TGF-β INHIBITORS ARE NOVEL DRUGS -IN DIABETIC NEPHROPATHY A REVIEW

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ABSTRACT

TGF-β INHIBITORS are novel drugs in treatment of Diabetic Nephropathy.

KEYWORDS: TGF-β, Diabetic Nephropathy, Podocyte, Mesangial cells.

Hypothesis

TGF-β Inhibitors are novel drugs they prevent thickening of glomerular basement membrane, glomerular hypertrophy, prevent podocytopathy, decreases vascular sensitivity, increases endogenous NO production vasodilators, inhibit collagen formation and reduce extracellular matrix, reduces CTGF AND VEGF, Inhibit GLUT 1 transporter, reduces fibronectin level, reduces hyperglycaemia induced heparin sulphate proteoglycan, inhibit apoptosis in mesangial cells in retina, decreases IL-8, MCP-1 in Kidney, decreases local expression of complement system and modulate immune-modulatory pathway by decreasing NF-κB thus TGF-β can be used in Treatment of diabetic complication especially Diabetic Nephropathy and Diabetic Retinopathy.

My Contribution in the review

• In this review the above hypothesis and probable benefit TGF-β Inhibitors are well supported From the available data and study.
• All the scattered data and study about TGF-β role in pathogenesis Diabetic Nephropathy is taken into consideration to support the hypothesis.
• Signalling pathway and role of protein micro-machinery from basic to pathology is added
• All the researcher and Scholar have done the best and I give credit to them but after going through various search engines and articles so many articles huge data is generated which is not helpful, the review article provides comprehensive and core material.
• The advantages of TGF –β Inhibitors are well elaborated in 1 to 21 points with strong study support and evidence.
• Search for – TGF-BETAβ INHIBITORS IN DIABETIC NEHROPATHY in various search engines generates data as follows as on date 9/7/17.
  • Google - About 3,83,000 results (0.68 seconds).
  • Google scholar generates- About 28,400 results (0.10 sec).
  • Pubmed- Items: 1 to 20 of 411 ARTICLES
  • Captome- 1-10 of 8536965 records for [TGF-β INHIBITORS IN DIABETIC NEPHROPATHY - Pharmacology (ADME)](no advanced settings applied).
  • Out which 37 articles are included my review highlight important role of TGF –β and all possible benefits of TGF-β Inhibitors.

INTRODUCTION
"TGF-BETA (transforming growth factor-β) super- β family cytokines, including TGF-β, activin, nodal, BMPs (bone morphogenetic proteins) and anti-M‘ullerian hormone, are multifunctional proteins that regulate cellular growth, differentiation, apoptosis and morphogenesis. (Kuratomi et al 2005:461).

A] FAMILY OF TGF-BETA: (note on p.309)
"Family comprises TGF-BETA s, activins, and bone morphogenetic proteins (BMPs)/ growth and differentiation factors that are structurally-related, secreted cytokines" (Böttinger 2007: 309).

TGF-BETA are pleiotropic molecules that regulate cell proliferation, differentiation, apoptosis, migration, and adhesion of many different cell types." (Böttinger 2007: 309).

"TGF-BETA-family ligands have crucial roles in embryogenesis, tissue repair, and in maintaining tissue homeostasis" (Böttinger 2007: 309).
PATHOPHYSIOLOGICAL ROLE (note on p.309)
"TGF-BETA signalling has been connected to numerous developmental disorders and many human diseases including cancer, fibrosis, and autoimmune diseases" (Böttinger 2007:309).

TGF-BETA LIGAND ROLE (note on p.309)
"TGF-BETA recruit signalling across the plasma membrane into the cell by inducing heteromeric complexes of type I and type II receptors with serine/threonine kinase activity" (Böttinger 2007:309).

E] RECEPTOR FAMILY (note on p.309)
"The family of receptors comprises 5 type II receptors and 7 type I receptors, also named activin receptor-like kinases (ALKs)" (Böttinger 2007:309).

F] SIGNALLING (note on p.309)
"On ligand-induced heteromeric complex formation, the constitutively active type II receptor kinase phosphorylates the type I receptor on specific serine and threonine residues in the juxtamembrane region, leading to initiation of type I receptor kinases" (Böttinger 2007:309).

"The ligand-induced initiation of TGF-BETA-receptor complexes leads to the recruitment and activation of major TGF-BETA signal transducers of the Smad family where activated type I receptor kinases quickly interact with and phosphorylate receptor-regulated Smads (R-Smads) at their extreme C-terminal serine residues" (Böttinger 2007:309).

"Phosphorylated R-Smads form heteromeric complexes with a common-partner Smad4 and translocate to the nucleus, where these heteromeric complexes can bind to DNA to control transcription of target genes through communication with co-activators and co-repressors" (Böttinger 2007:310).

