

8-1-2008

Molecular ablation of transforming growth factor beta signaling pathways by tyrosine kinase inhibition: the coming of a promising new era in the treatment of tissue fibrosis.

Joel Rosenbloom
Thomas Jefferson University

Sergio A. Jimenez
Thomas Jefferson University

Follow this and additional works at: <https://jdc.jefferson.edu/medfp>

 Part of the [Rheumatology Commons](#)

[Let us know how access to this document benefits you](#)

Recommended Citation

Rosenbloom, Joel and Jimenez, Sergio A., "Molecular ablation of transforming growth factor beta signaling pathways by tyrosine kinase inhibition: the coming of a promising new era in the treatment of tissue fibrosis." (2008). *Department of Medicine Faculty Papers*. Paper 203.
<https://jdc.jefferson.edu/medfp/203>

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's [Center for Teaching and Learning \(CTL\)](#). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Department of Medicine Faculty Papers by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.

Published in final edited form as:

Arthritis Rheum. 2008 August ; 58(8): 2219–2224. doi:10.1002/art.23634.

Molecular ablation of TGF- β signaling pathways by tyrosine kinase inhibition: The coming of a promising new era in the treatment of tissue fibrosis

Joel Rosenbloom, M.D., Ph.D. and Sergio A. Jimenez, M.D.

Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA 19107, USA

Keywords

Fibrosis; Systemic Sclerosis; Nephrogenic Systemic Fibrosis; Pulmonary Fibrosis; Transforming Growth Factor- β ; Imatinib Mesylate; Tyrosine Kinases; Collagen; Fibroblasts

Fibrotic diseases. A therapeutic challenge

There are numerous human diseases including systemic sclerosis; pulmonary, liver, and kidney fibrosis; and the newly recognized nephrogenic systemic fibrosis, which are characterized by abnormal and exaggerated deposition of collagen in the affected organs (reviewed in refs. 1-6). Tissue fibrosis causes disruption of normal organ architecture, and ultimately leads to their dysfunction and failure. The extent and rate of progression of the fibrotic process largely determine the course, response to therapy, and prognosis of these diseases. Although their etiology and pathogenesis are diverse and have not been completely elucidated despite intensive investigations, it is apparent that a common feature is the accumulation of abundant fibrous tissue and the presence of large numbers of fibroblasts displaying an activated phenotype. This phenotype is characterized by a notable elevation in the expression of the genes encoding type I and type III collagens and fibronectin, the initiation of expression of α -smooth muscle actin, and the reduction in expression of genes encoding extracellular matrix-degradative enzymes. Regardless of the etiologic event, the resulting alterations in the biosynthetic activity of extracellular matrix producing cells are crucial in the pathogenesis of fibrotic diseases. Indeed, it is the persistent activation of the genes encoding various collagens in these cells which distinguishes controlled repair, such as that occurring during normal wound healing, from the uncontrolled fibrosis which is the hallmark of the fibrotic diseases. However, despite numerous recent advances in the molecular biology of the events responsible for the regulation of genes encoding collagens and other extracellular matrix proteins, there is limited knowledge regarding the intimate mechanisms responsible for the pathologic increase in their expression in fibrotic diseases.

The wide spectrum of organs affected by the fibrotic diseases and the large number of individuals suffering their devastating effects pose one of the most serious health problems in current medicine and represent an enormous burden on health services and resources causing severe economic consequences. Despite the high frequency and the diversity of organs affected by the fibrotic diseases, there is currently no effective treatment. The recent elucidation of

crucial regulatory pathways involved in the fibrotic response has provided a sound basis for the development of novel and effective means of therapy. In particular, the identification of various important intracellular transduction pathways involved in the transcriptional activation of the genes encoding collagens and other proteins responsible for the fibrotic process has been of substantial importance. The delineation of the critical role of transforming growth factor- β (TGF- β) in the development of exaggerated tissue fibrosis, and the identification of the specific cellular receptors, kinases and intracellular mediators which participate in the cellular response to TGF- β in the earliest stages of tissue fibrosis, have been the most significant. Unfortunately, the current lack of approved drugs capable of modifying these important mechanisms indicates that the recently acquired knowledge regarding the pathogenesis of the fibrotic process has not been translated into effective therapies in this important area of human health.

