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Targeting the TGFβ signalling pathway in disease

Rosemary J. Akhurst[1] and **Akiko Hata**[2]

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

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

Abstract

Many drugs that target transforming growth factor-β (TGFβ) 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 TGFβ signalling pathway is a drug target, the potential clinical applications of TGFβ inhibition, the issues arising with anti-TGFβ 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 (TGFβ) superfamily of cytokines, which consists of TGFβs, 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 organisms[1]. The TGFβs *per se* are involved in many cellular processes, including growth inhibition, cell migration, invasion, epithelial-mesenchymal transition (EMT), extracellular matrix (ECM) remodelling and immune-suppression[2]. However, although normally dynamically regulated and involved in maintenance of tissue homeostasis, TGFβs are often chronically over-expressed in disease states, including cancer, fibrosis and inflammation, and this excessive production of TGFβ drives disease progression by modulating cell growth, migration or phenotype. The TGFβ 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 TGFβ-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 fibrosis[3]. In addition, the immune-modulatory activities of TGFβ have implications in many diseases, including cancer, cardiovascular disease, asthma, rheumatoid arthritis and multiple sclerosis[4].

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

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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

TGFβ 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 individuals[5-9]. This makes the pathway a particular challenge for drug development. Nevertheless, over the past decade several drugs targeting the TGFβ 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 TGFβ 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 fibrosis[10]. Additionally, there are novel reports of targeting TGFβ 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 TGFβ during tumorigenesis, which is characterized by tumour-suppressing activity of TGFβ at an early stage of cancer and tumour-promoting activity at later stages[13-16], few focus specifically on drug targets, drug classes and possible therapeutic applications beyond the oncology arena. The translation of anti-TGFβ therapies has been pursued most intensively for oncology; however, this Review also discusses the potential of the TGFβ signalling pathway as a target for non-neoplastic disease therapies and addresses the associated challenges in the development and application of these strategies.

The TGFβ family

The vertebrate genome contains more than 30 pleiotropic ligands that belong to the TGFβ superfamily, including TGFβs, BMPs, GDFs, activins, inhibins, Nodal and AMH[1]. TGFβ has a conserved motif of nine cysteine residues, eight of which form a tight cysteine knot, with the ninth being crucial for homodimerization[2]. Aberrant expression and activity of many of the ligands of the TGFβ superfamily are associated with developmental defects and human diseases[17]. Here we focus on TGFβs as there are currently several clinical trials underway involving therapies targeting TGFβ signalling, whereas other members of the TGFβ superfamily are under-represented in current trials.

Three highly homologous isoforms of TGFβ exist in humans: TGFβ1, TGFβ2 and TGFβ3. They share a receptor complex and signal in similar ways but their expression levels vary depending on the tissue[18], and their functions are distinct as demonstrated by the phenotypes of knockout mice[19-23]. Each TGFβ ligand is synthesized as a precursor, which forms a homodimer that interacts with its latency-associated peptide (LAP) and a latent TGFβ-binding protein (LTBP), forming a larger complex called the large latent complex (LLC). The TGFβ activation process involves the release of the LLC from the ECM, followed by further proteolysis of LAP to release active TGFβ to its receptors[2]. Matrix metalloproteinase 2 (MMP2) and MMP9 are known to cleave latent TGFβ. In addition to MMPs, thrombospondin 1 (THBS1) is known to activate latent TGFβ[24]. Alternatively, upon mechanical stretch, αVβ6 integrin can activate TGFβ by binding to the RGD motif present in LAP and inducing the release of mature TGFβ from its latent complex[25,26].

TGFβ signalling

Proteolytic cleavage, interaction with integrins or pH changes in the local environment are known to activate latent TGFβ and free active TGFβ for binding to its receptors at the cell membrane. TGFβ 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 TGFβ superfamily members to signalling receptors also exist. Each subfamily of the TGFβ 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. TGFβ 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 TGFβ receptor type I (TβRI; also known as TGFBR1 and ALK5) leads to phosphorylation of receptor-specific SMAD (R-SMAD) proteins. SMAD2 and SMAD3 are substrates of TβRI, 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 responses[27] (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 identified[28] (Fig. 1). Therefore, the canonical TGFβ-SMAD pathway modulates gene expression both transcriptionally and post-transcriptionally to propagate the physiological and pathological activities of TGFβ. In the non-canonical pathway, the activated TGFβ receptor complex transmits a signal through other factors, such as tumour necrosis factor (TNF) receptor-associated factor 4 (TRAF4), TRAF6, TGFβ-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-κB (NF-κB) (Box 2; Fig. 2). Thus, cellular responses to TGFβ 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 TGFβ signalling pathway, TGFβ 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 TGFβ signalling from suppressing growth to inducing cellular plasticity[29]. 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 TGFβ and other signalling pathways vary considerably but are essential to define the activities of TGFβ in propagating spatially and temporally specific outputs[6,31,32].

Biological actions of TGFβ

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

Inhibition of cell proliferation

TGFβ strongly inhibits the growth of many cell types, including epithelial, endothelial, haematopoietic and immune cells[37,38]. TGFβ also has pro-apoptotic and differentiation-inducing actions on epithelial cells; together, these actions result in tumour suppression in the context of cancer[34]. TGFβ 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 phase[39]. Conversely, TGFβ 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 determination[40]. In oncology, many tumours attenuate TGFβ 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, TGFβ 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 TGFβ signalling during tumorigenesis, the contribution of TGFβ 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 TGFβ signalling, as well as complex interactions between this pathway and other ligands of the TGFβ family, such as BMPs (Box 3). This has been confirmed by *in vitro* studies, demonstrating the contradictory effects of TGFβ in the regulation of vascular cells[36,41]. Furthermore, the TGFβ pathway often exhibits contrasting effects in different vascular cell types, such as endothelial versus vascular smooth muscle cells[36]. The promiscuous and cell type-specific action of the TGFβ pathway on vascular cells makes the application of targeted TGFβ signalling therapies for cardiovascular disease a particular challenge.

