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Targeting Transforming Growth Factor-β (TGF-β) in Cancer and Non-Neoplastic Diseases

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Abstract

Transforming growth factor-β (TGF-β) superfamily is a key player in the regulation of a wide variety of physiological processes from development to pathogenesis. Since the discovery of the prototypic member, TGF-β, almost three decades ago, there have been tremendous advances in our understanding of its complex biology. TGF-β misregulation has been implicated in the pathogenesis of a variety of diseases, including cancer with a direct role in facilitating metastasis, fibrosis and inflammation. Consequently, TGF-β is currently explored as a prognostic candidate biomarker of tumor invasiveness and metastasis; and it offers an attractive target for cancer therapy. Several anti-TGF-β approaches, such as TGF-β antibodies, antisense oligonucleotides and small molecules inhibitors of TGF-β type 1 receptor kinase, have shown great promise in the preclinical studies. Here, we consider why the TGF-β signaling pathway is a drug target, the potential clinical applications of TGF-β inhibition, the issues arising with anti-TGF-β therapy and how these might be adopted using personalized approaches with a special care for patient selection and timing of therapy so that we may bring forward all the potentials of targeting this pathway for therapeutic uses in both cancer, preferentially in combination therapy, and non-neoplastic diseases.

Keywords

Transforming Growth Factor-β (TGF-β), Monoclonal Antibodies (MoAbs), Antisense Oligonucleotides (ASO), Small Molecule Receptor Kinase Inhibitors (SMIs)

1. Introduction

The transforming growth factor-β (TGF-β) superfamily was discovered in a hunt for autocrine factors secreted

from cancer cells that promote transformation of normal fibroblast [1]. In 1978 De Larco and Todaro described the partial purification of Sarcoma Growth Factors (SGFs) and their ability to induce anchorage-independent growth in normal rat kidney cells [2]. The first member of the transforming growth factor-β (TGF-β) superfamily of secreted polypeptide factors, TGF-β1, was then discovered two years later when Roberts *et al*. [3] and Moses *et al*. [4] independently purified TGF-β as one component of SGF. These findings initiated the birth of the TGF-β signaling field and since then, the family has grown considerably and TGF-β superfamily was defined as a key class of secreted morphogens [5] [6].

The human TGF-β family comprises more than 30 factors that can be divided into two distinct branches using two distinct downstream pathways. Factors such as activin, nodal, lefty, myostatin, and TGF-β are clustered in one family branch, and bone morphogenetic proteins (BMPs), anti-muellerian hormone (AMH, also known as MIS), and various growth and differentiation factors (GDFs) are grouped into the other branch [7]-[9]. TGF-βs control a plethora of cellular functions, and their activity is critical for regulating numerous developmental and homeostatic processes. In addition, TGF-β plays a dual role in cancer, despite being normally dynamically regulated and involved in maintenance of tissue homeostasis. TGF-βs are often chronically over-expressed in disease states, including cancer, fibrosis and inflammation, and this excessive production of TGF-β drives disease progression by modulating cell growth, migration or phenotype. That is why TGF-β signaling pathway has been the focus of research for the past three decades and has therefore become a popular target for drug development not only for oncologists but also for cardiovascular surgeons to prevent neointimal hyperplasia, and for nephrologists and pneumologists in the treatment of fibrosis [10]. The present review focuses on the potential clinical application for TGF-β inhibition in cancer as well as non-neoplastic diseases presented and classified in terms of drug targets. We introduce this review aiming to gather as most recent updates as possible in this field together with the basics of TGF-β signaling pathway so that we may bring forward all the potentials of targeting this pathway for therapeutic uses in both cancer and non-neoplastic diseases.

2. TGF-β Signaling

Most members of this cytokine family exist in variant forms. TGF-β the prototypic member of its superfamily exists in humans in three highly homologous isoforms: TGF-β1, TGF-β2 and TGF-β3. The bioactive cytokine molecule is a dimer composed of a polypeptide chain that is cleaved from a precursor. The active TGF-β dimer signals by bringing together two pairs of receptor serine/threonine kinases known as the type I (TβR-I) and type II receptors (TβR-II), respectively in a heterotetrameric complex composed of a pair of each receptor. 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 signaling phosphorylating Smad transcription factors. Receptors of the TGF-β branch of the cytokine family phosphorylate Smads 2 and 3, whereas those of the other branch such as BMP receptors phosphorylate Smads 1, 5, and 8. Once activated the receptor substrate Smads (RSmads) shuttle to the nucleus and form a complex with Smad4, a binding partner common to all RSmads. Entering the nucleus, R-Smad-Co-Smad complex additionally recruits transcriptional coactivators, corepressors, and chromatin remodeling factors (co-factors) (**Figure 1**) [9] [11]-[13].

