micro- and macro-metastases in mouse models of head and neck SCC and mammary cancer *in vivo*^{140,141}. It might therefore be wise to use TGFβ inhibitors in combination with cytotoxic drugs to coax tumour cells out of their quiescent niche while simultaneously targeting those that respond proliferatively to TGFβ inhibition using chemotherapy. This strategy may be highly beneficial for ‘flushing out’ dormant disseminated tumour cells, as alluded to by Carlos Arteaga many years ago¹⁴². A further cautionary note is warranted, however, on the basis of two reports indicating that TGFβ may decrease the cancer-initiating cell population of diffuse type gastric carcinoma¹⁴³ and breast carcinoma¹⁴⁴ despite having little or no effect on cellular proliferation. Finally, TGFβ inhibitors might act on the stem cell niche by recruiting bone marrow mesenchymal stem cell-derived myofibroblasts that home in on the primary tumour, contribute to the tumour microenvironment as cancer-associated fibroblasts and consequently promote tumour progression¹⁴⁵. Clearly there are tissue- and cell type-specific effects of TGFβ inhibition that can influence the action of TGFβ on the cancer stem cell and its niche¹⁴⁶. Understanding the differential molecular mechanisms that elicit these

variable responses will be critical to a judicious choice of treatment with TGF β inhibitors or their derivatives. As TGF β inhibitors are not directly cytotoxic, the use of these inhibitors in combination with cytotoxic chemotherapeutics may be particularly efficacious. The activation of TGF β signalling in response to chemotherapeutics may drive the generation of cancer stem cells (via EMT), resulting in their chemoresistance¹³⁴. This event may be targeted with TGF β inhibitors, as demonstrated by the synergistic activity of doxorubicin and TGF β inhibitor combination therapy on breast cancer growth and metastasis¹⁴⁷. Studies in multiple myeloma also suggest that TGF β inhibitors could potentiate the cytotoxic effects of melphalan and dexamethasone¹⁴⁸. *In vitro*, the exposure of multiple myeloma cells to differentiated versus immature MC3T3-E1 pro-osteoblastic cells potentiated chemotherapy-induced multiple myeloma cell death. As TGF β inhibition acts within the bone microenvironment to elicit osteoblastic differentiation^{148,149}, this combinatorial approach holds great promise for the treatment of multiple myeloma and other bone metastatic cancers. Likewise, in a mouse model of serous gastric cell carcinoma, Ki26894 had an additive effect with a fluorouracil analogue in reducing tumour growth¹⁵⁰. Finally, another mechanism whereby TGF β inhibition can augment conventional therapies is in enhancing drug delivery to the tumour. There are reports that TGF β inhibition can reduce interstitial tumour pressure⁴⁴, which enhances the delivery of SMIs, and regulates vascular leakiness, which enhances the delivery of nanoparticle-encapsulated drugs, particularly in highly fibrotic and drug-refractile tumour types such as pancreatic cancer¹⁵¹.

Adoptive T cell therapy involves the harvesting and *ex vivo* expansion of autologous tumour-specific CTLs followed by their reintroduction into the patient to stimulate tumour killing¹⁵². Used most extensively in the treatment of MetM and lung cancer, this therapy often fails owing to the apoptosis of re-grafted CTLs. Preclinical studies suggest that failure may be due to the direct effects of TGF β on CTLs¹⁵³, and strategies to prevent such failure include the use of genetically modified CTLs with reduced TGF β responsiveness. Transduction of CTLs with a virus encoding a DNRII¹⁵⁴ has reached Phase I clinical trials, and recent preclinical data indicate that combining CTL therapy with T β RI-targeting SMIs may also significantly improve T cell survival and antitumour T cell cytotoxicity¹⁵⁵. Augmenting adoptive T cell therapy with SMIs may be a particularly attractive application of T β RI SMIs as patients need not be exposed to genetically engineered T cells. Moreover, patients might only require short-term exposure to the drug for efficacy in this application, thus avoiding the side effects of long-term SMI drug exposure, such as inflammation¹⁹, cardiovascular complications¹²⁶, bone and/or cartilage problems¹²⁷, subphyseal hyperostosis as well as chondrocyte hypertrophy and/or hyperplasia, and reducing the risk of developing SMI drug resistance¹⁵⁶.

Another clinical application with great promise is augmenting radiotherapy by inhibiting the TGF β path-way^{10,81,157}. Radiation not only physically activates latent TGF β *in vitro* but also induces the biological release of this growth factor as part of a stress response¹⁵⁸. Several groups have reported the positive role of TGF β in supporting the DNA damage repair pathway, particularly through activation of p53 and phosphorylation of ataxia telangiectasia mutated (ATM) after radiation therapy¹⁵⁹. Barcellos-Hoff's group demonstrated that LY2109761 and ID11 both attenuate radiation-induced activation of p53 and ATM in breast cancer cells *in vitro* and *in vivo*, thus preventing DNA repair and accentuating the cytotoxic effect of radiation⁸¹. Even short-term dosing with T β RI inhibitors might provide a considerable therapeutic advantage in potentiating radiotherapy, with the added benefit that the local activation of pro-tumorigenic stroma and tissue fibrosis — a major complication of radiation therapy¹⁰ — may also be suppressed by these drugs. In partnership with Genzyme, this group is currently undertaking a Phase I trial of fresolimumab in combination with radiotherapy for metastatic breast cancer. Eli Lilly is also undertaking a Phase I/IIa trial to test the safety and efficacy of LY2157299 in combination

with temozolomide-based radiochemotherapy in patients with newly diagnosed malignant glioma¹⁵⁷.

Myelodysplastic syndrome

Myelodysplastic syndrome (MDS) is characterized by abnormal myeloid and/or erythroid differentiation of bone marrow cells that results in various anaemias and cytopaenias. In one-third of MDS cases, a high-risk group of patients can progress to leukaemia. However, refractory cytopaenias are the major cause of morbidity and mortality in sufferers. It was recently shown that reduced expression of SMAD7, an inhibitor of T β RI, was a common and significant event observed in CD34⁺ myeloid progenitor cells in the bone marrow of patients with MDS¹⁶⁰. Indeed, low levels of myeloid SMAD7 expression were seen in most patients with MDS, regardless of the risk for progression to leukaemia. Downregulation of SMAD7 expression sensitized myeloid precursors to TGF β such that even very low levels of the ligand elicited an increase in TGF β responsiveness, as defined by P-SMAD2 levels and enhanced immune-suppressive effect, thus providing another opportunity to utilize TGF β inhibitors for therapeutic utility in human disease.

Treatment of primary CD34⁺ haematopoietic stem cells with LY2157299 suppressed the activation of T β RI by its ligand. Moreover, in a liver-specific TGF β 1-overexpressing transgenic mouse model of MDS that exhibits severe anaemia, LY2157299 decreased P-SMAD2 levels in the bone marrow and significantly increased the haematocrit of these mice. Importantly, in ten out of ten primary bone marrow cultures from patients with MDS, administration of LY2157299 significantly increased erythroid (burst-forming unit (BFU-E)) and myeloid (colony-forming unit (CFU); granulocytic monocytic) colony numbers *in vitro*, harbouring great promise for the treatment of patients with MDS¹⁶⁰.