Induction of epithelial-mesenchymal transition and the myofibroblast phenotype

TGFβ can induce an EMT of both epithelial and endothelial cells. This has consequences for disease progression in both cancer and fibrosis[3]. 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 TGFβ-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 gene[33]. 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 phenotype[3]. 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 tumour[42], 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 resistance[43]. Blocking the TGFβ 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 delivery[44].

In fibrotic conditions, excessive TGFβ production induced in the diseased state contributes to EMT elaboration, which can further exacerbate fibrosis, as seen in pulmonary[45,46], cardiac[47] and renal[48,49] fibrosis, and in arterial restenosis following surgical trauma[50].

TGFβ can also promote a proliferative and/or migratory phenotype on smooth muscle cells that can aggravate some vascular diseases, including neointimal formation following vascular surgery[51-53].

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 TGFβ is widely documented to increase collagen synthesis and deposition by fibroblasts, TGFβ has become a central therapeutic target for different types of fibrosis. TGFβ 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 TGFβ-SMAD signalling pathways. There is a reciprocal regulation of TGFβ by the ECM: latent TGFβ 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 TGFβ; this, in turn, leads to the activation of TGFβ 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 TGFβ 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 *Tgfbr2* demonstrate both direct and indirect actions of TGFβ on effector T cells[4].

TGFβ has potent growth-suppressing activity on most precursor cells of the immune system, particularly T and B cells of the adaptive arm. TGFβ is a potent suppressor of T cell proliferation[55] and an inducer of B cell apoptosis[56]. 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 TGFβ1 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 TGFβ-induced *Id3* (Ref. 57). TGFβ suppresses inflammatory T_H1 and T_H2 cell differentiation while stimulating suppressor T_{Reg} cells. Overall, TGFβ-mediated suppression of effector $CD8^+$ cytolytic cells and T_H cells, together with TGFβ dependence for suppressive T_{Reg} cell differentiation, results in the hyper-inflammatory phenotype seen in *Tgfb1*$^{-/-}$ mice.

During tumour progression, excess TGFβ suppresses immune surveillance by attenuating the antitumour functions of $CD8^+$ T cells, $CD4^+$ T cells and dendritic cells. $CD4^+$ T cell-specific ablation of TGFβ signalling in transgenic mice expressing dominant negative TβRII (DNRII; also known as CD4-ΔTβRII and CD4-ΔTGFBR2) led to the development of autoimmunity[58] 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 littermates[58]. Tumour-derived TGFβ also blocks the

differentiation of antigen-presenting dendritic cells[59] and modifies chemokine receptor expression to blunt dendritic cell chemotaxis[60], further suppressing immune surveillance.

In addition to having a predominant immune-suppressive function, TGFβ counterintuitively may have a pro-inflammatory role through its effects on T_H17 cells and cells of the innate immune system. TGFβ, together with interleukin-6 (IL-6), was reported to be an essential player in driving pro-inflammatory T_H17 lineage differentiation[61-63]. 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 disorders[55]. 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' cell[64]. Second, the role of TGFβ in regulating the balance between T_H1 and T_H17 differentiation is in dispute. Despite the widespread acceptance of a role for TGFβ in T_H17 differentiation[61-63], more recent studies have suggested that TGFβ is totally dispensable for the generation of these cells[65,66].

With respect to cells of the innate immune system, TGFβ directly suppresses NK cell-mediated production of IFNγ (which is required for the tumour killing activity of NK cells) through transcriptional effects of SMAD3 on the IFNγ promoter[67]. It also 'polarizes' macrophages[68] and neutrophils[69] 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 TGFβ). These molecules can exacerbate the local diseased state, resulting in solid tumour progression or inflammation associated with fibrosis or atherosclerosis[4].

In summary, the regulation of the immune system by TGFβ 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 TGFβ signalling

Virtually every component of the TGFβ 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 Pharma[70-74], ligand-competitive peptides from Digna Biotech[75-78], antibodies that target ligands, receptors or associated proteins spearheaded by Genzyme[79-81], and SMIs against TGFβ receptor kinases developed by many companies, with Eli Lilly having an active clinical programme in Phase II development[82]. 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 TGFβ2, 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 tumour[74]. More recently, the company has started developing intravenous delivery approaches for pancreatic cancer, which appear to be effective in mouse models[73] and were recently shown to be safe in humans[84].

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 trial[87].

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 con venient 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 carcinoma[93] and at 1 mg per ml for the fibrotic disorder focal segmental glomerulosclerosis[92]. Lerdelimumab[88,89] and metelimumab[90], despite passing safety tests, failed to show efficacy in fibrotic models of corneal scarring and systemic sclerosis, respectively, and were therefore discontinued[90]. 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 cancer[95]; 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 trials[96]. 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 TGFβ 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 TβRI-targeted SMI into clinical trials for oncology is Eli Lilly with LY2157299 (Ref. 82) (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 surgery[50].