3. Therapeutic Targeting of TGF-β Signalling in Cancer

TGF-β has been studied in multiple of cancer types. Some cancers may show specific mutation of TGF-β signaling components; for example, in colon, gastric, biliary, pulmonary, ovarian, esophageal, and head and neck carcinomas receptor mutations were highly represented with microsatellite instability (MSI) and some pancreatic cancers may show Smad4 mutations. Other cancer types such as breast and endometrial tumors occasionally show mutation of TGF-β signaling components. Rather, TGF-β growth response is lost either by changes in the profile of other active signaling networks or the relative availability of transcriptional co-repressors or co-activators that bind to and modulate the canonical Smad pathway [14].

TGF-β has dual action in cancer. Normally, it exerts protective or tumor suppressive effects on normal epithelial cells or during the early growth-sensitive stages of tumorigenesis that cancer cells must elude for malignant evolution. However, later in tumor development when carcinoma cells become refractory to TGF-β-mediated growth inhibition, the tumor cell responds by stimulating pathways with tumor progressing effects. At late

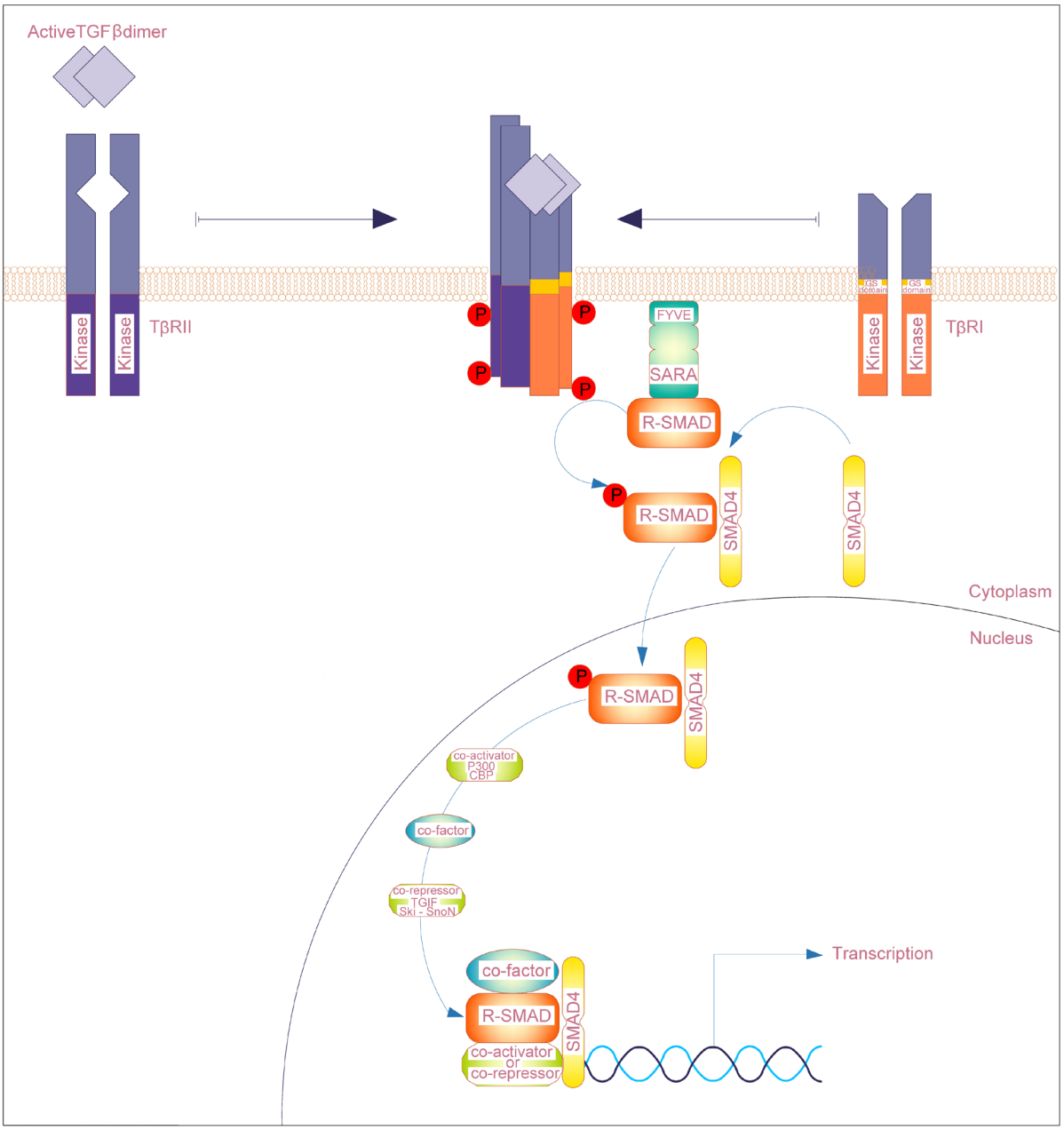

Figure 1. TGF-*β* canonical (Smad-dependent) signaling pathway.

stages of malignancy, tumor progression is driven by TGF-*β* overload. The tumor microenvironment is a target of TGF-*β* action that stimulates tumor progression *via* pro-tumorigenic effects on vascular, immune, and fibroblastic cells acting as a significant stimulator of tumor progression, invasion and metastasis. Once the tumor cell has undergone certain genetic and/or epigenetic changes that attenuate the growth suppressive pathway of TGF-*β*, targeted over expression of TGF-*β*1 can drive malignant progression and metastasis [15] [16].

As a result of the wide variety of effects of TGF-*β* on tumorigenesis, blockade of TGF-*β* and its signaling pathway provides multiple therapeutic opportunities. The objective of TGF-*β* inhibition is to target its tumor promoting properties, both cell autonomous and microenvironmental, while avoiding inhibition of its tumor suppression arm. In spite of the serious concerns that apply to targeting a pleiotropic cytokine pathway, anti-TGF-*β* compounds have been developed that show efficacy in preclinical studies and clinical trials, and there is some powerful evidence highlighting the potency of inhibiting TGF-*β* signaling as a way of controlling tumor pro-