Regulation of TGF- β function and signaling

Since the discovery of the potent profibrotic and immunomodulatory activities of TGF- β , this growth factor has been recognized as one of the most important molecules in the pathogenesis of systemic sclerosis and other fibroproliferative diseases, and a plethora of publications have described studies focused on this subject (reviewed in 7). Three functionally and structurally similar isoforms of TGF- β exist in humans and they play important roles in embryonic development, immune responses, and regulation of tissue repair following injury. One of the most important effects of TGF- β is the stimulation of extracellular matrix synthesis as evidenced by a remarkable increase in the production of numerous molecules including collagens type I, III, V, and VI, as well as, of other relevant proteins such as fibronectin and smooth muscle actin, a molecular marker of activated myofibroblasts. TGF- β also decreases the synthesis of collagen-degrading metalloproteinases and stimulates the production of protease inhibitors such as tissue inhibitor of metalloproteinases-1. Small amounts of TGF- β appear to sensitize fibroblasts to its own effects and maintain them in a persistently activated state involving an autocrine mechanism that causes further production of TGF- β . Bioactive TGF- β in a dimeric form binds to a constitutively active serine/threonine transmembrane kinase known as the TGF- β II receptor (T β RII). The signaling events that follow are extremely complex and the number of intracellular molecules and pathways involved in the process continues to expand (reviewed in 8-10). The classic pathway of TGF- β signal transduction into the cell nucleus involves the ligand-bound T β RII which recruits the TGF- β I receptor (T β RI) and then transphosphorylates it on three to five serine and threonine residues in a short (30 amino acid) regulatory sequence known as the GS region as shown in Figure 1. Signaling from the phosphorylated T β RI receptor to the nucleus then occurs through the Smad family of proteins. Smad2 or Smad3, two of the five receptor activated Smads (RSmads), bind to the activated TGF- β receptor complex and become phosphorylated by the activated T β RI at two serine residues near their C-end. Phosphorylation allows these proteins to form a complex with the co-Smad, Smad4, which is a cytoplasmic protein involved in the translocation of the Smad complex across the nuclear membrane into the nucleus. Once in the nucleus, Smad3/Smad4 complexes act as transcription factors, binding with the help of intranuclear proteins that act as transcriptional partners to specific DNA binding sites in the promoter regions of target genes and activating their expression. In contrast, Smad2 complexes do not appear to directly bind to DNA promoter sites but instead exert their effect through other unidentified transcription factors or co-activator proteins.

Numerous recent studies have provided additional details regarding the complex pathways of TGF- β modulation of expression of extracellular matrix genes and many participating proteins such as FKBP12, SARA, smurfs, co-activators and co-repressors, caveolin1, and others, have been identified. Furthermore, it has become apparent that important TGF- β effects may be mediated by protein cascades independent of Smad2/Smad3 signaling and that these pathways may become activated in a cell-specific and context-dependent manner. For example, it has

been shown that there are seven different T β RI molecular species, known as activin receptor-like kinases or Alks, encoded in the human genome which exert different and often opposing effects or are only functional in specific cell types. The typical TGF- β signaling cascade involving the RSmads, Smad2 and Smad3, is initiated by the Alk-5 T β RI, whereas another T β RI, Alk-1, initiates activation of the cascade involving RSmads 1, 5, or 8. Although it was initially believed that the Alk-1/Smad1, 5, or 8 cascade was activated only by the binding of a member of the family of bone morphogenetic proteins (BMPs) or that it was only active in endothelial cells, it has now been established that TGF- β can also signal through this pathway in fibroblasts (11). To further add to the complexity of the TGF- β activation and signaling cascades, it has been recognized that there are numerous other non-Smad mediated events involved in TGF- β functions and effects (12). Of particular relevance to the present discussion is the recent finding that one of these non-Smad pathways results in the activation of the non-receptor tyrosine kinase, c-Abl (13), apparently through the sequential action of phosphatidylinositol 3-kinase (PI3K) and p-21 activated kinase 2 (PAK2) as shown in Figure 1.