An alternative approach to suppress TGFβ 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 TGFβ pathway, with the caveat that gene therapy is still far from being widely accepted as a therapeutic approach[106].

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. TGFβ-resistant CTLs were found to have a functional advantage over unmodified CTLs in clearing TGFβ-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 cells[108-111]. This approach is in a Phase I clinical trial for advanced HER2-positive lung malignancy, labelled the HERCREEM trial (ClinicalTrials. gov identifier: NCT00889954).

Finally, Renova has developed a recombinant TGFβ3 ligand as an anti-scarring agent on the basis of the hypothesis that this ligand has activity that is independent of and antagonistic to TGFβ1 (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 TGFβ

Pre-existing drugs that have been extensively used for other applications may act, in part, by inhibiting TGFβ. 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 TGFβ 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 TGFβ[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 TGFβ levels, which — in certain cases such as arteriosclerosis — correlates with disease suppression[119].

Therapeutic uses of TGFβ signalling inhibition

Cancer

TGFβ 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 TGFβ 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, TGFβ 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 TGFβ ligands.

A major challenge in developing TGFβ 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 TGFβ drug development have been the inadvertent inhibition of the tumour-suppressing arm of TGFβ signalling in cancer[120-122] 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 TGFβ-mediated growth inhibition would not be a major issue[96,124,125]. However, clinical trials to date[82] have not revealed the cardiac valvulopathy[126] or hyperostosis and chondrocyte hypertrophy and hyperplasia[127] 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 TβRI but develop only self-limiting and mostly non-malignant skin lesions[128]. 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 dabrafenib[129], 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 skin[132]. Intriguingly, one study of keratoacanthoma that appeared in sorafenib-treated patients showed somatic *TGFBR1* missense mutations[133], one of which was also identified as a causative germline mutation for MSSE[128].

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 therapy[134]. 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 cancer[135], 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 cancer[135], glioblastoma[136-138] and chronic myeloid leukaemia[139] — 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 markers[137]. 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 trial[137]. 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 ago[142]. 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 carcinoma[143] and breast carcinoma[144] 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 progression[145]. 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 niche[146]. Understanding the differential molecular mechanisms that elicit these

variable responses will be critical to a judicious choice of treatment with TGFβ inhibitors or their derivatives. As TGFβ inhibitors are not directly cytotoxic, the use of these inhibitors in combination with cytotoxic chemotherapeutics may be particularly efficacious. The activation of TGFβ signalling in response to chemotherapeutics may drive the generation of cancer stem cells (via EMT), resulting in their chemoresistance[134]. This event may be targeted with TGFβ inhibitors, as demonstrated by the synergistic activity of doxorubicin and TGFβ inhibitor combination therapy on breast cancer growth and metastasis[147]. Studies in multiple myeloma also suggest that TGFβ inhibitors could potentiate the cytotoxic effects of melphalan and dexamethasone[148]. *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 TGFβ 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 growth[150]. Finally, another mechanism whereby TGFβ inhibition can augment conventional therapies is in enhancing drug delivery to the tumour. There are reports that TGFβ inhibition can reduce interstitial tumour pressure[44], 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 cancer[151].

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 killing[152]. 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 TGFβ on CTLs[153], and strategies to prevent such failure include the use of genetically modified CTLs with reduced TGFβ responsiveness. Transduction of CTLs with a virus encoding a DNRII[154] has reached Phase I clinical trials, and recent preclinical data indicate that combining CTL therapy with TβRI-targeting SMIs may also significantly improve T cell survival and antitumour T cell cytotoxicity[155]. Augmenting adoptive T cell therapy with SMIs may be a particularly attractive application of TβRI 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 inflammation[19], cardiovascular complications[126], bone and/or cartilage problems[127], subphyseal hyperostosis as well as chondrocyte hypertrophy and/or hyperplasia, and reducing the risk of developing SMI drug resistance[156].

Another clinical application with great promise is augmenting radiotherapy by inhibiting the TGFβ path-way[10,81,157]. Radiation not only physically activates latent TGFβ *in vitro* but also induces the biological release of this growth factor as part of a stress response[158]. Several groups have reported the positive role of TGFβ in supporting the DNA damage repair pathway, particularly through activation of p53 and phosphorylation of ataxia telangiectasia mutated (ATM) after radiation therapy[159]. 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 radiation[81]. Even short-term dosing with TβRI 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 therapy[10] — 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 glioma[157].

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 TβRI, was a common and significant event observed in $CD34^+$ myeloid progenitor cells in the bone marrow of patients with MDS[160]. 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 TGFβ such that even very low levels of the ligand elicited an increase in TGFβ responsiveness, as defined by P-SMAD2 levels and enhanced immune-suppressive effect, thus providing another opportunity to utilize TGFβ inhibitors for therapeutic utility in human disease.

Treatment of primary $CD34^+$ haematopoietic stem cells with LY2157299 suppressed the activation of TβRI by its ligand. Moreover, in a liver-specific TGFβ1-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 MDS[160].

Fibrosis

IPF is a progressive, chronic and irreversible lung disease occurring in older adults, and has an unknown cause[161]. 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 TGFβ plays a major part[16]. TGFβ1, 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. αVβ6 integrin, which binds and activates latent TGFβ1 and TGFβ3, is highly induced following lung injury or fibrosis[162]. TGFβ activity[163] 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, scarring[162] and reduced lung function. TGFβ also promotes pulmonary EMT that additionally contributes to the expansion of fibroblasts and myofibroblasts[164].