gression including metastasis [17]. Initial efforts to develop TGF-*β* signaling antagonists focused on inhibition of TGF-*β* ligand binding to T*β*RII receptor by the use of soluble TGF-*β* binding proteins (decorin, fetuin). However, these inhibitors have not provided sufficient specificity for therapeutic purposes [18]. Further advances have generated new classes of inhibitors with greater specificity. The major classes of TGF-*β* inhibitors include: 1) ligand traps including monoclonal antibodies that target ligands, receptors or associated proteins (MoAbs), 2) antisense oligonucleotides (ASO), 3) small molecule receptor kinase inhibitors (SMIs), and 4) peptide aptamers (**Figure 2**).

- **Ligand Traps**

  Ligand traps serve as a sink for the excess TGF-*β* produced by tumor cells and fibroblasts during cancer progression, which increases with aggressiveness and tumor stage. Ligand traps include anti-ligand neutralizing monoclonal antibodies (MoAbs) and soluble decoy receptor proteins that incorporate the ectodomains from either T*β*RII or T*β*RIII/betaglycan protein.

  TGF-*β* monoclonal antibodies (MoAbs) that are typically used to disrupt TGF-*β* ligand binding to the T*β*RII receptor have been raised against individual ligands or may be designed to block all three isomers. The high degree of target specificity of MoAbs, their high binding affinity and long half-life and thus reduced frequency of dosing are all advantages that counterbalance inherent undesirable pharmaceutical properties, such as structural complexity, large molecular weight so poor tissue penetration, and physiologic barriers to intratumoral uptake. Examples include **CAT-152 (Lerdelimumab)** [19] a recombinant human IgG4 that neutralizes TGF-*β*2 following glaucoma surgery, **CAT-192 (Metelimumab)** [20] a recombinant human IgG4 that neutralizes TGF-*β*1, and **GC-1008 (Fresolimumab)** a pan-specific monoclonal antibody that binds all three TGF-*β* isoforms and reduces their biological activity [18], 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 in Phase I trials for metastatic melanoma (MetM) plus renal cell carcinoma and for the fibrotic disorder focal segmental glomerulosclerosis [10] [21]. Lerdelimumab and Metelimumab, despite passing safety tests, failed to show efficacy in fibrotic models of corneal scarring and systemic sclerosis, respectively, and were therefore discontinued [19] [22].