DOWNSTREAM (note on p.309,310)
"Smad2 and Smad3 act downstream of the TGF-BETA, activin, or nodal type I receptors ALK4, ALK5, and ALK7" (Böttinger 2007:309) "Smad1, Smad5, and Smad8 act downstream of BMP type I receptors ALK2, ALK3, and ALK6" (Böttinger 2007:310).

ALK1 (note on p.310)
"ALK1 is a type I receptor for TGF-BETA that activates Smad1, Smad5, and Smad8 in neurons and endothelial cells dependent on ALK5 kinase activity" (Böttinger 2007: 310).
SMAD IMPORTANT COMPONENT OF TGF-BETA SIGNALLING (note on p.237)

Smad distribution)(note on p.2822)

"Smad proteins are dire components of the TGF-BETA signalling pathways." (Stroschein et al 2001:2822) "Absence of TGF-BETA, the two highly homologous receptor-associated Smads, Smad2 and Smad3, are dispersed mostly in the cytoplasm." (Stroschein et al 2001:2822) "β1 TGF stimulates fibroblast proliferation, collagen α1α1 production, and smooth muscle actin (SMA) expression, prevents matrix degradation and eventually leads to scar formation" (Zhang et al 2012:237).

Smad–phosphorlation

"TGF-BETA1 stimulus, Smad-2 and -3 are phosphorylated at serine residues in the carboxyl termini by the type I receptor" (Yang 2003:3167) "phosphorylation of Smad-2/3 induces their association with common partner Smad-4, and they subsequently translocate into the nuclei, where they control the transcription of TGF-BETA1-responsive genes" (Liu et al 2016:8, Yang 2003:3167).

Smad (note on p.10736) "phosphorylation of Smad2 and phosphorylation of Smad3 are regulated separately" (Zhu et al 2005:10736).

"Inside the nuclei, activated Smad can interact with general transcriptional co-activators, resulting in transcriptional activation. Alternatively, they form transcriptionally inactive complexes with co-repressors" (Yang 2003:3167).

"TGF-β1, as a fundamental mediator of fibrogenesis, plays a critical role in the renal tubular EMT process by a Smad-dependent pathway, resulting in TIF" (Liu et al 2016:8).

Smad INTERACTION (NOTE ON P.2822)

"In the nucleus, the Smad complexes interact with numerous cellular partners and participate in diverse downstream activities. The Smads can bind to the TGF-BETA-responsive promoter DNA either directly through the N-terminal Mad homology-1 (MH1) domain or in conjunction with other sequence-specific DNA binding proteins such as the FAST and Milk family of proteins" (Stroschein et al 2001:2822) "C-terminal" (Stroschein et al 2001:2822) "MH2 domains, Smad proteins interact with general or promoter-specific transcriptional activators to activate transcription of various TGFtarget genes "associate with transcriptional corepressors such as TGIF"
G] INHIBITORY Smads (note on p.310)  
"Inhibitory Smads interact with and recruit Smurf (Smad ubiquitination regulatory factor) ubiquitin ligases to ligand receptor complexes, commencing Smurf-induced receptor degradation via proteosomal and lysosomal pathway" (Böttinger 2007:310).

H] Smad2 and smad3 (note on p.612)  
"Smad2 has also been revealed to mediate production of matrix metalloproteinase 2, whereas the induction of c-fos, Smad7, and TGF-BETA 1 auto-induction are dependent on Smad3” (Nakagawa et al 2004:612).

"Smad2/Smad4 complex binds directly to the SBE sequences in the SnoN promoter to activate its expression, while Smad3/Smad4 inhibits SnoN expression through binding to an inhibitory element." (Zhu et al 2005:10739).

"Smad2 and Smad3 play unlike roles in regulation of SnoN transcription through direct binding to the positive and negative regulatory elements in the SnoN promoter." (Zhu et al 2005:10742).

PROANGIOGENIC AND ANTIANGIOGENIC (note on p.605)  
"Smad3 has a proangiogenic role and stimulates VEGF-A expression, whereas Smad2 has an anti-angiogenic role in mediating TSP-1 and sFlt-1 expression." (Nakagawa et al 2004: 606)  
"Smad2 was critical in the production of anti-angiogenic factors in response to TGF-BETA1. Both TSP-1 and sFLT-1 were induced through Smad2-dependent pathways." (Nakagawa et al 2004: 612).

Smad 3 (note on p.312)  
"knockout of the major signal transducer Smad3 in mice prevents renal defects and fibrosis induced by experimental ureteral obstruction or diabetes mellitus" (Böttinger 2007: 312).

"Smad3 also is required for fibrogenic activation of vascular smooth muscle cells by angiotensin II" (Böttinger 2007: 312).