Pirfenidone, a novel compound that inhibits TGFβ 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 group[165]. 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 TGFβ-based therapies for IPF include gene transfer of a soluble TβRII construct (as a ligand decoy), which attenuated injury and fibrosis in bleomycin-induced IPF in mice[166]. P144 (disitertide; Digna Biotech), a synthetic peptide that attenuates TGFβ activity and is derived from the

extracellular domain of betaglycan, was also shown to reduce carbon tetrachloride-induced liver injury in mice[78]. P17, another Digna Biotech anti-TGFβ peptide, has been shown to be efficacious in attenuation of injury and fibrosis in bleomycin-induced IPF in mice[167]. 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-TGFβ therapies in clinical trials for IPF include the pan TGFβ-neutralizing antibody GC-1008 (Genzyme) and the αVβ6 integrin-blocking antibody STX-100 (Stromedix)[168,169].

Renal fibrosis has long been thought to be driven by excess TGFβ, which results in renal scarring and, ultimately, kidney failure[170]. 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 TGFβ1 (from the liver) develop progressive liver and renal fibrosis[170]. Interestingly, although mice overexpressing active TGFβ1 develop progressive renal injury, latent TGFβ1 also has a protective role in renal fibrosis through negative effects on inflammation[49]. TGFβ1 mediates progressive renal fibrosis by stimulating the synthesis of ECM production while inhibiting its degradation[49]. TGFβ1 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 IPF[171]. Blockade of TGFβ1 with neutralizing TGFβ antibodies prevents or ameliorates renal fibrosis *in vivo* and *in vitro*, demonstrating the functional role of TGFβ1 in EMT and renal fibrosis. A number of therapeutic interventions that block the action of TGFβ have resulted in various degrees of improvement in kidney structure and function in preclinical studies; such interventions include TGFβ ASOs, a neutralizing anti-TGFβ antibody, a soluble TGFβ receptor, blockade of TGFβ activation by decorin[172], an SMI of TGFβ receptors, delivery of the inhibitor protein SMAD7 (Ref. 173) and a THBS1-blocking peptide that interferes with TGFβ activation[97]. A Phase I/II trial with GC-1008, a pan-TGFβ-neutralizing antibody, exhibited encouraging efficacy in patients with focal segmental glomerulosclerosis[92], and Eli Lilly is undertaking trials of its own anti-TGFβ1 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 failure[36]. 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 TGFβ 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 TGFβ signalling pathway in this pathogenesis. TGFβ, a potent stimulator of collagen production by cardiac fibroblasts, is induced in response to cardiovascular injury. The TGFβ-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]. TGFβ 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 fibrosis[77], and losartan can reverse fibrosis in a mouse model of hypertrophic cardiomyo pathy[174]; however, no drug targeting the TGFβ pathway has yet reached clinical trials for this application. A recent study demonstrated that miR-21, which is regulated by SMADs upon TGFβ 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 lesions[175]. 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 TβRI and TβRII in sclerodermal fibroblasts suggests that increased production of type I collagen by autocrine TGFβ signalling leads to aberrant ECM deposition and scarring[36]. Therapeutic approaches to scleroderma have included inhibition of TGFβ activity in sclerotic tissue. Unfortunately, CAT-192, a TGFβ1-neutralizing antibody, did not show evidence of efficacy in a study on the treatment of patients with early-stage systemic scleroderma[90]; 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 TGFβ1 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 standardized[176].

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. TGFβ1 is a major player in the early development of intimal hyperplasia in arteries and peripheral vein grafts. The exact mechanism of action of TGFβ signalling in intimal hyperplasia and subsequent graft failure is unclear, but it is speculated that TGFβ1 contributes at multiple steps, including EMT, promotion of fibroblast, endothelial and vascular smooth muscle cell proliferation, increased collagen synthesis and deposition, and induction of fibrosis[177]. Soluble forms of the small, leucine-rich proteoglycans decorin and fibromodulin, which possess TGFβ-antagonist activity, exhibit potent intimal hyperplasia-suppressing effects in cultured human saphenous vein, offering the potential for therapeutic benefit after coronary artery bypass surgery[178-180]. The novel pyrrole-imidazole polyamide drug class, targeted to suppress *TGFB1* gene transcription, showed efficacy in reducing neointimal hyperplasia and stimulating re-endothelialization of carotid arteries in a preclinical model of arterial injury[50].

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 TGFβ signalling[11]. Normal fibrillin 1-containing microfibrils interact with the large latent TGFβ complex (LLC) to control the release of mature, active TGFβ[181]. Mutated fibrillin 1 fails to sequester latent TGFβ, leading to the promiscuous activation of TGFβ[11]. Thus, MFS highlights the critical role of microfibrils in regulating local concentrations of TGFβ and in maintaining the homeostasis, morphogenesis and function of various organs[181]. 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 TGFβ antagonists, including a TGFβ-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 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 TGFβ2 (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 effects[184]. Tranilast, another incidental TGFβ inhibitor, has also been used successfully to reduce re-occurrence of corneal fibrosis, or primary pterygium, following corneal surgery[185]. It is possible that topical application of more-specific TGFβ inhibitors might also be used in treating corneal haze and conjunctival scarring (Table 1).

Challenges and considerations for TGFβ blockade

Individualized responses to TGFβ blockade

Many diseases being tackled with TGFβ 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 TGFβ 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 TGFβ signalling pathway genes[8,41,186]. Moreover, in mouse models of cancer, asthma and vascular development, outcomes of reduced TGFβ1 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-TGFβ drug treatment on the basis of surrogate markers of TGFβ involvement in the disease process (such as increased TGFβ 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 TGFβ 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 PMNCs[189-192] to TGFβ inhibition *ex vivo*.