TGF- β as a target of antifibrotic therapies

The crucial role that TGF- β plays in the initiation and progression of tissue fibrosis and the recognition of its participation in the pathogenesis of numerous fibrotic diseases has focused substantial attention on this growth factor as a target for the development of anti-fibrotic therapies. Indeed, several strategies have been developed to block TGF- β effects, including soluble T β RII fragments, decorin, tranilast, TGF- β -neutralizing antibodies, threonine kinase inhibitors, RNA expression inhibitors such as anti-sense expression vectors, small inhibitory RNA (siRNA) or blocking oligonucleotides, and reduction of TGF- β gene transcription with pirfenidone. However, despite the intensive investigation *in vitro* as well as *in vivo* in various animal models of fibrosis and in some studies in human subjects, these approaches have either not been effective or are still in the experimental/clinical trial stage and, therefore, are not presently available for clinical use.

Tyrosine kinase inhibitors

Protein kinase inhibitors are a relatively new class of therapeutic agents that are capable of potent modulation of several cellular phenotypes in oncologic and in chronic inflammatory diseases. Of particular interest in the present context are results of numerous recent studies demonstrating that the non-receptor kinase c-Abl participates in some of the TGF- β downstream signaling responsible for the development of a profibrotic phenotype response in a variety of cell types. The initial observations leading to this important discovery appeared in the hematologic literature with three reports describing simultaneously a remarkable reduction of bone marrow fibrosis in patients receiving treatment for chronic myelogenous leukemia with the c-Abl inhibitor, imatinib mesylate (14-16). Several subsequent studies confirmed the marked improvement in myelofibrosis following imatinib administration. Imatinib is the prototypical drug of the novel class of non-receptor kinase inhibitors. It is a phenylaminopyrimidine-derived small molecule capable of exerting a potent inhibition of several tyrosine kinases including BCRc-Abl, c-Abl, C-kit, and the platelet derived growth factor receptor (PDGFR) at micromolar concentrations. Although the initial kinetic analysis of the mechanisms involved in the potent inhibition of BCRc-Abl kinase by imatinib indicated that this drug binds to the ATP-binding pocket of c-Abl and efficiently blocks its tyrosine kinase activity, further studies have shown that the binding occurs near the ATP-binding pocket resulting in a profound conformational change of the kinase active site domain stabilizing it in an inactive conformation. Following the initial studies describing inhibition of bone marrow fibrosis by imatinib, several investigators examined the effects of the drug on *in vitro* and *in vivo* models of fibrotic diseases. A landmark study by Daniels et al. (17) examined the effects

of imatinib on TGF- β signaling in fibroblasts and on the development and progression of bleomycin-mediated lung fibrosis in mice. The results showed that TGF- β stimulated c-Abl kinase and that imatinib abolished the fibrogenic response of fibroblasts to exogenous TGF- β in a Smad2/Smad3 independent manner. Furthermore, they showed that *in vivo* administration of imatinib in mice abrogated the biochemical and morphological changes of bleomycin-induced lung fibrosis. Several subsequent reports explored in further detail the mechanisms of TGF- β signaling inhibition by imatinib and confirmed the potent effects of the drug in abrogating experimentally induced fibrotic processes such as liver, renal, and myocardial fibrosis (18-20). A more recent *in vitro* and *in vivo* study examined the effects of imatinib on collagen gene expression by dermal fibroblasts cultured from patients with systemic sclerosis and demonstrated that c-Abl appeared to have a novel and important function in TGF- β -induced fibrotic responses. Furthermore, this study showed that c-Abl inhibition with imatinib abrogated the development of cutaneous fibrosis induced by bleomycin in mice, a process which mimics the skin fibrosis of systemic sclerosis (21). Similar observations were recently published by Distler et al. (22), confirming the potent inhibitory effect of imatinib on the development of scleroderma-like cutaneous alterations in mice receiving dermal injections of bleomycin. Although numerous studies have conclusively demonstrated the participation of c-Abl in the regulation of genes associated with the fibrotic process, the molecular events occurring downstream from c-Abl and the intimate mechanisms of its participation in the regulation of gene expression remain elusive.