SMAD 3 (note on p.10739)  
"CGACGG box is a novel Smad3-dependent inhibitory element (SIE) and that Smad3/Smad4 inhibits TGF-BETA-induced transcription of the SnoN gene by binding to the SIE." (Zhu et al 2005: 10739).
"Smad2 and Smad3 knockout fibroblasts indicate a broad role for Smad3, but not Smad2, as a critical mediator of fibrogenic signaling by TGF-BETA in mesenchymal cells" (Böttinger 2007: 312).

**SMAD 4 -MUTATION OF SMAD 4 IN HUMAN CANCER CELLS** (note on p.10742)

Mutations of Smad4 found in human cancer cells," (Zhu et al 2005: 10742).

"Human cancer cells with these Smad4 mutations, the snoN promoter may be activated to a higher level in the presence of TGF-BETA due to the lack of negative regulation by Smad3/Smad4 and an additional stimulation by Smad3. Since numerous tumour cells also show an elevated level of TGF-BETA1 as they progress to a more malignant stage" (Zhu et al 2005: 10742).

"**Smad6 and Smad7** share a conserved Mad homology 2-, dwarfin type (MH2) domain with R-Smads and Co-Smads and constitute the subclass of inhibitory Smads" (Böttinger 2007:310).

**SMAD 7** (note on p.611)

"Smad7 obstructed the expression of both VEGF-A and TSP-1 in response to TGF-BETA, consistent for a role for Smad 2 and/or Smad 3 in their regulation" (Nakagawa et al 2004:611).

Smad transcriptional co-repressors are exceptional regulatory components within the nuclei during the final stage of TGF-BETA1 signalling; hence, their abundance and activity allow the cell to make the decision whether to proceed with the transcription of TGF-BETA1-responsive genes." (Yang 2003:3168) "Smad transcriptional co-repressors in regulatory TGF-BETA1 signalling, the regulation and relative implication of their expression in fibrotic kidney are completely unknown" (Yang 2003:3168) role of Smad in kidney unknown (Yang2003: p.3168).

**Degradation**

"Some activated Smads can also be targeted for degradation by the ubiquitin-dependent proteasome "(Stroschein et al 2001: 2822).
Unwanted TGF-BETA response is prevented by SnoN and Ski
"Plenty of Smad transcriptional co-repressors SnoN and Ski are evidently reduced in the fibrotic kidney induced by unilateral ureteral obstruction (UOO) in mice." (Yang 2003:3168)

Reduction of Smad in fibrotic kidney Smad and Ski (note on p.3174) "In normal kidney, plentiful SnoN and Ski are present in the nuclei of renal cells, which could protect and prevent unwanted TGF-BETA1 response, in case Smad find their way into the nuclei." (Yang 2003).

Smad loss in animal model (note on p.3174)
"loss of Smad co-repressors takes place gradually, which closely replicates the course of renal fibrosis in this model" (Yang 2003:3174) "diminishing of Smad co-repressors in the unhealthy kidney would create an classical "profibrotic" environment in which TGF-BETA1 signal" (Yang 2003:3174).

Reduction of Smad in obstructive injury and renal injury
"Reduction of Smad transcriptional co-repressors Ski and SnoN in the fibrotic kidney after obstructive injury." (Yang 2003:3173) Smad loss leads to fibrosis (note on p.3175) loss of Smad transcriptional co-repressors in the fibrotic kidney is one of the crucial events that have greatest influence on the final outcome of TGF-BETA1 signalling and renal fibrogenesis." (Yang 2003:3175).

Smad co-suppressor types
"Three Smad transcriptional co-repressors, namely Ski (Sloan-Kettering Institute proto-oncogene), SnoN (Ski-related novel gene, non Alu-containing), and TGIF (TG-interacting factor), have been identified" (Yang 2003:3168).

SnoN
"SnoN is a member of the Ski family of onco-proteins. It was identified based on sequence homology with v-Ski, the transforming protein of the Sloan-Kettering virus " (Zhu et al 2005: 10732).

SnoN HUMAN (note on p.2823)
"Human SnoN is a universally expressed transcriptional co-repressor of 684 amino acids (Nomura et al. 1989) that interacts with Smad2, Smad3, and Smad4 to antagonize TGF-β Signalling" (Stroschein, Bonni, Wrana, & Luo, 2001).
INTERACTION
"SnoN interacts directly with Smad2, Smad3, and Smad4 and represses their capacities to activate TGF target genes by disrupting the formation of an active heteromeric Smad complex, engaging a transcription co-repressor complex to the TGF-Beta-responsive promoters, and hindering the binding of transcriptional co-activator p300/CBP." (Zhu et al. 2005:10731).

ACTIVATION OF SnoN (note on p.10732)
"Initiation of SnoN transcription by TGF-BETA requires the binding of the Smad proteins to the SnoN promoter which is "determined the cis and trans-acting elements involved." (Zhu et al. 2005: 10732) "longer induction of SnoN expression by TGF correlates with a protracted phosphorylation and activation of Smad2." (Zhu et al 2005: 10736).