  1D11 is another pan-neutralizing monoclonal antibody whose action in suppressing lung metastasis and angiogenesis was demonstrated to be through significant increase in the anti-tumor response of CD8+ T-cells, reduced IL-17 levels in the tumor microenvironment and enhanced tumor apoptosis [23]. 1D11 can rescue bone loss as well reducing expression of PTHrP and its regulator Gli2 [24]. **2G7** is another MoAb in preclinical trials which has shown efficacy in inhibiting breast cancer metastasis, increasing NK cells activity and preventing radiation induced acceleration of metastases [25]-[27]. **LY2382770**, a TGF-*β*1 ligand-selective blocking MoAb has progressed to Phase II trials for kidney fibrosis [10].

  IMC-TR1, a T*β*RII-blocking antibody, has just entered clinical trials for breast and colon cancer [28]. **PF-03446962** is an anti-T*β*RI MoAb which competes highly efficiently with the binding of the T*β*RI ligands BMP9 and TGF-*β* to T*β*RI. This antibody inhibits endothelial cell sprouting and can serve as an anti-angiogenesis agent. A Phase II clinical trial of PF-03446962 in patients with advanced malignant pleural mesothelioma is recruiting patients now [29]. **STX-100 (Stromedix)**, an anti-integrin *β*6 (aV*β*6) antibody that prevents the activation of TGF-*β*, has been used efficaciously in preclinical studies of fibrosis and cancer; it is in a Phase II trial for fibrosis [30].

  An alternative approach to avert TGF-*β* signaling is the employment of recombinant Fc-fusion proteins containing the soluble ectodomain of either T*β*RII (T*β*RII-Fc) or the type III receptor, betaglycan. Hu *et al*. (Hu *et al*., 2010) evaluated the systemic administration of type II receptor T*β*RII-Fc coupled to an oncolytic adenovirus (Ad.sT*β*RII-Fc) on breast cancer bone metastases in a nude mouse model. Their study demonstrated that intravenous delivery of **Ad.sT*β*RII-Fc** resulted in viral replication and expression of T*β*RII-Fc in skeletal tumors as well as a signification reduction of primary tumor growth and osteolytic bone destruction. **Disitertide (P144)** shows an alternative ligand trap approach using peptide mimetics of T*β*RIII that blocks ligand binding to receptors. It has completed a Phase IIa clinical trial for scleroderma and skin fibrosis, showing safety and efficacy when topically applied to skin [31]-[34].

  LSKL (Leu-Ser-Lys-Leu), a peptide antagonist of TGF-*β* activation, binds to a conserved sequence in the LAP region of the latent complex and has demonstrated efficacy in reducing TGF-*β* signalling *in vitro*. This antagonist is based on thrombospondin and specifically blocks TGF-*β* activation [35].

