Novel therapeutic approach for kidney fibrosis

DAVID HARRIS
30/09/17
Treat to help slow decline in kidney function and reduce hypertension risk*

- Lifestyle changes
  - Smoking cessation
  - Dietary salt restriction
  - Moderate alcohol consumption
  - Maintain BMI between 18.5 and 24.9 kg/m² through diet and exercise
  - Avoid more than two caffeinated drinks per day
- Blood pressure: assess and maintain blood pressure <130/80 mmHg with ACE inhibitor or ARB
- Cholesterol: maintain total cholesterol level <4.0 mmol/L with diet and statin
- Blood glucose (for patients with concurrent diabetes): aim for HbA₁c <7.0 %
- Avoid nephrotoxic drugs and episodes of acute kidney injury
Liraglutide: Renal Outcomes


GLP-1ra
Canagliflozin: Renal Outcomes

A Hospitalization for Heart Failure
Hazard ratio, 0.67 (95% CI, 0.52–0.87)

B Death from Any Cause
Hazard ratio, 0.87 (95% CI, 0.74–1.01)

C Progression of Albuminuria
Hazard ratio, 0.73 (95% CI, 0.67–0.79)

D Composite of 40% Reduction in eGFR, Requirement for Renal-Replacement Therapy, or Death from Renal Causes
Hazard ratio, 0.60 (95% CI, 0.47–0.77)

Integrated CANVAS Program

SGLT2 inhibitor
Tolvaptan in early-stage ADPKD

A Total Kidney Volume

C Kidney Function

Torres et al. NEJM 2012
Non diabetic CKD

*not attractive for pharma*

Population heterogeneity
Absence of reliable biomarkers for subgroup selection
Absence of reliable surrogates (efficacy biomarkers)
Large subject numbers
Long follow-up
Some novel therapies in human CKD

Pirfenidone: study withdrawn
Nox1/4 inhibitor – negative trial
Anti-CTGF antibodies FG3019: studies terminated
SSAO/ VAP1 inhibitors: phase 1 clinical trial concluded, not reported
Curcumin – phase 3 trial completed, not reported
Tranilast and analogues FT011: in phase 1b clinical trial
Alpha-lipoic acid: recruiting
Tie2 Rec activator - angiopoietin receptor, tyrosine kinase inhibitor: in development
JAK-STAT inhibitors: in development
LOX inhibitors: in development

Anti TGF-β Ab (LY2382770) – negative trial
FGS trial terminated

N=36
DB, randomised
TGF-β1 mAb for DN trial terminated

Voelker J et al. JASN 2017;28:953-962

N=416 DB, randomised phase 2
BMP7 and TGFβ1 are better predictors of major renal endpoints than eGFR+UACR

Wong MG et al. Kidney Int 2013;83:278-84
**TRANSLATION OF SMALL MOLECULAR ANTI-FIBROTICS**

N=63, mainly for respiratory (IPF), liver (NASH, NAFLD), and skin (scleroderma, keloid) diseases

N=11 for renal disease

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pirfenidone</td>
<td>multiple</td>
<td>diabetic nephropathy (DN, phase 2, completed)</td>
</tr>
<tr>
<td>F-351</td>
<td>p38 (α,γ) inh</td>
<td>(liver &amp; kidney fibrosis (phase 1b/11)</td>
</tr>
<tr>
<td>Atrasentan</td>
<td>sel ETAr inh</td>
<td>DN (phase 11, SONAR)</td>
</tr>
<tr>
<td>GKT-137831</td>
<td>NOX1 &amp; NOX4 inh</td>
<td>DN (phase 11)</td>
</tr>
<tr>
<td>Bardoxolone</td>
<td>NRF2-KEALi act</td>
<td>CKD &amp; DN (phase 111, terminated—safety)</td>
</tr>
<tr>
<td>Baricitinib</td>
<td>JAK1 &amp; JAK2 inh</td>
<td>DN (phase 11)</td>
</tr>
<tr>
<td>Emricasan</td>
<td>pan-caspase inh</td>
<td>severe renal impairment (phase 1)</td>
</tr>
<tr>
<td>Beraprost</td>
<td>prostacyclin analogue</td>
<td>primary glom disease (phase 11b/111)</td>
</tr>
<tr>
<td>CTP-499</td>
<td>pan-PDE inh</td>
<td>DN (phase 11, completed)</td>
</tr>
<tr>
<td>Pyridoxamine</td>
<td>metabolite of vit B6</td>
<td>DN (phase 11, completed)</td>
</tr>
<tr>
<td>Bindarit</td>
<td>CCL3, -7, -8 inh</td>
<td>DN (phase 11, completed)</td>
</tr>
</tbody>
</table>