SnoN (note on p.2823)
"TGF stimulation, a speedy degradation of SnoN occurs, most likely intervened by Smad3 and to a lesser extent, Smad2. The removal of the inhibitory SnoN may be decisive for activation of TGF signaling as this allows the Smads to activate transcription of TGF responsive genes" (Stroschein et al 2001: 2823).

SnoN - feedback regulator
"Transcriptional co-regulator, SnoN, is a critical and multipurpose regulator of TGFβ-induced transcription and responses. SnoN controls TGFβ-mediated responses by acting as a transcriptional co-repressor or transcriptional co-activator. SnoN associates with Smad2/3 and Smad4 and is engaged to TGF β responsive genes, thus influencing their transcription. Inducing SnoN degradation, TGFβ stimulates SnoN transcription; once expressed, SnoN acts as a negative feedback inhibitor of TGF signaling." (Huang et al 2014:1) TGF-β1/Smad signalling induces the degradation of SnoN by the ubiquitin-proteasome pathway (UUP)" (Liu et al 2016:2) "Nuclear transcriptional co-repressor SnoN is recognized as an significant negative regulating factor of TGF-β1/Smad signalling pathway, which could impede the activation of TGF-β1 target genes and interfere with the biological effects of TGF-β1/Smad signalling pathway by interacting with the downstream Smad proteins" (Liu et al 2016: 8).
NEGATIVE ROLE (note on p.2832)
"SnoN is an key negative regulator of TGF signalling that functions to maintain the repressed state of TGF target genes in the absence of ligand and in the negative feedback control of TGF signalling." (Stroschein et al 2001: 2832).

SnoN NEGATIVE FEEDBACK MECHANISM NOT CLEAR (note on p.10743)
"SnoN is a co-repressor of the Smad proteins in epithelial cells. Upon induction by TGF-BETA, the newly synthesized SnoN may impede the transcriptional activity of the Smad proteins and turn off the expression of some TGF target genes in a negative-feedback manner. Nevertheless, the molecular details of this negative feedback mechanism have not been understood" (Zhu et al 2005: 10743).

"TGFβ1 signalling induces the degradation of SnoN by the ubiquitin-proteasome pathway (UPP). SnoN expression is altered under many pathological circumstances including wound healing, liver regeneration, and obstructive nephropathy" (Huang et al 2014:2).

"Both SnoN and Ski could block Smad mediated activation of TGF-BETA1-responsive promoter and exhibited additive effect in abolishing the profibrotic actions of TGF-BETA1." (Yang 2003:3167) "Nuclear transcription co-repressor Ski-related novel protein N (SnoN) is one of the most key factors that negatively regulate the TGF-β1/Smad signalling pathway. SnoN links with Smads to block the transduction of TGF-β1 signalling.

DEGRADATION (note on p.2832)

"SnoN is rapidly degraded by the ubiquitin-dependent proteasome in a Smad3-dependent manner, allowing activation of TGF target genes" (Zhu et al 2005: 10732) (Liu et al 2016: 2).

OVEREXPRESSION OF SnoN (note on p.10731)
"Overexpression of SnoN significantly impairs the ability of cells to undergo growth inhibition in response to TGF-BETA"(Zhu et al 2005:10731)" prolonged upregulation of SnoN expression plays a dire role in TGF-BETA-induced oncogenic transformation of
fibroblast cells 

"Overexpression of Smad7 competently blocked TGF-BETA-inducible transcription from the SnoN promoter" (Zhu et al 2005, p. 10734).

**SnoN (note on p.2822) in cancer**

"High levels of Ski or SnoN are detected in numerous types of human cancer cells" (Stroschein et al 2001:2822).

**SnoN ONCOGEIC PROTEIN IN CANCER (note on p.10742)**

SnoN is considered an oncoprotein due to its capacity to induce transformation in chicken embryo fibroblasts and its high level of expression in cancerous but not normal human cells" (Zhu et al 2005:10742).

"Up-regulation of SnoN expression occurs during certain phases of embryonic development in tissues with a high degree of proliferative activity, as well as in adult cancer cells, including those derived from breast cancer, Oesophageal cancer, lung cancer, and rhabdomyosarcoma" (Zhu et al 2005: 10732).

"In Oesophageal cancer and in numerous ovarian cancer cells (J. Gray, personal communication), amplification of the 3q26 region of the human chromosome that comprises the SnoN gene has been spotted, and this may partly account for the increased production of SnoN. In numerous lung cancer cells, the upsurge in SnoN expression occurs at the level of transcription (Q. Zhu and K. Luo, unpublished observation" (Zhu et al 2005: 10732).