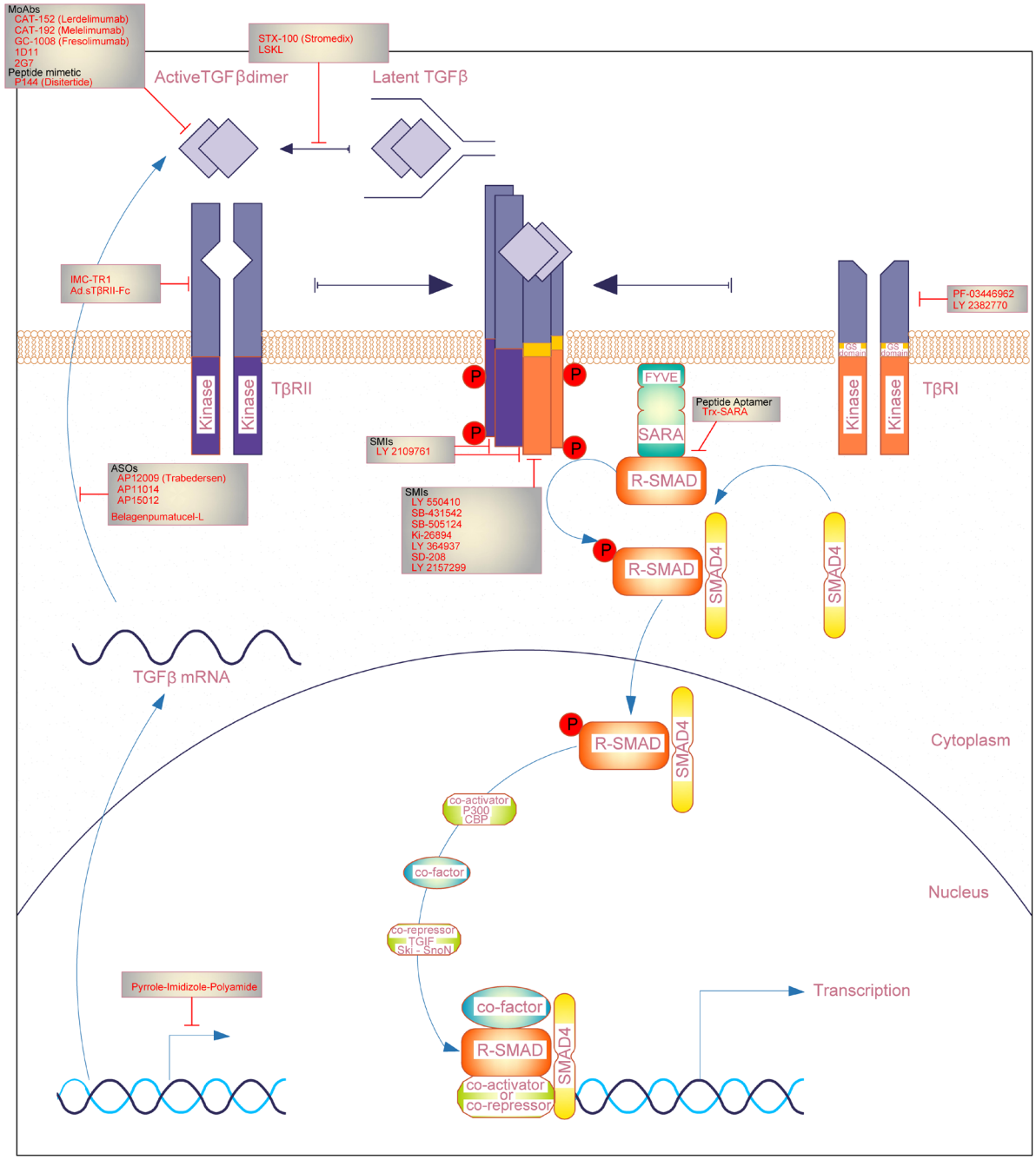

Figure 2. Therapeutic approaches targeting TGF-*β* signaling.

- **Antisense Oligonucleotides (ASOs)**
  TGF-*β* antisense oligonucleotides (ASO) as single strands of RNA (13 - 25 nucleotides in length) that are complementary to a chosen sequence of TGF-*β* mRNA. They prevent TGF-*β* protein translation of TGF-*β* mRNA strands through complementary nucleic acid hybridization and accelerated mRNA degradation by RNase H. In general, because multiple copies of a protein are produced by each mRNA molecule, targeting mRNA rather than the protein itself is potentially a more efficient approach to modulate protein function by altering its levels [36]. ASOs have some limitations which need to be taken into account when using them. These limitations include unpredictable RNA binding affinity, possible non-specific/off target effects and technical challenges of sequence design and delivery of relatively large molecules into the target cell as they

cannot cross the cellular membrane. Still, their target specificity and high binding affinity are advantages that push the continual research aiming to the successful clinical application of this technology.

AP12009 (Trabedersen) an ASO specifically targeting human TGF-*β*2 RNA has demonstrated a significant reduction in tumor growth, vascularization, and metastasis in pancreatic cancer which warrant further clinical development which made this drug progress to a Phase III clinical trial for oncology applications. Earlier clinical trials of Trabedersen in glioblastoma suggested that the effect of this drug might be through alterations in host immunity, since intra-tumoral injection of Trabedersen into a patient with multiple brains tumors not only led to regression of the target tumor, but to reduction of tumors in the contralateral brain hemisphere. One of the challenges of this drug is delivering it directly to the tumour to avoid the off-target toxicity. In the case of glioblastoma, this was achieved using intrathecal catheter delivery directly into the tumour. More recently, the manufacturing company has started developing intravenous delivery approaches for pancreatic cancer, which appear to be effective in mouse models and were recently shown to be safe in humans [10] [37]-[39]. **AP11014** (ASO targeting human TGF-*β*1) and **AP15012** are other two antisense molecules in pre-clinical trials for treatment of non-small cell lung cancer, prostate carcinoma and CRC; and MM, respectively [18] [40].

An anti-TGF-*β*2 antisense strategy has also been used to generate augmented tumour vaccines. **Belagenpumatucel-L** is such a drug, in which an ~900-nucleotide TGF-*β*2 antisense construct is transfected into allogeneic non-small-cell lung cancer (NSCLC) cells, which are then used as a tumour vaccine. This tumour vaccine has superior activity compared to conventional tumour vaccination approaches allowing progression to a Phase III clinical trial [10] [41].