Fibrosis

IPF is a progressive, chronic and irreversible lung disease occurring in older adults, and has an unknown cause¹⁶¹. The main histological features of IPF are heterogeneous parenchyma, with areas of fibrosis and honeycombing alternating with areas of less-affected or normal parenchyma. IPF is characterized by a progressive reduction in lung function, with an estimated 20% survival prospect after 5 years, making it more lethal than many cancers. The progressive fibrotic reaction in IPF is associated with an epithelium-dependent fibroblast activation, in which TGF β plays a major part¹⁶. TGF β 1, which is secreted by alveolar epithelial cells in patients with IPF, drives the process by promoting the migration, proliferation and differentiation of resident mesenchymal cells. α V β 6 integrin, which binds and activates latent TGF β 1 and TGF β 3, is highly induced following lung injury or fibrosis¹⁶². TGF β activity¹⁶³ then promotes activation and differentiation of fibroblasts into myofibroblasts, which are specialized contractile cells that cause aberrant ECM deposition, leading to the destruction of lung architecture, scarring¹⁶² and reduced lung function. TGF β also promotes pulmonary EMT that additionally contributes to the expansion of fibroblasts and myofibroblasts¹⁶⁴.

Pirfenidone, a novel compound that inhibits TGF β activity *in vitro*, decreased the rate of decline in vital lung capacity and marginally increased progression-free survival in patients with IPF. Pooled data from two concurrent Phase III clinical trials in IPF indicated improvement in pulmonary function in the pirfenidone-treated group¹⁶⁵. Currently, there are no US Food and Drug Administration (FDA)-approved drugs for IPF, and pirfenidone is the first such drug to be approved for IPF in Europe. Other approaches to develop TGF β -based therapies for IPF include gene transfer of a soluble T β RII construct (as a ligand decoy), which attenuated injury and fibrosis in bleomycin-induced IPF in mice¹⁶⁶. P144 (disitertide; Digna Biotech), a synthetic peptide that attenuates TGF β activity and is derived from the

extracellular domain of betaglycan, was also shown to reduce carbon tetrachloride-induced liver injury in mice⁷⁸. P17, another Digna Biotech anti-TGF β peptide, has been shown to be efficacious in attenuation of injury and fibrosis in bleomycin-induced IPF in mice¹⁶⁷. Although P144 has been used clinically for skin fibrosis, drug delivery is an issue for the clinical development of P144 for IPF. However, Digna Biotech has recently partnered with Flamel Technologies to investigate the use of a proprietary drug delivery platform for this application (see the press release: 'Flamel Technologies and Digna Biotech Announce Multiple Product Development Agreement'). Other anti-TGF β therapies in clinical trials for IPF include the pan TGF β -neutralizing antibody GC-1008 (Genzyme) and the α V β 6 integrin-blocking antibody STX-100 (Stromedix)^{168,169}.

Renal fibrosis has long been thought to be driven by excess TGF β , which results in renal scarring and, ultimately, kidney failure¹⁷⁰. With the increasing incidence of diabetes and associated kidney damage in affluent countries, this is a clinical application of growing importance. Mice overexpressing an active form of TGF β 1 (from the liver) develop progressive liver and renal fibrosis¹⁷⁰. Interestingly, although mice overexpressing active TGF β 1 develop progressive renal injury, latent TGF β 1 also has a protective role in renal fibrosis through negative effects on inflammation⁴⁹. TGF β 1 mediates progressive renal fibrosis by stimulating the synthesis of ECM production while inhibiting its degradation⁴⁹. TGF β 1 also mediates renal fibrosis by inducing the transformation of tubular epithelial cells into myofibroblasts through EMT in a similar way to the process seen in IPF¹⁷¹. Blockade of TGF β 1 with neutralizing TGF β antibodies prevents or ameliorates renal fibrosis *in vivo* and *in vitro*, demonstrating the functional role of TGF β 1 in EMT and renal fibrosis. A number of therapeutic interventions that block the action of TGF β have resulted in various degrees of improvement in kidney structure and function in preclinical studies; such interventions include TGF β ASOs, a neutralizing anti-TGF β antibody, a soluble TGF β receptor, blockade of TGF β activation by decorin¹⁷², an SMI of TGF β receptors, delivery of the inhibitor protein SMAD7 (Ref. 173) and a THBS1-blocking peptide that interferes with TGF β activation⁹⁷. A Phase I/II trial with GC-1008, a pan-TGF β -neutralizing antibody, exhibited encouraging efficacy in patients with focal segmental glomerulosclerosis⁹², and Eli Lilly is undertaking trials of its own anti-TGF β 1 monoclonal antibody, LY2382770, in diabetic kidney disease.

Cardiac fibrosis is a pathological feature that is common to a number of forms of heart disease, including myocardial infarction, ischaemic, dilated and hypertrophic cardiomyopathies and congestive heart failure³⁶. The cellular basis of cardiac fibrosis is the aberrant accumulation of collagens and other ECM proteins, which impair ventricular function and predispose to cardiac arrhythmias. Because TGF β has pleiotropic effects in the cardiovascular system and as cardiac fibrosis is a multifactorial disease, the development of an effective therapy will require a detailed understanding of the role of the TGF β signalling pathway in this pathogenesis. TGF β , a potent stimulator of collagen production by cardiac fibroblasts, is induced in response to cardiovascular injury. The TGF β -SMAD pathway activates the transcription of several key fibrotic genes, such as those encoding connective tissue growth factor (CTGF), fibronectin, collagens and plasminogen activator inhibitor 1 (PAI1)³⁶. TGF β reduces collagenase production and stimulates the expression of tissue inhibitor of metalloproteinases (TIMPs), resulting in an overall inhibition of ECM degradation and leading to excessive ECM accumulation. P144 has been investigated in a preclinical model of cardiac fibrosis⁷⁷, and losartan can reverse fibrosis in a mouse model of hypertrophic cardiomyopathy¹⁷⁴; however, no drug targeting the TGF β pathway has yet reached clinical trials for this application. A recent study demonstrated that miR-21, which is regulated by SMADs upon TGF β activation, is consistently induced by cardiac stress. As miR-21 plays a part in tumorigenesis by promoting cell proliferation, increased expression

of miR-21 might contribute to the progression of fibrotic lesions¹⁷⁵. ASOs against miR-21 might therefore become a novel therapeutic approach for treating cardiac fibrosis.

Scleroderma

Scleroderma (progressive systemic sclerosis) is a systemic autoimmune disorder characterized by skin sclerosis, calcinosis and changes in microvasculature. Increased expression of T β RI and T β RII in sclerodermal fibroblasts suggests that increased production of type I collagen by autocrine TGF β signalling leads to aberrant ECM deposition and scarring³⁶. Therapeutic approaches to scleroderma have included inhibition of TGF β activity in sclerotic tissue. Unfortunately, CAT-192, a TGF β 1-neutralizing antibody, did not show evidence of efficacy in a study on the treatment of patients with early-stage systemic scleroderma⁹⁰; however, GC-1008 is now in a Phase I clinical trial for patients with diffuse systemic sclerosis. Furthermore, topical application of Digna Biotech's P144 peptide inhibitor of TGF β 1 has shown some efficacy in reducing skin fibrosis in a Phase II clinical trial for systemic sclerosis (see the press release: 'Flamel Technologies and Digna Biotech Announce Multiple Product Development Agreement'), with the caveat that clinical end points for quantifying skin fibrosis have not yet been standardized¹⁷⁶.

Restenosis following coronary artery bypass and angioplasty

The development of fibromuscular intimal hyperplasia following angioplasty and coronary artery bypass surgery is a major clinical problem and can lead to coronary artery graft failure. The success of coronary artery reconstructive procedures is limited by the high incidence of restenosis secondary to intimal hyperplasia. TGF β 1 is a major player in the early development of intimal hyperplasia in arteries and peripheral vein grafts. The exact mechanism of action of TGF β signalling in intimal hyperplasia and subsequent graft failure is unclear, but it is speculated that TGF β 1 contributes at multiple steps, including EMT, promotion of fibroblast, endothelial and vascular smooth muscle cell proliferation, increased collagen synthesis and deposition, and induction of fibrosis¹⁷⁷. Soluble forms of the small, leucine-rich proteoglycans decorin and fibromodulin, which possess TGF β -antagonist activity, exhibit potent intimal hyperplasia-suppressing effects in cultured human saphenous vein, offering the potential for therapeutic benefit after coronary artery bypass surgery¹⁷⁸⁻¹⁸⁰. The novel pyrrole-imidazole polyamide drug class, targeted to suppress *TGFBI* gene transcription, showed efficacy in reducing neointimal hyperplasia and stimulating re-endothelialization of carotid arteries in a preclinical model of arterial injury⁵⁰.