The light at the end of the tunnel for patients with systemic sclerosis and other fibrotic diseases?

There are four interesting articles published in this issue of Arthritis and Rheumatism which corroborate the potential importance of imatinib in the therapy of a spectrum of fibrotic diseases. In the paper by Pannu et al., studies with skin biopsies from 8 normal individuals and from 8 patients with systemic sclerosis and with cultured fibroblasts from these biopsies demonstrated activation of a novel pathway of TGF- β signaling in fibroblasts involving Alk-1 and Smad1 leading to the transcriptional activation of the connective tissue growth factor gene (CTGF/CCN-2). Furthermore, Pannu et al. failed to find any differences in the amounts of phosphorylated Smad3 between normal fibroblasts and fibroblasts cultured from skin of systemic sclerosis patients. These results are in contrast to those described by other investigators who postulated that the excessive tissue fibrosis in systemic sclerosis was mediated by activation of the Alk-5/Smad3 pathway. Of substantial relevance to this discussion was the observation that this pathway of TGF- β signaling and the consequent activation of CTGF/CCN-2 were abolished by addition of imatinib to the cultured fibroblasts. The important role of c-Abl in the activation of the Alk-1/Smad1 pathway and in the phosphorylation of Smad1 was further confirmed employing a specific c-Abl siRNA which completely abolished Smad1 phosphorylation and, remarkably, normalized the excessive collagen production in systemic sclerosis fibroblasts. Although these findings indicate that Smad1 phosphorylation is a downstream effect mediated by c-Abl, the mechanisms involved are not known. Since phosphorylation and activation of all RSmads is accomplished through specific kinase domains of Alk receptors, the participation of c-Abl in the phosphorylation of Smad1 is puzzling and requires further investigation. The second paper, by van Daele et al., describes studies performed with lung fibroblasts obtained from bronchial biopsy specimens from a patient with systemic sclerosis and pulmonary fibrosis in whom the lung involvement progressed despite treatment with twice monthly intravenous cyclophosphamide and low dose corticosteroids. Imatinib prevented proliferation of cultured fibroblasts induced by PDGF as well as TGF- β stimulation of collagen type I gene transcription *in vitro*. Significantly, treatment of this patient with imatinib resulted in improvement in skin tightness with a reduction of Rodnan skin score (RSS) from 18 before treatment to 12 three months after initiation of imatinib and stabilization

of pulmonary function and computerized tomography (CT) scanning findings. A third study by Distler et al. showed that 20 week treatment with imatinib caused remarkable improvement in the pulmonary function and CT findings of a patient with mixed connective tissue disease associated with rapidly progressive pulmonary fibrosis. The fourth report by Kay and High found that treatment with imatinib decreased fibrosis and resulted in relatively rapid improvement of skin changes and knee joint contractures in two patients with nephrogenic systemic fibrosis (NSF). NSF is a recently recognized fibrotic disease characterized by severe cutaneous and visceral fibrosis occurring almost exclusively in patients with renal insufficiency following administration of gadolinium containing contrast agents for magnetic resonance imaging. Severe and progressive joint contractures causing disabilities occur frequently in these patients. The cases reported by Kay and High showed substantial clinical improvement as well as histopathological evidence of reduction of collagen biosynthesis following treatment with imatinib. These four papers provide valuable information regarding the novel role of c-Abl in tissue fibrosis and, more importantly, bring closer the promise of “light at the end of the tunnel” for patients affected by these diseases as stated in a recent editorial by Wolheim (23).