- **Small Molecule Receptor Kinase Inhibitors (SMIs)**

  These inhibitors are ATP mimetics designed to be specific for inhibition of phosphorylation of R-SMADs by blocking T*β*RI receptor kinase site. The representative SMI compound is **LY550410** and contains set of heteroaryl rings that have the key functionality necessary for potent binding to the kinase-domain ATP binding site [17]. In spite of the fact that ligand traps and ASOs limit the bioavailability of the active TGF-*β* ligands, they fail to directly block receptor signaling. Small molecule inhibitors of the TGF-*β* receptor kinases have an advantage here, besides their great advantages in tissue penetration and delivery, although reduced drug specificity of kinase inhibitors compared to ASOs or monoclonoal antibodies may be a challenge besides the need for multiple administrations. Yet, the targeting of receptor kinases by small molecules has been an abundant area of experimental drug development in the last few years precisely because of economical and easy drug production, stability and the practicality of drug delivery by the oral route [15].

  SB-431542, an SMI of T*β*RI, has been shown to block TGF-*β* induced transcription of fibronectin and collagen in renal epithelial carcinoma cells, as well as inhibit the proliferation of glioma and osteosarcoma cells [42]. Tanaka *et al*. [43] reported a microenvironment-mediated anti-tumor effect of SB-431542 treatment *in vitro* through induction of dendritic cell (DC) maturation. Another inhibitor **SB-505124** has found to be 3 - 5 times more potent (Byfield *et al*., 2004; Katz *et al*., 2013). However, both drugs are pharmacokinetically unstable and non-specific, a major hurdle to *in vivo* studies. **Ki26894**, also a T*β*RI/ALK5 kinase inhibitor, has been shown to block TGF-*β* signaling, invasion, and motility of the bone metastatic breast cancer cell line, MDA-MB-231-D [44] [45] **LY364937** another SMI that demonstrated a role in reducing in bone and lung metastases [46] **SD-208** also prevented the development of osteolytic bone metastases [47]. **LY2109761**, a dual inhibitor of T*β*RI/II has shown promising effects on inhibiting the formation of metastases in several short term mouse tumor models, including breast, colon, and pancreatic cancer. However, in a long term drug dosing tumor cells acquired biochemical resistance suggesting that long term suppression of a signaling pathway may not be efficacious when used as monotherapy [15] [48]. **LY2157299**, another SMI selective for T*β*RI, is currently tested in four clinical trials; all of them are still recruiting patients which are either in Phase Ib/II or Phase II testing LY2157299 either as a monotherapy or in combinations with gemcitabine, Temozolomide-based radiochemotherapy, or Lomustine).

- **Peptide Aptamers**

  Aptamers are small peptide molecules containing a target-binding and a scaffolding domain that stabilize and interfere with the function of the target. Aptamers may therefore be designed specifically against R-Smads, and against multimeric transcriptional complexes containing Smads and other transcription factors, transcriptional co-activators, or co-repressors. This approach therefore lends itself to design of more specific targets downstream of the receptor, and thus has the potential for targeting specific subsets of TGF-*β* res-

ponses. **Trx-SARA**, a peptide aptamer designed to bind to Smad2 and Smad3 is an example. Treatment with Trx-SARA has been reported to reduce the levels of Smad2/3 in complex with Smad4 after TGF-*β* stimulation. Furthermore, Trx-SARA treatment has been shown to inhibit TGF-*β*-induced EMT in NMuMG murine mammary epithelial cells *in vitro* [49]. No clinical trials have been undertaken with peptide aptamers.

- **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 TGF-*β*1 gene promoter to attenuate gene expression through preventing transcription factor binding. 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]-[52].

  Gene transfer of antagonizing signaling molecules, such as the inhibitory **SMAD7** is another approach to suppress TGF-*β* signaling applied to treat or prevent various pathological conditions, including colonic and hepatic fibrosis, vascular remodelling and diabetic kidney disease [10] [53].

  Avotermin, a recombinant TGF-*β*III ligand, has been developed as an anti-scarring agent on the basis of the hypothesis that this ligand has activity that is independent of and antagonistic to TGF-*β*1. 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 [10].