**UBIQUITIN LIGASES**

Arkadia is a nuclear protein with 989 amino acid residues, with a typical C-terminal RING domain. Arkadia appears to efficiently enrich TGF-1β signalling through instantaneous down-regulation of two different types of negative regulators, namely, Smad7 and SnoN, which are critical substrates of Arkadia and may play an vital role in determining the strength of TGF-1β family signalling in target cells. "(Huang et al., 2014).

"Upon stimulation of TGF-β1 signalling, Arkadia binds to phosphorylated Smad2/3 (p-Smad2/3) and brings degradation of Smad7 and SnoN/Ski, permitting transcription of TGF-β1 target genes" (Liu et al 2016: 2).

"Ubiquitin ligases, such as Smurf2 and Arkadia, mediate the capacity of TGF-BETA1β to induce ubiquitination and consequent degradation of SnoN" (Huang et al 2014: 2).
TGF-β1/Smad signaling brings the degradation of SnoN by the ubiquitin-proteasome pathway (UUP)" (Liu et al 2016: 2).

"Smad ubiquitin regulatory factor 2 (Smurf2) is an E3 ubiquitin ligase that controls transforming growth factor- TGF-1β/Smad signaling and is connected in a wide variety of cellular responses" (Huang et al 2014: 2)

I] Canonical Pathway (Note On P.310)
"Canonical Smad pathways, TGF-BETA may signal through mitogen activated protein kinases, phosphoinositide 3-OH kinases, small guanosine triphosphatases, and other mediators" (Böttinger 2007: 310).

J] INDUCERS OF TGF BETA (note on p.311) (note on p.312)
"TGF-BETA is induced by angiotensin II and is required for induction of hypertrophy in tubular epithelial cells and of matrix synthesis in mesangial cells" (Böttinger 2007: 311).

"TGF-BETA also is induced directly by a wide range of products of metabolism, including glucose, advanced glycation products, free fatty acids, reactive oxygen species, and others" (Böttinger 2007: 312).

"Mechanical stretch and shear stress increase TGF-BETA release and activation in renal and vascular cells" (Böttinger 2007: 312).

K] TGF-BETA UPREGULATION IN CKD (note on p.311)
"TGF-BETA 1 also is up-regulated in glomerular diseases and experimental and human diabetic nephropathy. Amplified expression of TGF-BETA and TGF-BETA receptors is a hallmark of virtually all human and experimental CKD" (Böttinger 2007: 311).

All three isoform are fibrogenic (Yu Ling 2003).

L] FUNCTIONAL ROLE FOR TGF-BETA (note on p.312)
"TGF-BETA inhibition decreases functional and pathologic abnormalities in experimental renal disease, suggesting a dire role for TGF-BETA in CKD and renal fibrosis" (Böttinger 2007: 312).
"TGF-BETA 1 develop progressive glomerulosclerosis and tubulointerstitial fibrosis, indicating that persistently amplified TGF-BETA activity is enough to induce progressive renal disease in mice" (Böttinger 2007: 312).

“Apoptosis of glomerular epithelial cells (podocytes) is induced by TGF-BETA 1 and/or Smad7, and heads activation of mesangial cells and mesangial matrix deposition in TGF-BETA 1 transgenic mice" (Böttinger 2007: 312).

**TGF-BETA IN EPITHELIAL CELLS** (note on p.313)

"TGF-BETA itself may act on epithelial cells to bring apoptosis in renal tubular epithelial cells and glomerular podocytes and thereby may promote further epithelial injury, subsequent increased phagocytic activity and alteration in epithelial mesenchymal cell communication" (Böttinger 2007: 313).

**TGF-BETA and extracellular matrix** (note on p.312)

"TGF-BETA largely controls messenger RNA levels and promoter activities of extracellular matrix genes, including COL1A1, COL1A2, COL3A1, COL5A2, COL6A1, COL6A3, COL7A1, and non-collagenous matrix genes such as fibronectin, proteoglycans, and others" (Böttinger 2007: 312).

"TGF-BETA controls transcription of several extracellular matrix (ECM) genes through Smad3-dependent mechanisms in which Smad3 act together with activator protein 1 complexes or Sp1, respectively, on CAGA motif Smad binding elements." (Böttinger 2007: 312).

**CTGF** (note on p.313)

"Connective tissue growth factor, a secreted cysteine rich domain protein, is induced by TGF-BETA in mesenchymal cells, including hepatic stellate cells (HSCs), mesangial cells, and fibroblasts, and may be vital for maximal matrix synthesis by CTGF-BETA ." (Böttinger 2007: 313).

**TGF-BETA and endothelial action** (note on p.612)

"TGF-BETA1 also regulates angiogenesis by direct effects on endothelial cell proliferation and also by modifying expression of the VEGF receptor on the endothelial cell." (Nakagawa et al 2004: 612).
M] Smad independent pathway (note on p.312)
"Smad-independent pathways may have a role in modulation of ECM gene expression by TGF-BETA." (Böttinger 2007: 312).