Patient selection for TGFβ inhibitors in oncology

The simplest biomarker for patient selection for TGFβ inhibitors in oncology is probably high circulating levels of TGFβ, as one major goal of this therapy is to reduce, but not totally ablate, TGFβ signalling[193]. As indicated above, non-invasive biomarkers for predicting patient responsiveness and efficacy of TGFβ 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 TGFβ 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 TGFβ 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 TGFβ 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 TGFβ 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 TGFβ 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 TGFβ. Molecular and histological analyses may also contribute to the prediction of tumour responses to TGFβ inhibition. The activation of alternative intracellular signalling pathways and transcription factor profiles has been associated with the switch from tumour suppression — by TGFβ — 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/EBPβ), a central transcription factor that binds within the SMAD transcription factor complex to elicit TGFβ-mediated cytostatic responses[196]. Upregulation of SIX1 in breast cancer has also been shown to be pivotal in the growth-suppressive to tumour-progressive switch[197], 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 TGFβ 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 TGFβ signalling pathway, which would then counter-indicate their combinatorial use with TGFβ inhibitors[199]. Indeed, the ability to specifically target the pro-tumorigenic versus the tumour-suppressive effects of TGFβ 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 TGFβ 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 phenotype[156], although this remains to be tested. Carefully restricting TGFβ inhibitors to short-term or intermittent usage should avoid these complications[82]. 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, TGFβ 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 TGFβ 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 TGFβ-SMAD3 signalling regulates glucose tolerance and energy homeostasis, and that blockade of the pathway may be used for regulation of diabetes and obesity[204]. The outlook for anti-TGFβ 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 TGFβ superfamily of growth factors

The basic framework of the canonical signal transduction pathways of three subfamilies of the transforming growth factor-β (TGFβ3) superfamily — TGFβ3s, 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 TGFβ, 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 negatively31,205. 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 TGFβ 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 TGFβ superfamily of ligands: TGFβs, activins/inhibins/Nodal and BMPs.

Molecular category	TGFp pathway*	Activin/inhibin/Nodal pathway*	BMP pathway*
Ligands	TGFβ1, TGFβ2, TGFβ3	Activin A, activin B, inhibin A, inhibin B, Nodal	BMP2, BMP4, BMP5, BMP6, BMP7, BMP8A, BMP8B, BMP9, BMP10
Type I receptors	TβRI(ALK5), ALK1 (ACVRLlorSKR3)	ALK4(ACVR1Bor ACTRIB), ALK7 (ACVR1C or ACTRIC)	ALK1 (ACVRL1, SKR3), ALK2 (ACVR1, ACTRI), ALK3 (BMPR1A), ALK6 (BMPR1B)
Type II receptors	TβRII	ACTRIIA, ACTRIIB	BMPR2, ACTRIIA, ACTRIIB
Type III receptors	TβRIII (betaglycan), endoglin, CRIPT03 (TDGF1P3)	CRIPT01 (TDGF1), CRIPT03 (TDGF1P3),TβRIII (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; Tβ3R, TGFβ receptor; TDGF, teratocarcinoma-derived growth factor.

Box 2

Non-canonical TGFβ signalling and crosstalk with other pathways

In addition to activating SMAD proteins, transforming growth factor-β (TGFβ) signalling can regulate the activity of a number of signalling molecules, such as TNF receptor-associated factor 4 (TRAF4), TRAF6, TGFβ-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-κB (NF-κB), to transmit a signal[6]. In addition, these non-canonical signals can crosstalk with the SMAD pathways and mutually modulate each other. Both canonical and non-canonical TGFβ signalling can also be influenced by other signalling pathways, such as the RAS, WNT, Hedgehog, Notch, tumour necrosis factor (TNF) and interferon pathways[6]. The exact nature of the crosstalk with other pathways and biological outcomes is complex and highly context-dependent[6]. 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 TGFβ activity.

Box 3

BMP-TGFβ-activin crosstalk in endothelial cells

Within the transforming growth factor-β (TGFβ) superfamily, the crosstalk between three subfamilies — activins/inhibins/Nodal, TGFβ3s and bone morphogenetic proteins (BMPs) — is well established during development and postnatal homeostasis of various organs[206-207]. In vertebrates, the BMP-SMAD1/SMAD5/SMAD8 and activin–Nodal–TGFβ-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, TGFβ can signal not only via canonical TGFβ receptor type I (TβRI)-SMAD2/SMAD3 but also via activin receptor-like kinase 1 (ALK1)-SMAD1/SMAD5/SMAD8 (Ref. 36). In contrast to TGFβ-TβRI signalling-mediated activation of SMAD2 or SMAD3, which leads to endothelium quiescence, TGFβ-ALK1 signalling induces SMAD1/SMAD5/SMAD8 activation and has been shown to stimulate endothelial cell migration, proliferation and tube formation, thus promoting angiogenesis[208]. 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 TGFβ-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 function[36]. In summary, the crosstalk among signalling pathways mediated by different TGFβ 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-β2 (TGFβ2)-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 chemotherapy[209]. 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 chemotherapy[74]. However, questions have been raised about this most recent study[210,211]. Wick and Weller[211] conceded that although trabedersen was clinically safe and that TGFβ 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 glioblastoma[212], 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-TGFβ 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 glioma[213], plus a new Phase I trial of IMC-TM1, an anti-TGFβ receptor type II (TβRII) 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 chemotherapy[87].