Most human cancers appear to have lost their growth-inhibitory arm while still responding to TGF-*β*. However, only about 10% of the tumors (mainly GI and head and neck tumors) appear to exhibit loss of expression of TGF-*β* receptors or Smads. In several tumor cell types, activation of cell cycle proteins such as CDK4, c-Myc, *β*-catenin and h-TERT occurs when TGF-*β* signaling is inactivated. Thus, those molecules could represent new functional targets for therapeutics of lethal cancers that evade TGF-*β*. In the intestine, the presence of TGF-*β*-signaling and the absence of Wnt signaling in the villus compartment result in rapid cell cycle arrest and differentiation. Thus, Tcf4 (affected by Wnt signaling) and Smad4 constitute a dominant switch between the proliferative progenitor and the transitional progenitor of differentiated epithelial cell. At all stages of CRC this switch is permanently reversed because TGF-*β* signaling is inactivated while Tcf4 is constitutively activated by mutations in the Wnt cascade, leading to aberrant crypt foci and the long lived adenomatous polyps. These observations make the Wnt signaling pathway a useful target in GI cancers. A vitamin D3 analog, **Seocalcitol**, has been known to be able to inactivate *β*-catenin, the key protein in the wnt signaling [40] [54]. Cross-talk between TGF-*β*/Smad and JAK/STAT signaling pathways has been reported. TGF-*β* can downregulate IL-6-induced phosphorylation of STAT3. **NSC 74859**, a STAT3-specific inhibitor, markedly inhibits STAT3 phosphorylation in HCCs with inactivation of the TGF-*β*/*β*2SP pathway, indicating that IL6/STAT3, can provide a novel approach to the treatment of specific HCCs [55] [56].

4. Therapeutic Targeting of TGF-*β* Signalling: Challenges and Opportunities

Major challenges in developing TGF-*β* inhibitors for cancer therapy are 1) individualized responses to TGF-*β* inhibition owing to innate genetic variation between individuals. It is well established that there is considerable phenotypic diversity in the range of responses to reduced TGF-*β* signaling *in vivo*, which are dictated by differential inheritance of germline genetic variants. It is therefore most rational, economical and safe to preselect patient populations before initiating anti-TGF-*β* drug treatment on the basis of surrogate markers of TGF-*β* (such as increased TGF-*β* ligand, P-SMAD levels peripheral blood mononuclear cells (PMNCs), and LIP/LAP ratio) [10] [57]. 2) The unintentional inhibition of the tumor-suppressing arm of TGF-*β* signaling in cancer [58] [59]. 3) The development of adverse side effects unrelated to cancer, such as widespread inflammation, autoimmunity or cardiovascular defects, reactions, although this problem has not yet materialized in the preclinical or clinical trials of systemic TGF-*β* blockers [14]. 4) Development of tumor drug resistance, which is inevitable for almost all anti-cancer drugs. 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 [48], although this remains to be tested. Carefully restricting TGF-*β* inhibitors to short-term or intermittent usage should avoid these complications. Combinatorial and/or sequential treatment with complementary drugs will also be important. 5) TGF-*β* inhibitors might enhance the progression of premalignant lesions and release isolated and disseminated tumor (stem) cells from dormancy by initiating proliferation and/or disrupting the stem cell niche [10] [14]. It might therefore be wise to use TGF-*β* inhibitors in combination with cytotoxic drugs to coax tumor cells out of their quiescent niche while simultaneously targeting those that respond proliferatively to

TGF-β inhibition using chemotherapy [60]. Finally, 6) TGF-β inhibitors might act on the stem cell niche by recruiting bone marrow mesenchymal stem cell-derived myofibroblasts that home in on the primary tumor, contribute to the tumor microenvironment as cancer-associated fibroblasts and consequently promote tumor progression [61]. Clearly there are tissue- and cell type specific effects of TGF-β inhibition that can influence the action of TGF-β on the cancer stem cell and its niche [62]. Understanding the differential molecular mechanisms that elicit these variable responses will be critical to a judicious choice of treatment with TGF-β inhibitors or their derivatives.

Thereby, opportunities lie in using TGF-β inhibitors in combination where TGF-β inhibition can enhance therapeutic efficacy of various cytotoxic agents as rapamycin [63] and doxorubicin [60] [64]. Ki26894 had an additive effect with a fluorouracil analogue in reducing tumour growth [65]. Augmenting adoptive T cell therapy with SMIs may improve T-cell survival and anti-tumor T-cell cytotoxicity with reduced side effects of long-term SMI drug exposure [66] [67]. Another clinical application with great promise is augmenting radiotherapy by inhibiting the TGF-β pathway. Ionizing radiotherapy (as a stress factor) is known to induce TGF-β in both the tumor and tumor microenvironment which results in enhanced DNA damage response [68]. 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 [69] [70].