N] INHIBITION OF TGF-BETA (note on p.313)
"Prostaglandin E2 inhibits TGF-BETA 1-induced collagen synthesis, indicating extensive signalling cross-talk in profibrotic signalling in HSCs" (Böttinger 2007: 313).

TGF-BETA INHIBITION (note on p.311)
"In experimental models of renal and pulmonary fibrogenesis, inhibition of TGF-BETA with neutralizing anti-TGF-BETA antisera ameliorate characteristic accumulation of extracellular matrix" (Böttinger 2007: 311).

TGF-BETAREDUCTED BY RAS INHIBITION (note on p.311)
"Importantly, inhibitors of the RAS reduce TGF-BETA expression in renal cells in vitro and in vivo" (Böttinger 2007: 311).

O] TARGETING TGF-BETA (note on p.315)
"Large-molecule TGF-BETA antagonists and small molecule inhibitors of the TGF-BETA - receptor type 1 kinase are under development for crucial indications in fibrotic diseases, diabetic nephropathy, and possibly metastatic cancers" (Böttinger 2007: 315).

P] DRUGS
1) "Pentoxifylline, a non-selective phospho-diesterase inhibitor, diminishes tubulointerstitial fibrosis by dual mechanisms, including inhibition of Smad3/-4- activated transcription, and blockade of profibrogenic effects of connective tissue growth factor (CTGF)" (Böttinger 2007: 315).
2) "The low-molecular-weight plant alkaloid halofuginone inhibits extracellular matrix accumulation in numerous animal models of fibrotic disorders" (Böttinger 2007: 315).
3) "Pirfenidone is an antifibrotic agent that reduces ECM deposition in many fibrosis models, possibly through reduction of TGF-BETA 1 synthesis" (Böttinger 2007: 315).
4) Fresolimumab, tranilast- reduces proteinuria. Pirfenidone – pilot study shows beneficial renal effect but trial was stopped due to adverse effect [Soni 2017; pg22].
4) OXYMATRINE (OM)
"Oxymatrine (OM) is an alkaloid extracted from the Chinese herb Sophora japonica and has been established to prevent fibrosis. Nevertheless, the anti-fibrosis effect of OM in DN is still unclear." (Liu et al 2016: 1).

"Oxymatrine (OM) is an herbal product derived from the root of Sophora flavescens. OM has a tetracyclic quinolizine structure, and its molecular formula is C15H24N2O. OM is recounted to have anti-inflammatory, anti-oxidative, anti-viral, anti-fibrotic and immunological regulation effects. In current years, OM has been used in China for the treatment of various human illnesses such as hepatitis B infections and liver fibrosis" (Liu et al 2016: 2).

"OM is a traditional Chinese herbal product. OM has multiple pharmacological effects and functions. OM could decreases liver fibrosis, pulmonary fibrosis, myocardial fibrosis and skin scar tissue fibrosis through inhibiting TGF-β1/ Smad signalling pathway." (Liu et al 2016: 10).

"OM could reverse the remarkable decrease of E-cadherin and SnoN and significantly increaseα-SMA, FN, TGF-β1, Arkadia, p-Smad2 and p-Smad3 and remarkably attenuate the ubiquitin-dependent degradation of SnoN induced by high glucose, which point that OM can inhibit EMT induced by high glucose via inhibiting TGF-β1/Smad signalling pathway by reducing ubiquitin-dependent degradation of SnoN." (Liu et al 2016: 10).

"OM can inhibit the high glucose-induced renal tubal EMT by decreasing the degradation of SnoN mediated by Arkadia and inhibiting activation of the TGF-β1/Smad signalling pathway. Thus, OM may be an effective therapeutic drug and SnoN may be a potential therapeutic target for the treatment of DN." (Liu et al., 2016).

"OM had an anti-fibrotic effect on liver fibrosis, pulmonary fibrosis, myocardial fibrosis and skin scar tissue fibrosis through inhibition of the TGF-β/ Smad signaling pathway.

Nevertheless, the molecular mechanism underlying its pharmacological effects and whether OM can protect against renal fibrosis in DN remains unclear" (Liu et al 2016: 3).
Q] HEPATOCYTE GROWTH FACTORS (note on p.316)
"Hepatocyte growth factor and BMP7 are thought to diminish EMT and fibrosis in the unilateral ureteral obstruction renal injury model by antagonizing TGF-BETA 1 signalling."
(Böttinger 2007: 316).

R] ANTAGONIST OF TGF BETA (note on p.316)
"The idea of using antagonists of TGF-BETA to inhibit progression of CKD and stabilize life-sustaining functions of the kidney is likely if the extraordinary trial is taken with safe administration over long periods of time can be met." (Böttinger 2007: 316).