Marfan syndrome

MFS is a connective tissue disorder that affects the musculoskeletal, ocular and cardiovascular systems. It is caused by mutations in the gene encoding an ECM protein, fibrillin 1 (*FBN1*)¹⁸¹. Growing evidence suggests that *FBN1* mutations perturb not only the general integrity and elasticity of tissues but also — probably more crucially — local TGF β signalling¹¹. Normal fibrillin 1-containing microfibrils interact with the large latent TGF β complex (LLC) to control the release of mature, active TGF β ¹⁸¹. Mutated fibrillin 1 fails to sequester latent TGF β , leading to the promiscuous activation of TGF β ¹¹. Thus, MFS highlights the critical role of microfibrils in regulating local concentrations of TGF β and in maintaining the homeostasis, morphogenesis and function of various organs¹⁸¹. Dilation of the aortic root, which leads to aortic rupture and sudden death, is a major clinical issue for patients with MFS and patients of the Loeys-Dietz spectrum who carry mutations in *TGFBR1*, *TGFBR2* and *SMAD3*. A mouse model of MFS carrying a heterozygous *Fbn1* mutation developed an aortic aneurism similar to that of patients with MFS. Administration of TGF β antagonists, including a TGF β -neutralizing antibody or the angiotensin II type 1 receptor (AT1) blocker losartan, successfully rescued both cardiovascular and non-cardiovascular manifestations of MFS^{12,182}. As losartan is already in widespread clinical use

for hypertension and has shown no adverse effects, this drug is currently being tested in Phase I/II clinical trials for MFS ([ClinicalTrials.gov](https://clinicaltrials.gov) identifiers: NCT00429364, NCT00593710, NCT00683124, NCT00723801, NCT00763893, NCT00782327 and NCT01145612) and may plausibly reduce this life-threatening manifestation of MFS.

Postoperative scarring in ocular conditions

Trabeculectomy is a surgical procedure designed to reduce intraocular fluid pressure in patients with glaucoma. However, postoperative scarring and fibrotic blockage of the 'filtering bleb' that drains excess ocular fluid are serious complications of this procedure. In preclinical experiments, administration of neutralizing antibodies against human TGF β 2 (CAT-152) exhibited promising inhibition of scarring after glaucoma surgery in rabbits without having any adverse effects. Initial clinical trials with CAT-152 ameliorated scarring in patients who received trabeculectomy for intractable glaucoma^{88,183}; however, Phase III clinical trials were unable to validate such beneficial effects¹⁸⁴. Tranilast, another incidental TGF β inhibitor, has also been used successfully to reduce re-occurrence of corneal fibrosis, or primary pterygium, following corneal surgery¹⁸⁵. It is possible that topical application of more-specific TGF β inhibitors might also be used in treating corneal haze and conjunctival scarring (Table 1).

Challenges and considerations for TGF β blockade

Individualized responses to TGF β blockade

Many diseases being tackled with TGF β inhibitors, including fibrosis, inflammation, autoimmunity and cancer, are complex in nature and show strong genetic predisposition owing to innate genetic variation between individuals. It is well established that there is considerable phenotypic diversity in the range of responses to reduced TGF β signalling *in vivo*, which are dictated by differential inheritance of germline genetic variants. This is illustrated by the large spectrum of clinical severity and disease manifestations in individuals with mutations in TGF β signalling pathway genes^{8,41,186}. Moreover, in mouse models of cancer, asthma and vascular development, outcomes of reduced TGF β 1 levels are strongly influenced by interacting genetic modifier loci^{5,9,186,187}. It is therefore most rational, economical and safe to preselect patient populations before initiating anti-TGF β drug treatment on the basis of surrogate markers of TGF β involvement in the disease process (such as increased TGF β ligand and P-SMAD levels, and specific disease characteristics) and contra-indications of possible adverse side effects (such as susceptibility to inflammation or certain vascular conditions). Peripheral blood may provide non-invasive markers that might be useful in this respect, including the ability to quantitatively screen patient responses to TGF β inhibition on the basis of measuring P-SMAD2 levels in peripheral blood mononuclear cells (PMNCs)¹⁸⁸. Similarly, potential adverse inflammatory effects could be predicted by examining specific immunological responses of PMNCs¹⁸⁹⁻¹⁹² to TGF β inhibition *ex vivo*.

Patient selection for TGF β inhibitors in oncology

The simplest biomarker for patient selection for TGF β inhibitors in oncology is probably high circulating levels of TGF β , as one major goal of this therapy is to reduce, but not totally ablate, TGF β signalling¹⁹³. As indicated above, non-invasive biomarkers for predicting patient responsiveness and efficacy of TGF β inhibitors have been developed on the basis of measuring P-SMAD2 levels in PMNCs. Indeed, PMNCs can be treated with drugs *ex vivo* to determine, and thus predict, individual patient responses to SMIs^{188,193}. As TGF β inhibitors certainly act to reduce tumour metastasis, the assay of circulating tumour cell number may also be a useful indicator for therapeutic response. Moreover, as many of the pro-tumorigenic effects of TGF β are mediated by immune system modulation, it might

be possible to monitor blood cell or plasma protein profiles for patient selection and for surrogate monitoring of patient responses.

In cancer (as well as non-malignant diseases), the outcome of reduced TGF β signalling may be highly dependent on the innate genetic background of the individual, especially when considering tumour microenvironment effects, such as immune surveillance. Elucidating specifically which genetic variants influence signalling output will not only be useful for dissecting the intricacies of this signalling pathway *in vivo*, but may also provide predictive markers for the outcome (desired or undesired) of TGF β signal inhibition. However, for cancer therapeutics, patient selection may be more complex, as the response of both the tumour cell and host (tumour microenvironment and normal patient tissue) to TGF β blockade needs to be considered. Tumour biopsy and genetic analysis (for example, loss of *TGFBR2*, *SMAD2* or *SMAD4*)^{194,195} might predict whether the tumour retains growth sensitivity to TGF β . Molecular and histological analyses may also contribute to the prediction of tumour responses to TGF β inhibition. The activation of alternative intracellular signalling pathways and transcription factor profiles has been associated with the switch from tumour suppression — by TGF β — to tumour progression. These include an increased ratio of liver-enriched inhibitory protein (LIP) to liver-enriched activating protein (LAP), which are isoforms of CCAAT/enhancer-binding protein- β (C/EBP β), a central transcription factor that binds within the SMAD transcription factor complex to elicit TGF β -mediated cytostatic responses¹⁹⁶. Upregulation of *SIX1* in breast cancer has also been shown to be pivotal in the growth-suppressive to tumour-progressive switch¹⁹⁷, as has downregulation of *DAB2* (Ref. 198). Any of these tumour markers, possibly in combination, may be used in the future to predict tumour responses to TGF β inhibition.

Finally, the effects of interactions with other anticancer drugs will need to be considered, as some drugs may resurrect the growth-inhibitory arm of the TGF β signalling pathway, which would then counter-indicate their combinatorial use with TGF β inhibitors¹⁹⁹. Indeed, the ability to specifically target the pro-tumorigenic versus the tumour-suppressive effects of TGF β on the tumour cell *per se* will require the development of next-generation drugs focusing on these downstream pathways. In the meantime, much research still remains to be undertaken to make inroads into the area of informed patient selection for oncology applications of TGF β inhibitors.