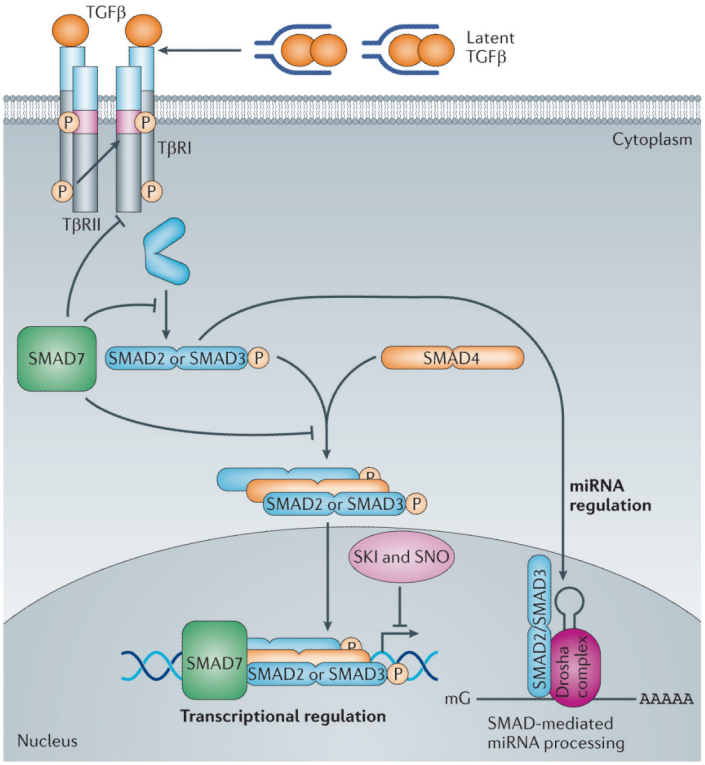

Figure 1. Schematic overview of the canonical, SMAD-dependent TGFβ signalling pathway

The transforming growth factor-β (TGFβ) ligands are synthesized as a large latent TGFβ complex consisting of mature dimeric TGFβ associated with its latency-associated peptide (LAP) and a latent TGFβ-binding protein (LTBP) (not shown). Upon activation, TGFβ dimers induce heteromeric complex formation between specific type II and type I receptors (such as TGFβ receptortype II (TpRII) andTpRI, respectively). Type II receptors then transphosphorylate the type I receptors, which propagate the signal into the cell by phosphorylating TGFβ 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 SBE[27]. The nuclear proteins SKI and SNO (also known as SKIL) antagonize the transcriptional regulation by SMADs. An inhibitory SMAD (I-SMAD), SMAD7, inhibits the TGFβ 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.

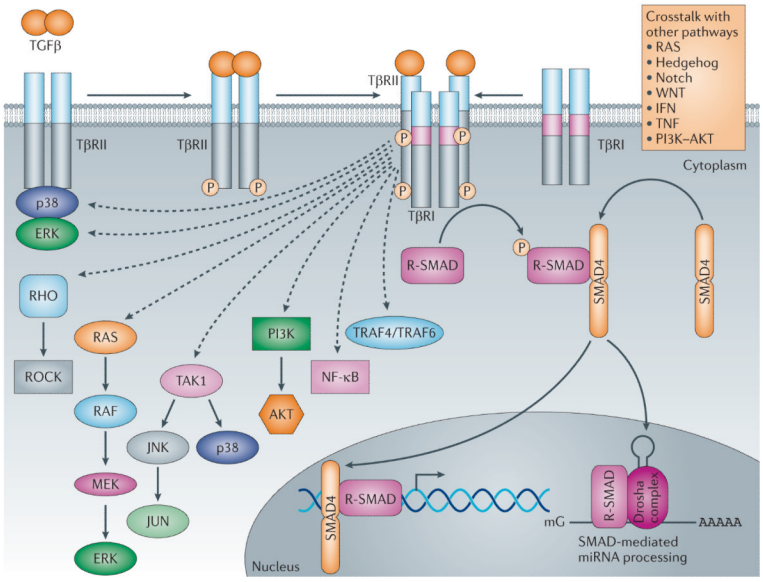

Figure 2. Schematic representation of non-canonical TGFβ signalling and crosstalk with other signalling pathways

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-KB (NF-kB). 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; TpR, TGFβ receptor. ‘mG’ and ‘AAAAA’ represent 5′ capping and 3′ polyadenylation of mRNAs, respectively.

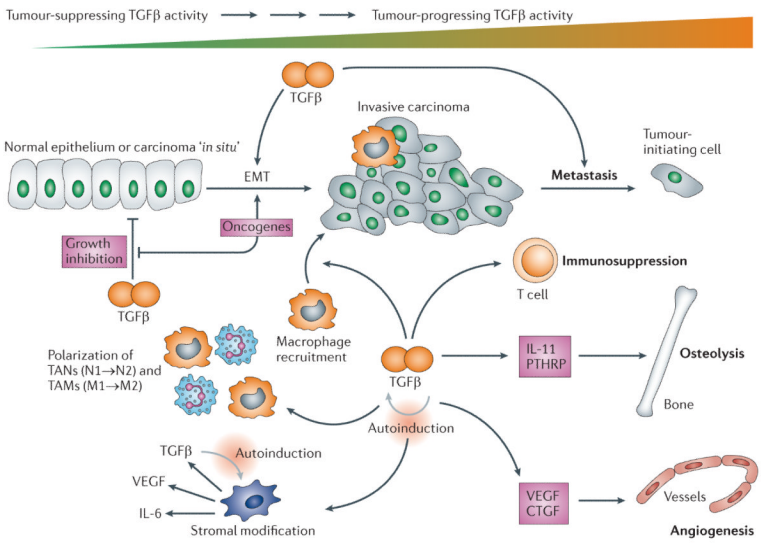

Figure 3. Biphasic activities of the TGFβ signalling pathway during tumorigenesis: from the tumour suppressor to the tumour promoter

Transforming growth factor-β (TGFβ) has biphasic actions during tumorig enesis, 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.