ADVANTAGES OF TARGETING TGF-BETABETA
1] Prevent retinal programme cell damage(PCD), apoptosis
Evidence and supporting study–TGF-β plays important role in PCD reduction of endogenous transforming growth factor beta (TGF-beta) prevents apoptotic PCD of neurons reduction of PCD caused by the neutralization of endogenous TGF-beta [Dünker N 2001].

2] Prevent vascular remodelling
Evidence and supporting study–TGF-β- Angiotensin II (Ang II) and transforming growth factor (TGF) beta1 play a part in vascular remodelling further more vascular smooth muscle TGF-beta receptors are regulated on the RNA level by TGF-beta and Ang II, and that Ang II dependent regulation of TGF-beta receptors is at least partially independent of endogenous TGF-beta. Stimulation of the transcriptional expression of TGF-beta receptors by Ang II may upsurge sensitivity of vascular smooth muscle cells to TGF-beta.[ Siegert A -1999].

Angiotensin II induces TGF-BETA in response with high glucose via Thrombospondin1.

Evidence and supporting study
Thrombospondin 1 mediates angiotensin II induction of TGF-beta activation in cardiac and renal cells under both high and low glucose conditions.[Zhou Yong 2006].

Further more Enhanced TGF-beta/Smad signalling in the early stage of diabetic nephropathy is independent of the AT1a receptor. [Okazaki Yuko].

Angiotensin II up-regulates transforming growth factor-beta type I receptor on rat vascular smooth muscle cells [Fukuda N, 2000].
ANG II stimulates TGF-beta expression in the kidney. AT1-receptor blocker may be potential drugs to interfere with this ANG II-mediated TGF-beta expression [Wolf G 1998] Drugs inhibiting TGF-BETA by RAAS blockade are reviewed [Soni NOP, 2017].

TGF-beta release were inhibited by the AT(1)-specific antagonist- losartan [Wolf G 1998].

3] Prevents vascular fibrosis  
Evidence and supporting study  
TGF-beta signalling in vascular fibrosis.[ Ruiz-Ortega Marta 2007].

4] Reduces Neutrophil chemotaxis  
Evidence and supporting study  
Chemotaxis of PMNs in response to TGF-beta isoforms is mediated by the interaction of the Arg-gly-Asp-ser sequence in the CBD of Fn with an integrin on the PMN cell surface, primarily the VLA-5 integrin. TGF-beta isoforms also provoked the release of cellular Fn from PMNs [Parekh T1994].

5] Reduces vascular reactivity and increases level of endogenous vasodilators  
Evidence and supporting study -TGF-beta1 can inhibit the production of potent vasodilators (such as nitric oxide) and stimulate the production of potent vasoconstrictors (such as endothelin). [Perrella M A1998] Thus inhibiting TGF-BETA 1 may reduce vascular reactivity and increase level of endogenous vasodilator.

6] Inhibiting TGF-BETA will reduce collagen formation in diabetes [Bollineni J S1993]  
Evidence and supporting study  

7] INHIBITION OF TGF –BETA Reduces PODOCYTOPATHY  
Evidence and supporting study  
Diabetic nephropathy, are associated with increased TGFβ1 signalling and thus TGFβ1 plays a vital role in the pathogenesis of podocytopathy [Herman-Edelstein Michal 2013].

TGF-β1 brings podocyte injury through Smad3-ERK-NF-κB pathway and Fyn-dependent TRPC6 phosphorylation [Yu Lixia, 2010].
8] TGF-β INHIBITORS –Inhibit the secretion of TGF-β A in mesangial cells which occur due to AGEs and in response to hyperglycaemia.

Evidence and supporting study
Murine mesangial cells stimulated with glycosylated protein secrete bioactive TGF-beta and demonstrate a disproportionate increase in the steady state levels of TGF-beta 2 mRNA [Pankewycz O G1994].

9] TGF-β INHIBITORS-REDUCES GLOMERULAR HYPERTROPHY
TGF-beta 1 may not only bring extracellular matrix synthesis, but may also contribute in the process of glomerular hypertrophy in response to injury. [Choi M E 1993].

10] TGF-β INHIBITORS-REDUCES CTGF AND VEGF
Evidence and supporting study Activated TGF-β/Smad signalling by podocytes may induce connective tissue growth factor (CTGF or CCN2) and vascular endothelial growth factor (VEGF) expression .TGF-β- induced CTGF and VEGF in mesangial matrix expansion in chronic progressive glomerular disease. Paracrine CTGF and VEGF may contribute to mesangial matrix accumulation in chronic glomerular disease and ultimately in the development of glomerulo-sclerosis. [Lee Hyun Soon 2012].