Drug resistance

In oncology, the development of tumour drug resistance is inevitable^{134,200,201}. It has been documented for standard chemotherapy, pathway-targeted therapies and is even common with anti-angiogenesis inhibitors^{200,201}. Acquired biochemical resistance of tumour cells to LY2109761 has been observed in a preclinical model of SCC and may have adverse consequences in driving a more stem cell-like phenotype¹⁵⁶, although this remains to be tested. Carefully restricting TGF β inhibitors to short-term or intermittent usage should avoid these complications⁸². Combinatorial and/or sequential treatment with complementary drugs will also be important. It is clear that oncologists will need an arsenal of different anticancer drugs to tackle cancer, in much the same way that antibiotics have been developed to combat infectious diseases.

Conclusion and future directions

In conclusion, TGF β signalling inhibitors are generally safe and may be efficacious in several clinical applications, especially in desperate cases such as end-stage cancer or IPF. The development of these drugs may offer further therapeutic opportunities. Counterintuitively, there have been reports suggesting that inhibition of the TGF β signalling pathway may be beneficial in autoimmune disorders, such as multiple sclerosis, through

downregulation of the T_H17 pathway^{202,203}. Recent studies have also suggested that TGF β -SMAD3 signalling regulates glucose tolerance and energy homeostasis, and that blockade of the pathway may be used for regulation of diabetes and obesity²⁰⁴. The outlook for anti-TGF β signalling therapy for numerous diseases appears bright. At least four companies are well on their way in clinical drug development, and further scientific and mechanistic studies are warranted in order to optimize patient selection and drug-dosing regimens for each disease application.

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Glossary

Epithelial-mesenchymal transition	(EMT). The transformation of a keratin-expressing epithelial cell into one with fibroblastic properties that express mesenchymal markers
Extracellular matrix	(ECM). Matrix that supports connective tissue and is composed of proteoglycans, hyaluronic acid and fibrillar proteins secreted from the cell and rich in bound growth factors
Fibrosis	The excess accumulation of fibroblasts and associated extracellular matrix
Metastasis	The dissemination of tumour cells and re-establishment of tumours at a secondary site
SMAD	Signal transduction component of the canonical transforming growth factor- β signalling pathway
microRNA	(miRNA). Small (20-23 nucleotides long) non-coding RNA involved in post-translational regulation of gene expression. miRNAs bind to the partially complementary sequence in the 3'-untranslated region (3'-UTR) of mRNAs and negatively regulate their expression either through translational inhibition or promotion of mRNA degradation.
Myofibroblast	A contractile fibroblast that expresses smooth muscle actin and myosin, and contributes to disease progression in cancer and fibrosis
Antisense oligonucleotides	(ASOs). Short chemically modified oligonucleotides complementary to a specific mRNA that can be used to cause specific knockdown of targeted gene expression
Tumour-initiating cells	(TICs). The putative cancer stem cells that have the ability to maintain tumour growth, differentiate into all cell types of a heterogenous tumour, and to re-establish secondary tumours with exceedingly high efficiency.

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Box 1**Canonical signal transduction pathway of the TGF β superfamily of growth factors**

The basic framework of the canonical signal transduction pathways of three subfamilies of the transforming growth factor- β (TGF β 3) superfamily — TGF β 3s, activins/inhibins/Nodal and bone morphogenetic proteins (BMPs) — is highly conserved. The ligand binds to a specific set of type I and type II receptors, which are both serine/threonine kinases, followed by signal transduction by SMAD proteins^{31,205}. Although each subfamily transmits the signal through a specific signalling pathway, the interaction among the TGF β , activins/inhibins/Nodal and BMP subfamilies is well recognized during development and in postnatal homeostasis of various organs (Box 3). Upon ligand binding and resultant heterotetrameric receptor complex formation, the constitutively active type II receptor phosphorylates the type I receptor, which in turn propagates a signal by phosphorylating the receptor-specific SMADs (R-SMADs)^{31,205}. Unlike type I and type II receptors, type III receptors do not possess kinase activity and are not required for signal transduction; however, they bind to specific ligands and modulate the signalling pathway either positively or negatively^{31,205}. Phosphorylation of R-SMADs at two serine residues within the extreme carboxyl terminus by type I receptor kinase activity promotes association with the common mediator SMAD (co-SMAD), SMAD4, resulting in nuclear accumulation and sequence-specific binding to DNA in concert with other DNA-binding transcription factors that bind distinct sequences adjacent to the SMAD-binding element (SBE)²⁷, and together these complexes modulate transcription. The inhibitory SMADs (I-SMADs), SMAD6 and SMAD7, antagonize R-SMAD activation by competing with R-SMADs for type I receptor interaction and/or by recruiting specific ubiquitin ligases or phosphatases to the activated receptor complex, thereby targeting it for proteasomal degradation or dephosphorylation, respectively. SMAD7 inhibits signalling from all branches of the TGF β superfamily, whereas SMAD6 is a specific inhibitor of the BMP signalling pathway. The table indicates the basic molecules in the signal transduction pathway, including three types of receptors and SMADs, for three subfamilies of the TGF β superfamily of ligands: TGF β s, activins/inhibins/Nodal and BMPs.

Molecular category	TGF β pathway*	Activin/inhibin/Nodal pathway*	BMP pathway*
Ligands	TGF β 1, TGF β 2, TGF β 3	Activin A, activin B, inhibin A, inhibin B, Nodal	BMP2, BMP4, BMP5, BMP6, BMP7, BMP8A, BMP8B, BMP9, BMP10
Type I receptors	T β RI(ALK5), ALK1 (ACVRL1orSKR3)	ALK4(ACVR1Bor ACTRIIB), ALK7 (ACVR1C or ACTRIC)	ALK1 (ACVRL1, SKR3), ALK2 (ACVR1, ACTRI), ALK3 (BMPRI1A), ALK6 (BMPRI1B)
Type II receptors	T β RII	ACTRIIA, ACTRIIB	BMPRI2, ACTRIIA, ACTRIIB
Type III receptors	T β RIII (betaglycan), endoglin, CRIPTO3 (TDGF1P3)	CRIPTO1 (TDGF1), CRIPTO3 (TDGF1P3),T β RIII (betaglycan)	RGMA, RGMB (DRAGON), RGMC (HJV or HFE2), endoglin
R-SMADs	SMAD2, SMAD3	SMAD2, SMAD3	SMAD1, SMAD5, SMAD8
Co-SMAD	SMAD4	SMAD4	SMAD4
I-SMADs	SMAD7	SMAD7	SMAD6, SMAD7

* Alternative protein names are listed in brackets. ACTR, activin receptor; ALK, activin receptor-like kinase; BMP bone morphogenetic protein; BMPR, BMP receptor; RGM, repulsive guidance molecule; T β 3R, TGF β receptor; TDGF, teratocarcinoma-derived growth factor.

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Box 2**Non-canonical TGF β signalling and crosstalk with other pathways**

In addition to activating SMAD proteins, transforming growth factor- β (TGF β) signalling can regulate the activity of a number of signalling molecules, such as TNF receptor-associated factor 4 (TRAF4), TRAF6, TGF β -activated kinase 1 (TAK1), p38 mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase (ERK), JUN N-terminal kinase (JNK), RHO GTPases, phosphoinositide 3-kinase (PI3K)–AKT and nuclear factor- κ B (NF- κ B), to transmit a signal⁶. In addition, these non-canonical signals can crosstalk with the SMAD pathways and mutually modulate each other. Both canonical and non-canonical TGF β signalling can also be influenced by other signalling pathways, such as the RAS, WNT, Hedgehog, Notch, tumour necrosis factor (TNF) and interferon pathways⁶. The exact nature of the crosstalk with other pathways and biological outcomes is complex and highly context-dependent⁶. However, some of the crosstalk has been found to modulate the function and stability of SMAD proteins through post-translational modifications, and to define cell type- and context-specific outcomes by inducing other factors that modulate TGF β activity.