Speculations and caveats

Several promising therapeutic agents have previously been tested in a variety of fibrotic states, but in most instances with disappointing results. There is great interest and expectation about the possibility that imatinib, a drug approved for use in chronic myelogenous leukemias and gastrointestinal stromal tumors (GIST), may also have a therapeutic effect for fibrotic diseases. Currently there is only limited experience with the use of imatinib for treatment of fibrotic diseases. Thus, given the disappointing results from clinical trials of several promising antifibrotic agents previously studied, it is essential that imatinib and similar drugs be shown to be effective in a large cohort of patients in well controlled studies prior to their widespread use to treat fibrotic diseases. Imatinib has been used extensively in the treatment of chronic myelogenous leukemia and GIST with spectacular results and relatively little toxicity considering the outstanding benefits achieved. However, one or more adverse reactions including congestive heart failure, edema, muscle cramps, diarrhea, anemia, neutropenia and thrombocytopenia occurred in a substantial proportion of the treated patients. It must, therefore, be demonstrated that long term use of imatinib is free of substantial side effects in patients with severe fibrotic diseases, in particular, regarding its cardiotoxicity given the reduced cardiac functional reserve of patients with systemic sclerosis, pulmonary fibrosis, and nephrogenic systemic fibrosis. We have reason to be encouraged, but there is only limited knowledge concerning the signaling pathways in which the pertinent tyrosine kinases participate in relation to stimulation of fibrosis. While it presently appears that many fibrotic reactions, irrespective of initiating events, terminate in a final common pathway susceptible to this therapeutic intervention, this optimistic view must be substantiated.

Acknowledgments

Supported by NIH Grant AR01916 to S.A.J. The authors thank Susan V. Castro, Ph.D. for her assistance in the preparation of the illustration and the manuscript.

Bibliography

1. Varga J, Abraham D. Systemic Sclerosis: A prototypic multi-system fibrotic disorder. *J Clin Invest* 2007;117:557–567. [PubMed: 17332883]
2. Jimenez SA, Derk CT. Following the molecular pathways toward and understanding of the pathogenesis of Systemic Sclerosis. *Ann Int Med* 2004;140:37–50. [PubMed: 14706971]
3. Gharakee-Kermani M, Phan SH. Molecular mechanisms of and possible treatment strategies for idiopathic pulmonary fibrosis. *Curr Pharm Des* 2005;11:3943–3971. [PubMed: 16305523]