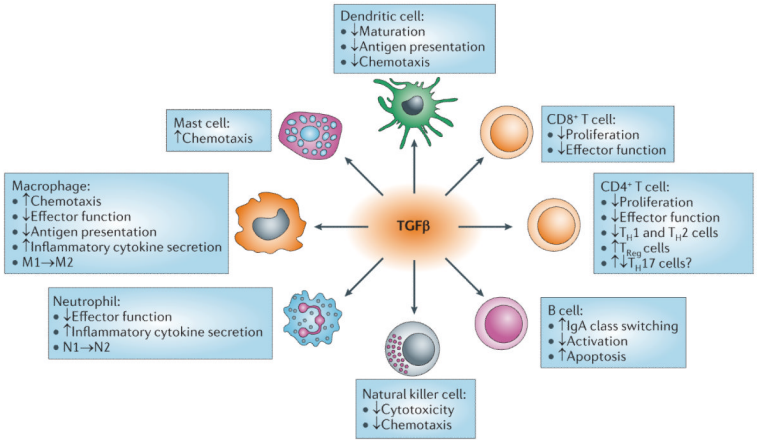

Figure 4. TGFβ effects on immune cells

Transforming growth factor-p (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.

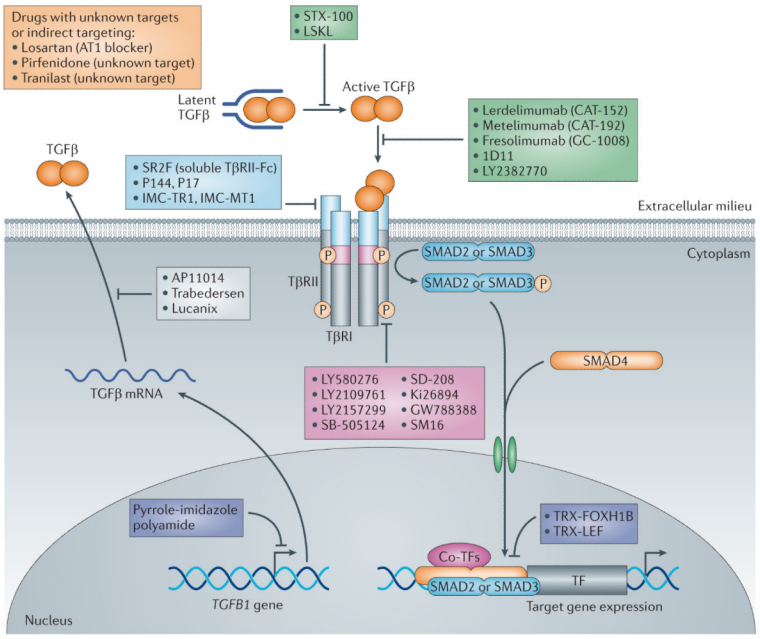

Figure 5. Schematic representation of therapeutic approaches for blocking TGFβ signalling

Transforming growth factor-p (TGFβ) signalling can be inhibited by: sequestering ligands using soluble receptor ectodomain constructs (ligand traps) derived from TGFβ receptortype II (TpRII) or TpRIII; using TGFβ-neutralizing antibodies; or with TpRII or TpRI kinase inhibitors. Furthermore, translation of TGFβ mRNA can be blocked by targeting TGFβ mRNA with antisense oligonucleotides, thus preventing the production of the ligand. Different small-molecule kinase inhibitors against TpRI have been developed to block its kinase activity. Peptide inhibitors against specific TGFβ ligands are also used. Other approaches block the transformation of TGFβ from the latent to the active form. Three molecules are shown that either affect TGFβ 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.

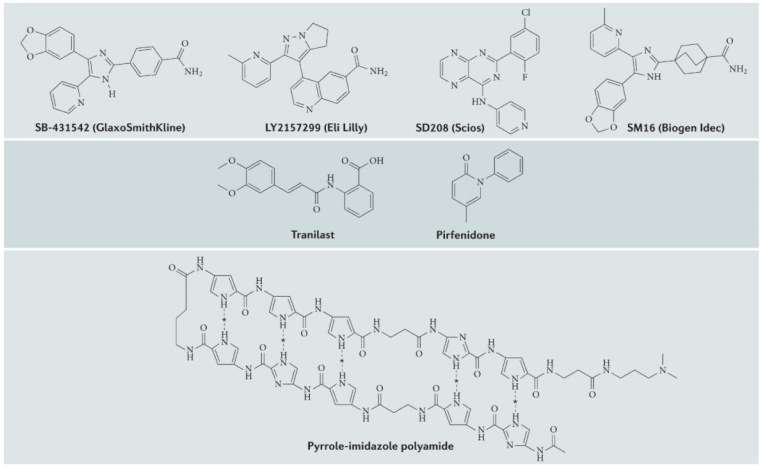

Figure 6. Structures of representative small-molecule inhibitors of TGFβ signalling