11] TGF INHIBITOR will inhibit GLUT1 in mesangial cells and prevent uptake of glucose which is responsible for injury.
Evidence and supporting study
TGF-beta 1 stimulates the glucose uptake by enhancing the GLUT1 mRNA expression in mesangial cells and the effect is antagonized by Rhein. [Liu Z, 1999] Rhein may be hopeful drug for diabetic nephropathy.

TGF-beta 1 stimulates glucose uptake by enhancing GLUT1 expression in mesangial cells. [Inoki K, 1999].

12] TGF INHIBITOR Reduces the fibronectin by interfering PKA.
Evidence and supporting study
13] TGF-BETA INHIBITOR – reduces hyperglycaemia induced heparan sulfate proteoglycan (HSPG)
Evidence and supporting study
TGF-beta 1 stimulates mesangial HSPG expression and production. Because these effects may be decreased by oligonucleotides antisense to TGF-beta 1 [Kolm V 1996].

14] TGF–beta inhibitors may prevent apoptosis in mesangial cells since high glucose induced apoptosis is mediated by TGF-BETA. [Khera Tarnjit K 2007].

15] TGF INHIBITORS INHIBIT–TGF-BETA1-INDUCED EPITHELIAL-TO-MESENCHYMAL TRANSITION
Evidence and supporting study
Epithelial-to-mesenchymal cell transformation (EMT) is the trans-differentiation of tubular epithelial cells into myofibroblasts, a key event in progressive chronic kidney disease in diabetes, resulting in fibrosis. TGF-beta-induced EMT is a crucial contributor to fibrotic scar formation as seen in DN [Hills Claire E].

16] TGF-BETA inhibitors can reverse partial thickening of Glomerular basement membrane and reverse Diabetic nephropathy in animal models.
Evidence and supporting study
Inhibiting renal TGF-beta activity can partially reverse the GBM thickening and mesangial matrix expansion in this mouse model of type 2 diabetes. Anti-TGF-beta regimens would be useful in the treatment of diabetic nephropathy. [Chen Sheldon 2003].

17] TGF-BETA inhibitors may reduce abnormal deposition of extracellular matrix (ECM) therein and expansion of the mesangial matrix (MM)
Evidence and supporting study
TGF-beta1 in vivo induces unusual deposition of fetal laminin alpha1, alpha2 and beta1 chains and collagen type IValpha1/alpha2 in the GBM. On the other hand, the TGF-beta1-mediated expansion of the mesangial ECM is dominated by the normal components. [Chai Qing 2003].
18] **TGF–Beta Inhibitors – reduces production of IL-8 and MCP-1**
Glomerular up-regulation of monocyte chemotactic protein-1 (MCP-1), followed by an influx of monocytes bring about extracellular matrix deposition is a common outcome of many types of glomerulonephritis.

**Evidence and supporting study**
TGF-beta1 induces IL-8 and MCP-1 through a connective tissue growth factor-independent pathway. [Qi Weier 2005].

19] **TGF–Beta Inhibitors – reduces local expression of complement system**
Recently targeting complement system Mannose Binding lectin (MBL) in Diabetic nephropathy has been reviewed. [Soni NOP 2017] Local complement system plays role in pathogenesis of Diabetic Nephropathy.

Also using Recombinant complementary Inhibitor CD 59 to treat Diabetic Nephropathy is Reviewed [Soni NOP 2017].

**Evidence and supporting study**
TGF-beta, chemokines and complement components play a role in numerous types of renal disease, suggest that TGF-beta is tangled in the regulation of local expression of chemokines and complement components by tubular cells. [Gerritsma J S 1998].

20] **TGF–Beta Inhibitors–Inhibits activation of HDAC 2**, recently HDAC inhibitor in Diabetic Nephropathy is reviewed [Soni NOP 2017] HDAC 2 plays important role in pathogenesis.

**Evidence and supporting study**
Hydrogen peroxide increased HDAC-2 activity, and the treatment with an antioxidant, N-acetyl-cysteine, almost completely reduced TGF-beta1-induced activation of HDAC-2. These findings suggest that HDAC-2 plays an important role in the development of ECM accumulation and EMT in diabetic kidney and that ROS mediate TGF-beta1-induced activation of HDAC-2. [Noh Hyunjin 2009].
21] TGF-BETA 1 Inhibitors reduces Nf-κB.

Evidence and supporting study
Decreased expression of TGF-beta (1) reduces chemokine production in association with reduced NF-kappa B DNA binding activity, suggesting that immune-modulatory pathways in the kidney [Qi Weier 2006].

CONCLUSION
All the above 1 to 21 points suggest TGF-beta inhibitors are Novel drugs and supports the hypothesis and prevent progression of CKD and stabilize life-sustaining functions of the kidney if the extraordinary challenge of directing their safe administration over long periods of time can be met.

Conflict of interest
The author declares there is no conflict of interest regarding the publication of this paper

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I give credit to all authors list in my work and I quote them appropriately and acknowledge them for Nobel work.
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