4. Tskada S, Parson CJ, Rieppel RA. Mechanisms of liver fibrosis. *Clin Chim Acta* 2006;364:33–60. [PubMed: 16139830]
5. Liu Y. Renal fibrosis: new insights into the pathogenesis and therapeutics. *Kidney Int* 2006;69:213–217. [PubMed: 16408108]
6. Mendoza FA, Artlett CM, Sandorfi N, Latinis K, Piera-Velazquez S, Jimenez SA. Description of 12 cases of nephrogenic fibrosing dermopathy and review of the literature. *Semin Arthritis Rheum* 2006;35:238–249. [PubMed: 16461069]
7. Blobe GC, Schiemann EP, Lodish HF. Role of transforming growth factor β in human disease. *N Engl J Med* 2000;342:1350–1358. [PubMed: 10793168]
8. Schmierer B, Hill CS. TGF β -SMAD signal transduction: molecular specificity and functional flexibility. *Nat Rev Mol Cell Biol* 2007;8:970–82. [PubMed: 18000526]Review
9. Massagué J, Gomis RR. The logic of TGF β signaling. *FEBS Lett* 2006;580:2811–20. [PubMed: 16678165]Review
10. Massagué J, Seoane J, Wotton D. Smad transcription factors. *Genes Dev* 2005;19:2783–810. [PubMed: 16322555]Review
11. Rahimi RA, Leof EB. TGF- β signaling: a tale of two responses. *J Cell Biochem* 2007;102:593–608. [PubMed: 17729308]Review
12. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF- β family signaling. *Nature* 2003;425:577–584. [PubMed: 14534577]
13. Wilkes MC, Leof EB. Transforming growth factor beta activation of c-Abl is independent of receptor internalization and regulated by phosphatidylinositol 3-kinase and PAK2 in mesenchymal cultures. *J Biol Chem* 2006;281:27846–27854. [PubMed: 16867995]
14. Beham-Schmid C, Apfelbeck U, Sill H, Tsybrovsky O, Höfler G, Haas OA, Linkesch W. Treatment of chronic myelogenous leukemia with the tyrosine kinase inhibitor STI571 results in marked regression of bone marrow fibrosis. *Blood* 2002;99:381–3. [PubMed: 11756197]
15. Hassserjian RP, Boecklin F, Parker S, Chase A, Dhar S, Zaiac M, Olavarria E, Lampert I, Henry K, Apperley JF, Goldman JM. STI571 (imatinib mesylate) reduces bone marrow cellularity and normalizes morphologic features irrespective of cytogenetic response. *Am J Clin Pathol* 2002;117:360–7. [PubMed: 11888075]
16. Hirose Y, Kiyoi H, Iwai M, Yokozawa T, Ito M, Naoe T. Successful treatment with imatinib mesylate of a CML patient in megakaryoblastic crisis with severe fibrosis. *Int J Hematol* 2002;76:349–53. [PubMed: 12463599]
17. Daniels CE, Wilkes MC, Edens M, Kottom TJ, Murphy SJ, Limper AH, Leof EB. Imatinib mesylate inhibits the profibrogenic activity of TGF-beta and prevents bleomycin-mediated lung fibrosis. *J Clin Invest* 2004;114:1308–16. [PubMed: 15520863]
18. Yoshiji H, Noguchi R, Kuriyama S, Ikenaka Y, Yoshii J, Yanase K, Namisaki T, Kitade M, Masaki T, Fukui H. Imatinib mesylate (STI-571) attenuates liver fibrosis development in rats. *Am J Physiol Gastrointest Liver Physiol* 2005;288:G907–13. [PubMed: 15618280]
19. Wang S, Wilkes MC, Leof EB, Hirschberg R. Imatinib mesylate blocks a non-Smad TGF-beta pathway and reduces renal fibrogenesis in vivo. *FASEB J* 2005;19:1–11. [PubMed: 15629889]
20. Leipner C, Grün K, Müller A, Buchdunger E, Borsi L, Kosmehl H, Berndt A, Janik T, Uecker A, Kiehnopf M, Böhrer FD. Imatinib mesylate attenuates fibrosis in coxsackievirus B3 (CVB3)-induced chronic myocarditis. *Cardiovasc Res*. 2008 Mar 7;Epub ahead of print
21. Ishida W, Bhattacharyya S, Hinchcliff M, Mori Y, Wu M, Takagawa S, Takehara K, Varga J. Novel Role of C-abl Tyrosine Kinase in Profibrotic TGF-Beta Responses: Selective Modulation By the Anticancer Drug Imatinib Methylete (gleevec). *Arthritis Rheum* 2006;54(Suppl):S776. Abstract 1975
22. Distler JH, Jüngel A, Huber LC, Schulze-Horsel U, Zwerina J, Gay RE, Michel BA, Hauser T, Schett G, Gay S, Distler O. Imatinib mesylate reduces production of extracellular matrix and prevents development of experimental dermal fibrosis. *Arthritis Rheum* 2007;56:311–22. [PubMed: 17195235]
23. Wollheim FA. Treatment of pulmonary fibrosis in systemic sclerosis: light at the end of the tunnel? *Arthritis Rheum* 2007;56:9–12. [PubMed: 17195185]