Box 3**BMP-TGF β -activin crosstalk in endothelial cells**

Within the transforming growth factor- β (TGF β) superfamily, the crosstalk between three subfamilies — activins/inhibins/Nodal, TGF β 3s and bone morphogenetic proteins (BMPs) — is well established during development and postnatal homeostasis of various organs²⁰⁶⁻²⁰⁷. In vertebrates, the BMP-SMAD1/SMAD5/SMAD8 and activin–Nodal–TGF β -SMAD2/SMAD3 signalling pathways execute antagonistic actions in different developmental contexts by inducing the expression of antagonistic factors, such as inhibitory SMADs (I-SMADs: SMAD6 and SMAD7). Some studies have shown that the common mediator SMAD (co-SMAD), SMAD4, is rate-limiting; therefore, when one of the two pathways is activated, it can negatively influence the other pathway by sequestering SMAD4. In endothelial cells, TGF β can signal not only via canonical TGF β receptor type I (T β RI)-SMAD2/SMAD3 but also via activin receptor-like kinase 1 (ALK1)-SMAD1/SMAD5/SMAD8 (Ref. 36). In contrast to TGF β -T β RI signalling-mediated activation of SMAD2 or SMAD3, which leads to endothelium quiescence, TGF β -ALK1 signalling induces SMAD1/SMAD5/SMAD8 activation and has been shown to stimulate endothelial cell migration, proliferation and tube formation, thus promoting angiogenesis²⁰⁸. BMP9 was shown to induce SMAD2/SMAD3 and SMAD1/SMAD5/SMAD8 phosphorylation via signalling mediated by BMP receptor 2 (BMPR2), activin receptor type II (ACTRII) and ALK1 or ALK2. Cross-activation of TGF β -specific and BMP-specific receptor-specific SMADs (R-SMADs) by a single ligand is believed to provide a mechanism for the ligand to fine-tune endothelial cell behaviour and function³⁶. In summary, the crosstalk among signalling pathways mediated by different TGF β family ligands exists in every tissue. However, the mechanism and the biological outcome of this crosstalk are highly species-, tissue- and context-dependent.

Box 4**Oncology trials to date**

A two-part clinical trial of GC-1008 for the treatment of advanced metastatic melanoma (MetM) and renal cell carcinoma (22 patients) found the drug to be safe and well tolerated with no dose-limiting toxicities (DTLs). Five patients achieved at least stable disease as assessed by RECIST (response evaluation criteria in solid tumours) criteria, and therefore received extended treatment. One patient achieved a partial response with a greater than 75% reduction in the target lesion. The only adverse effect was keratoacanthoma-like lesions in sun-damaged skin of two of the patients with MetM. However, these resolved on cessation of drug treatment and were not malignant⁹³. Despite these promising results, the pursuit of GC-1008 for oncology was terminated after Genzyme was acquired by Sanofi in late 2011.

Antisense Pharma has had success with trabedersen (AP12009) in glioblastoma, pancreatic cancer and colon cancer. Preclinical and clinical studies^{70,71,73} indicate that neutralization of transforming growth factor- β 2 (TGF β 2)-mediated immunosuppression, leading to activation of tumour-infiltrating natural killer cells, is the major mode of action. Intra-tumoural administration of trabedersen to glioblastoma led to shrinkage of the targeted tumour as well as tumours elsewhere in the brain. Three Phase I/II studies of trabedersen for recurrent or refractory high-grade glioma (glioblastoma) and anaplastic astrocytoma showed survival benefit compared with conventional chemotherapy²⁰⁹. A randomized, controlled Phase IIb study evaluating the efficacy and safety of two doses (10 and 80 mM) of trabedersen in comparison with standard therapy concluded that patients with glioblastoma on trabedersen had a threefold enhancement in cognitive function 2 and 3 years after therapy compared to standard chemotherapy⁷⁴. However, questions have been raised about this most recent study^{210,211}. Wick and Weller²¹¹ conceded that although trabedersen was clinically safe and that TGF β inhibitors, in general, show promise for cancer therapy, the conclusions drawn by Bogdahn *et al.*⁷⁴ were premature. Because of other advances in both neurosurgical procedures and first-line standard of care for patients with glioblastoma²¹², the SAPPHIRE Phase III trial of trabedersen was recently halted owing to patient recruitment issues (ClinicalTrials.gov identifier: NCT00761280). Nevertheless, the drug has undergone a Phase I/II trial for patients with advanced pancreatic cancer, MetM or metastatic colorectal carcinoma, and showed excellent safety and encouraging survival results (ClinicalTrials.gov identifier: NCT00844064)⁸⁴.

Eli Lilly's clinical small-molecule inhibitor LY2157299 was found to be safe and well tolerated in a Phase I glioblastoma trial⁸². Of 28 patients treated in a dose escalation study (14 days on/14 days off treatment), at least three patients showed antitumour effects with durable responses beyond 1 year. As a result, the Eli Lilly anti-TGF β signalling programme for oncology continues to be pursued with an ongoing Phase II trial of LY2157299, with or without gemcitabine, for hepatocellular carcinoma, glioblastoma and advanced pancreatic cancer, and with lomustine in patients with treatment-refractory malignant glioma²¹³, plus a new Phase I trial of IMC-TM1, an anti-TGF β receptor type II (T β RII) antibody.

NovaRx's belagenpumatucel-L (Lucanix) has completed an open-label clinical trial of 75 patients with non-small-cell lung cancer (NSCLC) with a median follow-up of 14.5 months (44 months for patients with stable disease). One-year, two-year and five-year survivals were 55%, 35% and 20%, respectively. Individuals who demonstrated an increase in both cellular and humoral immune reactivity had a significant survival advantage over individuals who showed an increase in only one measure of immunity

(32.5 months versus 11.6 months; $p = 0.015$). On the basis of these findings, an international, randomized Phase III trial to evaluate the efficacy of belagenpumatucel-L in a maintenance setting has been initiated for patients with stage III/IV NSCLC who have stable disease following frontline chemotherapy⁸⁷.

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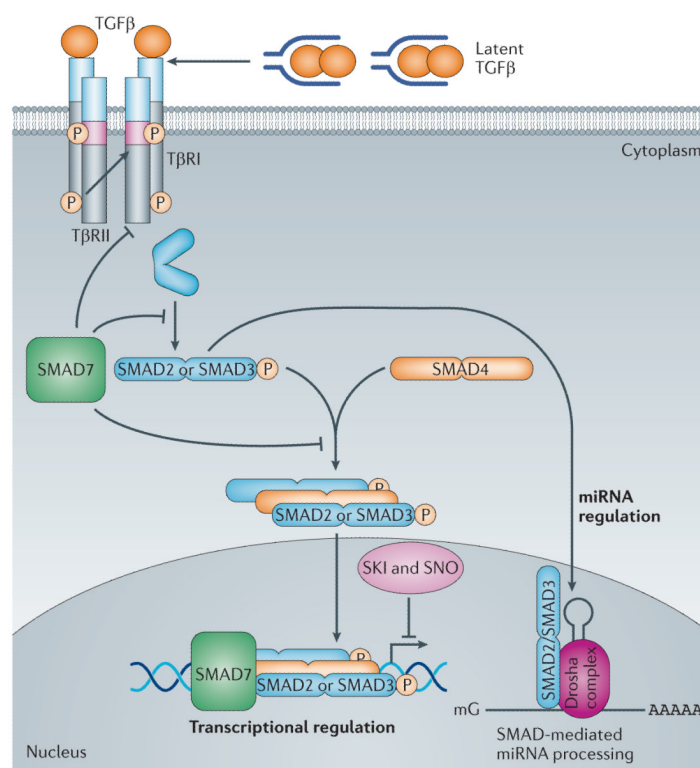