5. Clinical Applications of TGF-β Signaling Inhibition in Non-Neoplastic Diseases

- **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 due to reduced expression of Smad7. 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. Reduced expression of SMAD7 in hematopoietic cells led to increased TGF-β mediated gene transcription and enhanced sensitivity to TGF-β mediated suppressive effects. TGF-β is a myelosuppressive cytokine that has been implicated in the hematopoietic suppression as well as the autocrine production of other myelosuppressive cytokines (TNF, IL-6, and IFN γ) in MDS.
  LY2157299 (TβRI inhibitor) significantly increased erythroid (burst-forming unit (BFUE)) and myeloid (colony-forming unit (CFU); granulocytic monocytic) colony numbers *in vitro*, showing a great promise for the treatment of patients with MDS [71] [72].
- **Fibrosis**
  Idiopathic Pulmonary Fibrosis (IPF) is characterized by a progressive reduction in lung function, with an estimated 20% survival prospect after 5 years, making it more lethal than many cancers. The progressive fibrotic reaction in IPF is associated with an epithelium-dependent fibroblast activation, in which TGF-β plays a major part. **Pirfenidone**, a novel compound that inhibits TGF-β activity *in vitro* with still unknown mechanism, **Disitertide (P144)**, the synthetic peptide mimetics of TβRIII, **GC-1008 (Fresolimumab)**, the TGF-β-neutralizing antibody and **STX-100 (Stromedix)**, the αVβ6 integrin-blocking antibody are all anti-TGF-β therapies in clinical trials for IPF with pirfenidone being the first such drug to be approved for IPF in Europe [10].
  Renal fibrosis has long been thought to be driven by excess TGF-β, which results in renal scarring and, ultimately, kidney failure. TGF-β1 mediates progressive renal fibrosis by stimulating the synthesis of ECM production while inhibiting its degradation and by inducing the transformation of tubular epithelial cells into myofibroblasts through EMT [73]-[75]. A **GC-1008** and **LY2382770** trials exhibited encouraging efficacy in patients with focal segmental and diabetic kidney disease respectively [21].
- **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 TGF-β signalling leads to aberrant ECM deposition and scarring. Therapeutic approaches to scleroderma have included inhibition of TGF-β activity in sclerotic tissue with **GC-1008** and **Disitertide (P144)** showing efficacy in clinical trials phase I and II respectively [76] [77] after **CAT-192** failure to show evidence of efficacy in a study on the

treatment of patients with early-stage systemic scleroderma [22].

- **Restenosis Following Coronary Artery Bypass and Angioplasty**
  TGF-β1 is a major player in the early development of intimal hyperplasia in arteries and peripheral vein grafts through yet unknown mechanism but speculated to be multiple steps, including EMT, promotion of fibroblast, endothelial and vascular smooth muscle cell proliferation, increased collagen synthesis and deposition, and induction of fibrosis. The novel **pyrrole-imidazole polyamide** drug class showed efficacy in reducing neointimal hyperplasia and stimulating re-endothelialization of carotid arteries in a preclinical model of arterial injury [10] [50].
- **Diabetes and Obesity**
  Recent studies have also suggested that TGF-β-SMAD3 signaling regulates glucose tolerance and energy homeostasis, and that blockade of the pathway may be used for regulation of diabetes and obesity [78].

6. Conclusions

TGF-β signaling is involved in many normal physiological functions. TGF-β has a predominant role in a variety of cancer types during progression and metastasis as well as other non-neoplastic diseases. Increased levels of TGF-β in the tumor and tumor microenvironment with the potential to impede the metastatic potential of tumor cells while simultaneously having an impact on the tumor microenvironment, including angiogenesis, stromal activation and immunosuppression, provide a powerful rationale for evaluating TGF-β signaling inhibitors in cancer therapy.

TGF-β signaling 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 with a special consideration to the distinct limitation of each class in respect of delivery, specificity and toxicity and to the general limitations in respect of harmful off-target effects, drug individualized efficacy and contextual dependency on tumor type and clinical stage. Patient selection and timing of treatment are therefore pivotal criteria for treatment success. This personalized treatment can take place by using molecular diagnostic tools such as genetic screens and biomarkers.

7. Outlook

In order to prevent side effects and tumor drug resistance developed through long term application, future research should focus on sequential or combinatorial treatments containing anti-TGF-β drugs + ionized irradiation/chemotherapy. Concomitant targeting of several targets may be also effective due to its impact on deleterious cross-talks between those pathways.

Ultimately, a deep understanding of the interacting networks of signal pathway that regulate TGF-β outcome in tumor and host cells should allow the judicial choice of drug combination for each specific tumor type. The next several years promise to improve our understanding of approaching cancer therapy by further evaluation of TGF-β signaling inhibitors for clinical efficacy.

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