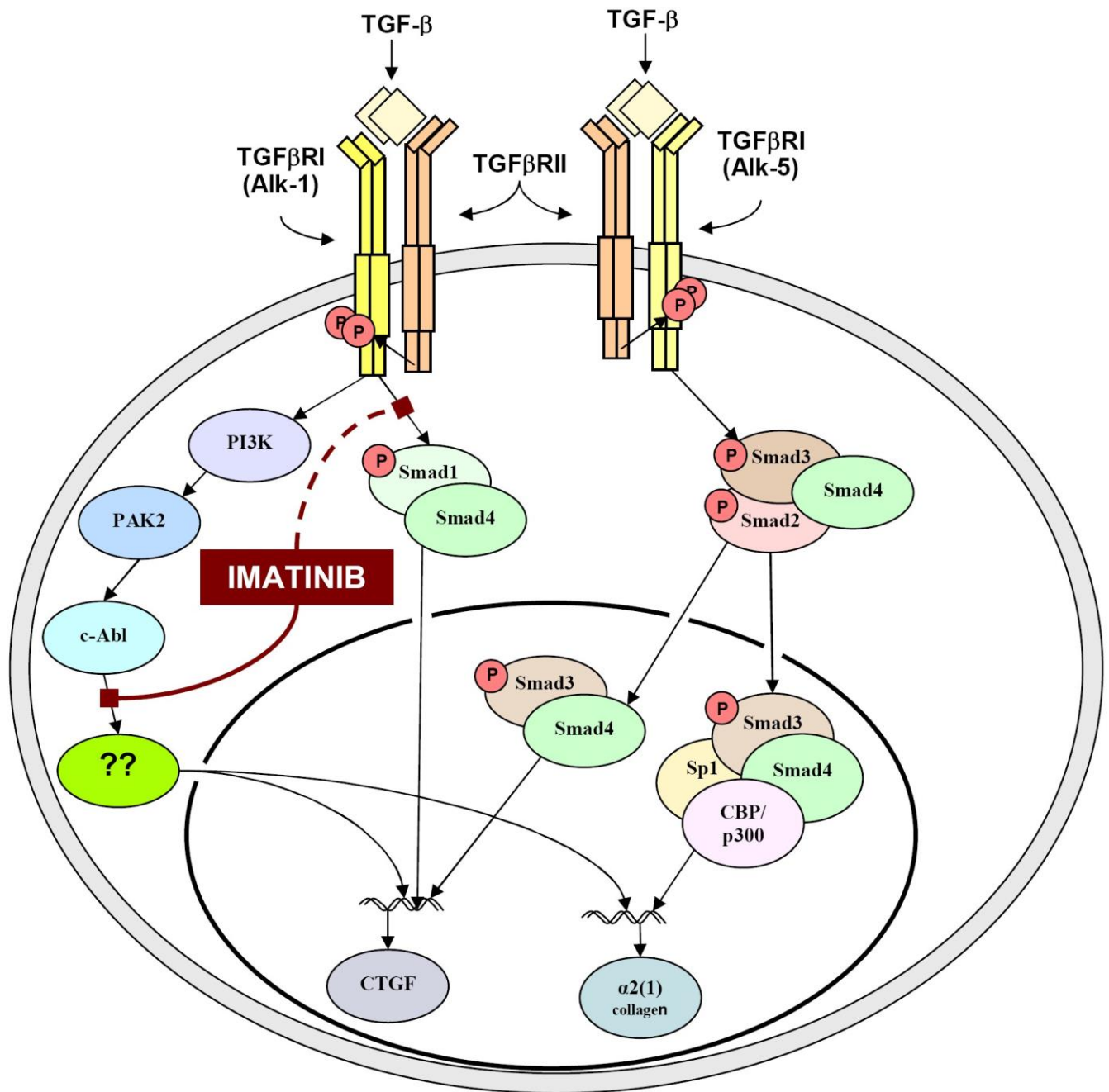


Figure 1. TGF-β signaling pathways critical for the fibrotic response

Illustrated are two canonical Smad pathways originating from two representative tetrameric receptors. After TGF-β binding, the TGFβRII receptor recruits a type I receptor (TGFβRI) and activates it by phosphorylation. Alk-5 then specifically phosphorylates receptor-regulated Smad 2 and Smad3 whereas Alk-1 phosphorylates Smad1 (Smad5 and Smad8, also activated by Alk-1, are not illustrated). The receptor Smads then complex with Co-Smad4 resulting in their transport to the nucleus where they cooperate with other factors to regulate transcription of critical genes, here represented by CTGF and α2(I) collagen. Also illustrated is a non-canonical pathway resulting in the activation of c-Abl. As pictured, imatinib blocks the activity of c-Abl, effectively inhibiting the fibrotic response although the events downstream of c-Abl

are presently unknown. Imatinib also blocks the phosphorylation of Smad1, but here also the pathway is unknown.