Figure 1. Schematic overview of the canonical, SMAD-dependent TGF β signalling pathway
 The transforming growth factor- β (TGF β) ligands are synthesized as a large latent TGF β complex consisting of mature dimeric TGF β associated with its latency-associated peptide (LAP) and a latent TGF β -binding protein (LTBP) (not shown). Upon activation, TGF β dimers induce heteromeric complex formation between specific type II and type I receptors (such as TGF β receptor type II (T β RII) and T β RI, respectively). Type II receptors then transphosphorylate the type I receptors, which propagate the signal into the cell by phosphorylating TGF β receptor-specific SMADs (R-SMADs: SMAD2 and SMAD3). They form heteromeric complexes with the common mediator SMAD (co-SMAD: SMAD4) and translocate to the nucleus. Once in the nucleus, the R-SMAD-co-SMAD complex preferentially associates with the genomic SMAD-binding element (SBE) in a sequence-specific manner. However, high-affinity binding of the R-SMAD-co-SMAD complex with the SBE generally occurs in concert with other DNA-binding transcription factors that bind to distinct sequences adjacent to the SBE²⁷. The nuclear proteins SKI and SNO (also known as SKIL) antagonize the transcriptional regulation by SMADs. An inhibitory SMAD (I-SMAD), SMAD7, inhibits the TGF β pathway through multiple mechanisms, including by mediating the degradation of the type I receptor, inhibiting phosphorylation of R-SMADs by the type I receptor kinase or inhibiting the formation of the R-SMAD-co-SMAD complex. In addition to regulating transcription, R-SMADs can modulate microRNA (miRNA) biogenesis by facilitating the processing of primary miRNA into precursor miRNA in the nucleus. The co-SMAD is not required for the regulation of miRNA biosynthesis by R-SMADs. ‘mG’ and ‘AAAAA’ represent 5’ capping and 3’ polyadenylation of mRNAs, respectively.

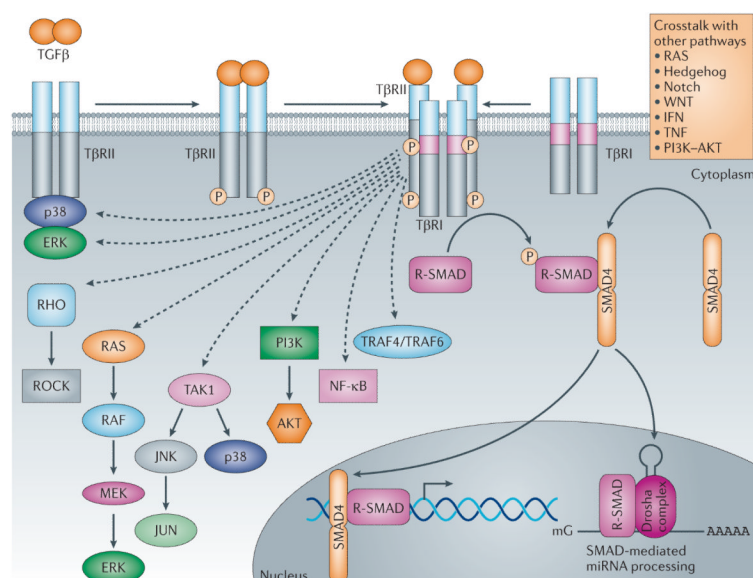


Figure 2. Schematic representation of non-canonical TGFβ signalling and crosstalk with other signalling pathways

In the non-canonical pathways, the activated transforming growth factor-β (TGFβ) receptor complex transmits a signal through other factors, such as TNF receptor associated factor 4 (TRAF4) or TRAF6, TGFβ-activated kinase 1 (TAK1), p38 mitogen-activated protein kinase (p38 MAPK), RHO, phosphoinositide 3-kinase (PI3K)-AKT, extracellular signal-regulated kinase (ERK), JUN N-terminal kinase (JNK) or nuclear factor-κB (NF-κB). TGFβ signalling can be influenced by pathways other than the canonical and non-canonical TGFβ signalling pathways, such as the WNT, Hedgehog, Notch, interferon (IFN), tumour necrosis factor (TNF) and RAS pathways. The crosstalk between TGFβ and other pathways defines the activities of TGFβ to propagate spatial- and temporal-specific signals. miRNA, microRNA; ROCK, RHO-associated protein kinase; R-SMAD, receptor-specific SMAD; TβRI, TGFβ receptor. 'mG' and 'AAAAA' represent 5' capping and 3' polyadenylation of mRNAs, respectively.

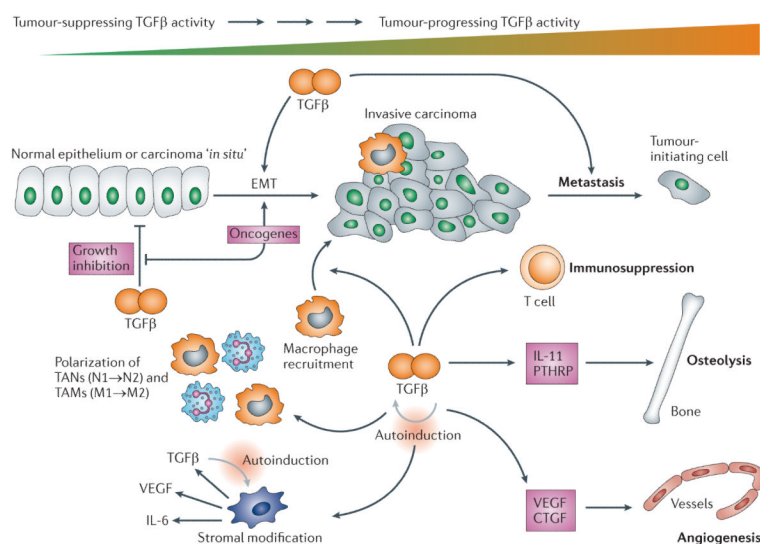


Figure 3. Biphasic activities of the TGFβ signalling pathway during tumorigenesis: from the tumour suppressor to the tumour promoter

Transforming growth factor-β (TGFβ) has biphasic actions during tumorigenesis, suppressing tumorigenesis at early stages but promoting tumour progression later on, which is the underlying paradigm for the action of TGFβ during disease progression in general and thus complicates the development of therapies targeting TGFβ signalling. The light grey arrows indicate a positive feedforward loop resulting in higher levels of TGFβ, which is a feature of non-neoplastic as well as neoplastic diseases. The current goal in cancer therapy is to downmodulate excessive levels of TGFβ ligands and to target the tumour-progressing versus the tumour-suppressing arm of TGFβ action; the latter goal will almost certainly require more-specific second-generation drugs. CTGF, connective tissue growth factor; EMT, epithelial-mesenchymal transition; IL, interleukin; PTHRP, parathyroid hormone-related protein; TAMs, tumour-associated macrophages; TANs, tumour-associated neutrophils; VEGF, vascular endothelial growth factor.

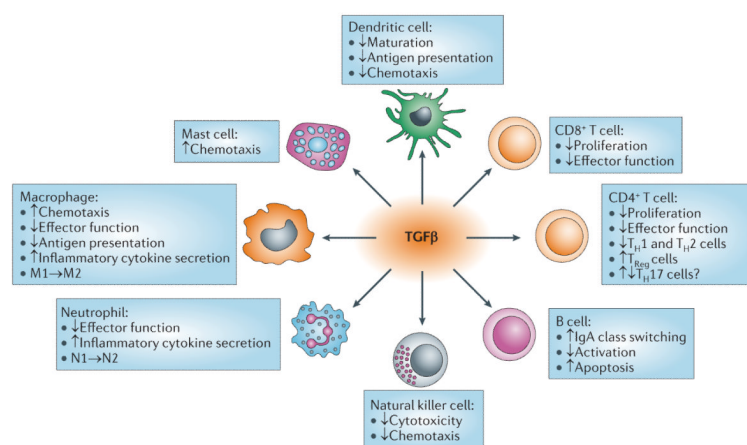


Figure 4. TGFβ effects on immune cells

Transforming growth factor- β (TGFβ) has effects on most immune cell types. The figure depicts the activity of TGFβ on immune cell subsets that is relevant to human diseases. M1→M2 and N1→N2 indicate polarization of macrophages and neutrophils, respectively, from type I to type II. IgA, immunoglobulin A; T_H, T helper; T_{Reg}, regulatory T.