Depicted are the molecular structures of a selection of small-molecule inhibitors identified to target the transforming growth factor-p (TGFβ) signalling pathway. SB-431542, LY2157299, SD208 and SM16 are all ATP mimetics that inhibit TGFβ receptortype I (TpRI; also known asTGFBRl) 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
Trabedersen (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
			Glioblastoma	Phase III	NCT00761280	Glioblastoma trials stopped in March 2012 owing to advances in standard of care and neurosurgery (Box 4)	
Belagen-pumatucel-L (Lucanix); NovaRx	Antisense gene-modified allogeneic tumour cell vaccine	TGFβ2	NSCLC	Phase III	NCT00676507	Well tolerated in 75 patients; survival advantage justifies further Phase III evaluation	85-87
Disitertide (P144); Digna Biotech	Peptide	Peptide based on TβRIIIthat 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 (CAI-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 (CAI-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	Phasel	NCT00464321	Completed and safe; plans to progress	92
			Systemic sclerosis	Phasel	NCT01284322	Still recruiting	-
						Study ongoing	-
						Completed, no results	-
			Myelofibrosis	Phasel	NCT01291784	See Box 4	93
			TPF	Phasel	NCT00125385	See Box 4	93

Drug; company	Type	Targets	Disease applications	Stage	Clinical trial identifiers	Summary of results	Refs
			Renal cell carcinoma	Phasel	NCT00356460	See Box 4	93
			Malignant melanoma	Phasel	NCT00356460	See Box 4	81
			Metastatic breast cancer (with radiotherapy)	Phasel	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	aVβ6 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	NCI10038320	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	-
			Advanced pancreatic carcinoma	Phase II	NCT01373164	Recruiting; comparison of gemcitabine with gemcitabine plus LY2157299	-
Dominant negative *TGFBR2*-modified CILs	Recombinant T cells	TβRII	Adoptive transfer of T cells expressing HER2 and Δ*NTGFBR2* for lung cancer (HERCREEM)	Phasel	NCT00889954	No update on clinical trials	-

Drug; company	Type	Targets	Disease applications	Stage	Clinical trial identifiers	Summary of results	Refs
Dominant negative *TGFBR2*-modified CILs	Recombinant T cells (a clinical grade retrovirus vector encoding dominant negative TβRII)	TβRII	TGFβ-resistant LMP2A-specific CILs for EBV-positive lymphoma	Phasel	NCT00368082	Preclinical efficacy in tumour killing of TGFβ-secreting EBV-positive lymphoma; no update on clinical trials	107
Avotermin (Juvista); Renova	Recombinant protein	TGFβ3	Scarring	Phase II	NCT004322111, NCT00656227	Thejuvista Phase II trial had not reached its primary or secondary efficacy end points as of February 2011	214
Pirfenidone; lnterMune	Small molecule, not TGFβ-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 TGFβ-specific	AT1	Marfan syndrome (MFS)	Phase I/II	NCT00723801, NCT00763893, NCT00782327	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 TGFβ-specific	Unknown	Corneal primary pterygium	Phase III	NCT01003613	Tranilast reduces myofibroblast proliferation of corneal myofibroblasts *in vitro* and may be a novel adjuvant therapy for corneal keloid	185, 215,216
IMC-TR1; lmClone Systems/ Eli Lilly	Humanized antibody	TβRII	Mammary and colon cancer	Phasel	NCT01646203	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–Barrvirus; IPF, idiopathic pulmonary fibrosis; LMP2A, an EBV-specific antigen; MetM, metastatic melanoma; NSCLC, non-small-cell lung cancer; oligo, oligonucleotide; Tβ3R, TGFβ3 receptor; TGFβ, transforming growth factor-β; *TGFBR2*, gene encoding TβRII.

Table 2

Summary of TGFp inhibitory drugs in preclinical development

Drugs; company	Type	Targets	Disease applications	Stage	Summary of results	Refs
AP11014; Antisense Pharma	Antisense oligo	TGFβ1 ligand	Prostate cancer, NSCLC, colorectal cancer	Preclinical	AP11014 significantly reduced TGFβ1 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 TGFβ1 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
1D11; R&D Systems	Mouse antibody	Mouse TGFβ1, TGFβ2 and TGFβ3 ligands	Breast cancer	Preclinical	Safe and efficacious in tumour metastasis in mice	79,80, 218
SR2F (academic only)	Ligand trap	TGFβ1, TGFβ3	Breast cancer	Preclinical	Very safe after lifetime exposure in mice; not progressing to clinical trial	125
Soluble TβR2-Fc; Genzyme	Ligand trap	TGFβ1, TGFβ3	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	TβRI 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	IPRI kinase	-	Preclinical	Extensively used *in vitro*; pharmacokinetically unstable *in vivo*	223-225
SD208, SD093; Scios	Small molecule	TβRI 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	TβRI kinase	Breast cancer	Preclinical	Not progressing to clinical trial	148,150
SM16; Biogen Idec	Small molecule	TβRI kinase	Mesothelioma	Preclinical	Not progressing to clinical trial	10,155, 228,229
GW788388; GlaxoSmithKline	Small molecule	TβRI 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	*TGFB1* gene promoter	Cutaneous and corneal scarring, arterial restenosis, kidney fibrosis	Preclinical	Preclinical efficacy in inhibition of *TGFB1* 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.