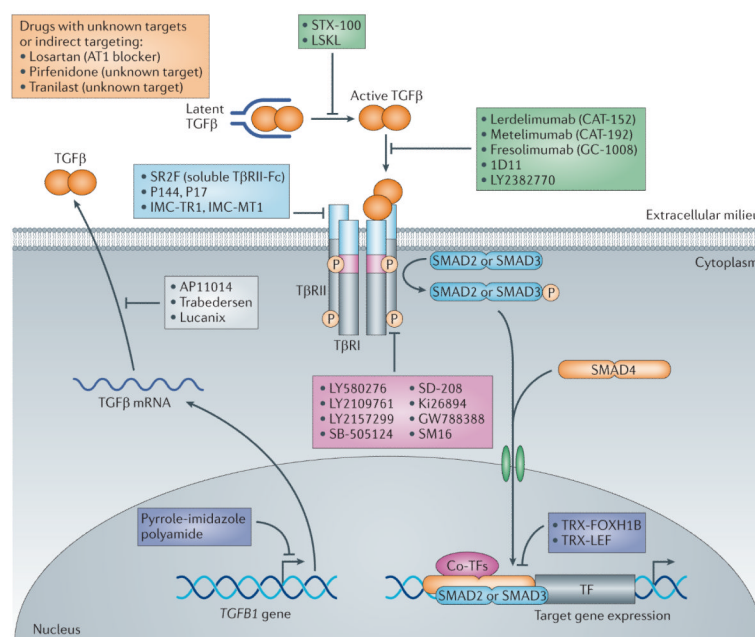


Figure 5. Schematic representation of therapeutic approaches for blocking TGF β signalling
 Transforming growth factor- β (TGF β) signalling can be inhibited by: sequestering ligands using soluble receptor ectodomain constructs (ligand traps) derived from TGF β receptortype II (T β RII) or T β RIII; using TGF β -neutralizing antibodies; or with T β RII or T β RI kinase inhibitors. Furthermore, translation of TGF β mRNA can be blocked by targeting TGF β mRNA with antisense oligonucleotides, thus preventing the production of the ligand. Different small-molecule kinase inhibitors against T β RI have been developed to block its kinase activity. Peptide inhibitors against specific TGF β ligands are also used. Other approaches block the transformation of TGF β from the latent to the active form. Three molecules are shown that either affect TGF β signalling indirectly (losartan) or that have an as-yet-unidentified target (tranilast and pirfenidone). All of these approaches decrease the initiation of intracellular receptor signalling pathways, such as phosphorylation of downstream receptor-specific SMADs (R-SMADs), and thereby blunt the transcriptional regulation of target genes. ATI, angiotensin II type 1 receptor; co-TFs, co-transcription factors; FOXH1B, forkhead box protein H1B; LEF, lymphoid enhancer-binding factor; LSKL, Leu-Ser-Lys-Leu peptide; TRX, thioredoxin.

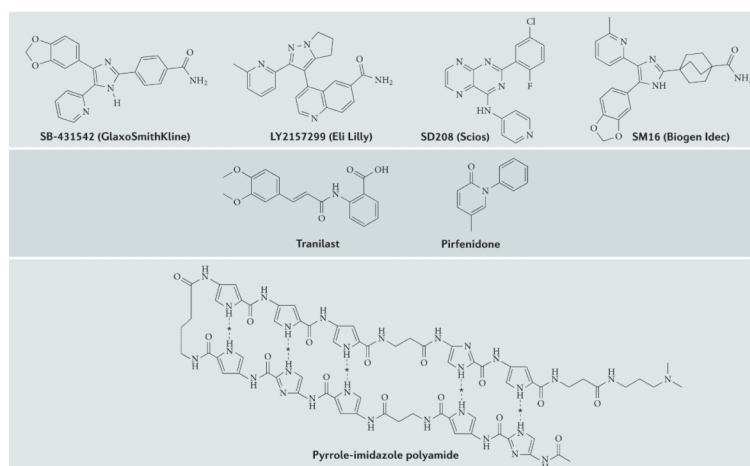


Figure 6. Structures of representative small-molecule inhibitors of TGF β signalling

Depicted are the molecular structures of a selection of small-molecule inhibitors identified to target the transforming growth factor- β (TGF β) signalling pathway. SB-431542, LY2157299, SD208 and SM16 are all ATP mimetics that inhibit TGF β receptortype I (TpRI; also known as TGFBR1) kinase activity. Pyrrole-imidazole polyamide blocks transcription of the *TGFB1* gene. Pirfenidone and tranilast have unknown molecular mechanisms of action. Dashed lines denote putative hydrogen bonding with bases in DNA; asterisks indicate positions where hydrogen bonds form with nucleotide residues of DNA within the *TGFB1* gene promoter.

Table 1
Summary of clinical trials for TGFβ inhibitory drugs

Drug; company	Type	Targets	Disease applications	Stage	Clinical trial identifiers	Summary of results	Refs
Trabectedin (AP12009); Antisense Pharma	Antisense oligo	TGFβ2 ligand	Glioblastoma	Phase I/IIb	NCT00431561	Safe	70,73,74
			Pancreatic cancer, MetM, colon cancer	Phase I	NCT00844064	Pancreatic cancer trials continue	84
Belagen-pumactucel-L (Lucanix); NovaRx	Antisense gene-modified allogeneic tumour cell vaccine	TGFβ2	Glioblastoma	Phase III	NCT00761280	Glioblastoma trials stopped in March 2012 owing to advances in standard of care and neurosurgery (Box 4)	85-87
			NSCLC	Phase III	NCT00676507	Well tolerated in 75 patients; survival advantage justifies further Phase III evaluation	
Disiertide (P144); Digna Biotech	Peptide	Peptide based on TβRIII that blocks ligand binding to receptors	Skin fibrosis in systemic sclerosis	Phase II	NCT00574613, NCT00781053	Preclinical efficacy in peritoneal fibrosis associated with peritoneal dialysis, renal and cardiac fibrosis, corneal haze and retinal AMD; safety and efficacy in Phase IIa clinical trial for scleroderma/skin fibrosis	75-78
Lerdelimumab (CA1-152); Cambridge Antibody Technology	Humanized antibody	TGFβ2 ligand	Reduction of scarring after glaucoma surgery	Phase III (complete)		Safe; ineffective in reducing scarring in Phase III trial	88,89
Metelimumab (CA1-192); Cambridge Antibody Technology	Humanized Antibody	TGFβ1 ligand	Diffuse systemic sclerosis	Phase I/II	NCT00043706	Ineffective when systemically administered in doses up to 10 mg per kg	90
Fresolimumab (GC-1008); Cambridge Antibody Technology/ Genzyme/ Sanofi	Humanized antibody	TGFβ1, TGFβ2 and TGFβ3 ligands	Focal segmental glomerulosclerosis	Phase I	NCT00464321	Completed and safe; plans to progress	92
			Systemic sclerosis	Phase I	NCT01284322	Still recruiting	-
						Study ongoing	-
						Completed, no results	-
			Myelofibrosis	Phase I	NCT01291784	See Box 4	93
			TPF	Phase I	NCT00125385	See Box 4	93

Drug; company	Type	Targets	Disease applications	Stage	Clinical trial identifiers	Summary of results	Refs
			Renal cell carcinoma	Phase I	NCT00356460	See Box 4	93
			Malignant melanoma	Phase I	NCT00356460	See Box 4	81
			Metastatic breast cancer (with radiotherapy)	Phase I	NCT01401062	Active and recruiting patients	-
			Relapsed malignant pleural, mesothelioma	Phase II	NCT01112293	Ongoing but not recruiting participants	-
LY2382770; Eli Lilly	Humanized Antibody	TGFβ1	Diabetic kidney disease (fibrosis)	Phase II	NCT01113801	Safety and efficacy in protecting kidney function in patients with diabetic kidney disease; still recruiting	-
SIX-100; Stromedix	Antibody	αVβ6 integrin	Fibrosis	Phase II	NCT01371305	Significant antifibrotic activity in preclinical models of lung, kidney and liver disease	168
LY2157299; Eli Lilly	Small molecule	TβRI kinase	Advanced-stage melanoma	Phase II	NCT00383320	See Box 4	82
			Recurrent glioblastoma	Phase II	NCT01582269	Recruiting; LY2157299 alone or with lomustine therapy versus lomustine alone in recurrent glioblastoma	213
			Glioblastoma	Phase II	NCT01220271	Recruiting; LY2157299 with temozolomide-based radiochemotherapy in newly diagnosed malignant glioma	212
			Hepato cellular carcinoma	Phase II	NCT01246986	Recruiting	-
Dominant negative <i>TGFβR2</i> -modified CILs	Recombinant T cells	TβRII	Advanced pancreatic carcinoma	Phase II	NCT01373164	Recruiting; comparison of gemcitabine with gemcitabine plus LY2157299	-
			Adoptive transfer of T cells expressing HER2 and Δ <i>NTGFR2</i> for lung cancer (HERCREEM)	Phase I	NCT00889954	No update on clinical trials	-

Drug; company	Type	Targets	Disease applications	Stage	Clinical trial identifiers	Summary of results	Refs
Dominant negative <i>TGFBR2</i> -modified CILs	Recombinant T cells (a clinical grade retrovirus vector encoding dominant negative T β RII)	T β RII	TGF β -resistant LMP2A-specific CILs for EBV-positive lymphoma	Phase I	NC T00368082	Preclinical efficacy in tumour killing of TGF β -secreting EBV-positive lymphoma; no update on clinical trials	107
Avotermin (Juvista); Renova	Recombinant protein	TGF β 3	Scarring	Phase II	NC T004322111, NC T00656227	The Juvista Phase II trial had not reached its primary or secondary efficacy end points as of February 2011	214
Pirfenidone; IntenMune	Small molecule, not TGF β -specific		IPF, glomerulosclerosis and diabetic kidney disease, pathological skin scarring	Phase III	Multiple trials	First drug approved for IPF in Europe	162
Losartan; Merck and Co.	Small molecule, not TGF β -specific	AT1	Marfan syndrome (MFS)	Phase I/II	NC T00723801, NC T00763893, NC T00782327	Reduction of aortic aneurysm in mouse model of MFS; clinical trials in progress to reduce aortic root dilation and cardiac muscle stiffness in patients with MFS	12,182
Tranilast; Kissei Pharmaceuticals	Small molecule, not TGF β -specific	Unknown	Corneal primary pterygium	Phase III	NC T01003613	Tranilast reduces myofibroblast proliferation of corneal myofibroblasts <i>in vitro</i> and may be a novel adjuvant therapy for corneal keloid	185, 215,216
IMC-TR1; ImClone Systems/ Eli Lilly	Humanized antibody	T β RII	Mammary and colon cancer	Phase I	NC T01646203	Preclinical efficacy against primary tumour growth and metastasis through multiple effects on tumour, stroma and immune cells	94

AMD, age-related macular degeneration; AT1, angiotensin II type 1 receptor; CTL, cytotoxic T lymphocyte; EBV, Epstein-Barr virus; IPF, idiopathic pulmonary fibrosis; LMP2A, an EBV-specific antigen; MetM, metastatic melanoma; NSCLC, non-small-cell lung cancer; oligo, oligonucleotide; T β 3R, TGF β 3 receptor; TGF β , transforming growth factor- β ; *TGFBR2*, gene encoding T β RII.

Table 2
Summary of TGF β inhibitory drugs in preclinical development

Drugs; company	Type	Targets	Disease applications	Stage	Summary of results	Refs
AP11014; Antisense Pharma	Antisense oligo	TGF β 1 ligand	Prostate cancer, NSCLC, colorectal cancer	Preclinical	AP11014 significantly reduced TGF β 1 secretion by 43-100% in different NSCLC, colon and prostate cancer cell lines	217
P17; Digna Biotech	Peptide	Peptide derived from Phage Display Technology that targets TGF β 1 binding to receptor	Liver and pulmonary fibrosis, metastatic lung cancer, angiogenesis, melanoma, immuno suppression, wet AMD	Preclinical	Preclinical efficacy in peritoneal fibrosis associated with peritoneal dialysis, lung fibrosis, corneal haze and retinal AMD	76
LSKL (academic only)	Peptide	Ihrombospondin	-	Preclinical	Preclinical efficacy in reducing renal injury and proteinuria in a murine model of diabetic nephropathy	97
ID11; R&D Systems	Mouse antibody	Mouse TGF β 1, TGF β 2 and TGF β 3 ligands	Breast cancer	Preclinical	Safe and efficacious in tumour metastasis in mice	79,80, 218
SR2F (academic only)	Ligand trap	TGF β 1, TGF β 3	Breast cancer	Preclinical	Very safe after lifetime exposure in mice; not progressing to clinical trial	125
Soluble T β R2-Fc; Genzyme	Ligand trap	TGF β 1, TGF β 3	Breast cancer	Preclinical	Safe and efficacious in suppressing metastasis in preclinical model of breast carcinoma; not progressing to clinical trial	96
LY580276, LY550410, LY364947, LY2109761 ^{*,*} ; Eli Lilly	Small molecule	T β R1 kinase	Cancer	Preclinical	LY2109761 is safe in long-term dosing of tumour-bearing mice, and efficacious in reducing metastasis and TICs in mouse cancer models	80,156, 219-222
SB-505124, SB-431542; GlaxoSmithKline	Small molecule	IPR1 kinase	-	Preclinical	Extensively used <i>in vitro</i> ; pharmacokinetically unstable <i>in vivo</i>	223-225
SD208, SD093; Scios	Small molecule	T β R1 kinase	Cancer	Preclinical	Efficacious in suppressing tumour metastasis in rodent models; programme discontinued after merger with Johnson & Johnson	146, 226,227
KI26894; Kirin Pharma ceuticals	Small molecule	T β R1 kinase	Breast cancer	Preclinical	Not progressing to clinical trial	148,150
SM16; Biogen Idec	Small molecule	T β R1 kinase	Mesothelioma	Preclinical	Not progressing to clinical trial	10,155, 228,229
GW798388; GlaxoSmithKline	Small molecule	T β R1 kinase	Fibrosis	Preclinical	Not progressing to clinical trial	230-232

Drugs; company	Type	Targets	Disease applications	Stage	Summary of results	Refs
GB1201, GB1203 (academic)	Pyrrole- imidazole polyamide	<i>TGFβ1</i> gene promoter	Cutaneous and corneal scarring, arterial restenosis, kidney fibrosis	Preclinical	Preclinical efficacy in inhibition of <i>TGFβ1</i> gene expression, which reduced corneal scarring and carotid artery restenosis	50, 102, 103

* Contrary to earlier reports^{4,16}, LY573636 is not a TGFβ-specific inhibitor. NSCLC, non-small-cell lung cancer; oligo, oligonucleotide; TβR, TGFβ receptor; TGFβ, transforming growth factor-β; TICs, tumour-initiating cells.