TGF-β: the master regulator of fibrosis

Meng X-M et al. NRN 2016;12:325-38
TGF-β causes tissue fibrosis through three major Signaling Pathways

Hypothesis: β-catenin/Foxo is the key target to dissociate profibrotic from anti-inflammatory and wound-healing effects of TGF-β
Inhibition of β-catenin/TCF should increase β-catenin/Foxo binding

Both TCF & Foxo bind to the Armadillo repeats 1-7 of β-catenin

ICG-001, a peptidomimetic small molecule, selectively inhibits β-catenin/TCF in a CBP-dependent manner
TGF-β and regulatory T cells are key regulators of inflammation.
Anti-fibrotic effect of β-catenin/Foxo
β-catenin/Foxo1 protects against TGF-β-induced profibrotic changes in vitro
rhTGF-β1+ICG-001 increases β-catenin/Foxo in UUO kidney

proximity ligation assay
Inhibition of β-catenin/TCF interaction by ICG-001 decreases kidney fibrosis in UUO
Inhibition of β-catenin/TCF by ICG-001 increases β-catenin/Foxo1 in kidney of UUO mice

proximity ligation assay
β-catenin/Foxo1 protects against kidney fibrosis in UUO

(A) Immunofluorescence images of various proteins in different treatment groups: Control, UUO, UUO+rhTGF-β1, ICG-001, and UUO+ICG-001. Treatments include control, UUO, and UUO supplemented with rhTGF-β1 and ICG-001.

(B) Quantitative analysis of protein expression showing bar graphs for vimentin, collagen I, collagen III, and collagen IV. The graphs illustrate the percentage area distribution of each protein under different conditions.
β-catenin/Foxo1/TCF in human diabetic nephropathy & kidney transplant

![Proximity ligation assay]

- **Normal**
  - β-catenin/Foxo1
  - β-catenin/TCF

- **Fibrosis**
  - β-catenin/Foxo1
  - β-catenin/TCF

- Diabetic Nephropathy (r=0.8223)
- Transplant kidney (r=0.7611)

- P<0.01

- Diabetic Nephropathy (r=0.9223)
- Transplant kidney (r=0.7643)

- P<0.01
Renal β-catenin/Foxo1/TCF in human CKD

Transplant

Diabetic nephropathy

Hypertension

IgA nephropathy

Proximity ligation assay
Treg-dependent anti-inflammatory effect of β-catenin/Foxo
Inhibition of β-catenin/TCF interaction by ICG-001 reduces inflammation via iTreg in UUO kidney

Infiltrated cells (10 HP)

*P < 0.05 vs control
# p < 0.05 vs untreated UUO
†p < 0.05 vs TGF-β1 treated UUO
Inhibition of β-catenin/TCF interaction by ICG-001 reduces macrophage infiltration via iTreg in UUO kidney

| Sham control | UUO | UUO+ TGF-β1 | UUO+ICG-001 | UUO+ TGF-β1+ICG-001 | Pc61+UUO+ TGF-β1+ICG-001 |

*P < 0.05 vs control
# p < 0.05 vs untreated UUO
† p < 0.05 vs TGF-β1-treated UUO
Inhibition of β-catenin/TCF interaction by ICG-001 decreases kidney fibrosis, in part by iTregs

Sham control

UUO

UUO + TGF-β1

UUO + ICG-001

UUO + TGF-β1 + ICG-001

Pc61 + UUO + TGF-β1 + ICG-001

*P < 0.05 vs control
# p < 0.05 vs untreated UUO
† p < 0.05 vs TGF-β1-treated UUO
rhTGF-β1 + ICG-001 reduces kidney fibrosis after unilateral ischaemia reperfusion, via Tregs
Inhibition of β-catenin/TCF interaction by ICG-001 prevents TGF-β1-induced distant organ fibrosis (liver)

# $p < 0.05$ vs untreated UUO
† $p < 0.05$ vs TGF-β1-treated UUO
Inhibition of β-catenin/TCF interaction by ICG-001 prevents TGF-β1-induced distant organ fibrosis (lung)

# $p < 0.05$ vs untreated UUO

† $p < 0.05$ vs TGF-β1-treated UUO
non-fibrotic wound-healing effect of β-catenin/Foxo in kidney injury
ICG-001 promotes non-fibrotic wound healing in rhTGF-β1–treated C1.1 cells

Scratch Assay

0 h 48 h

IF staining of E-cadherin / α-SMA

control TGF-β

TGF-β + ICG-001 ICG-001

* P < 0.01 vs. control; # P < 0.01 vs. TGF-β
Wound healing assay

WT

KO FoxO1

KO TCF1

48 h Control TGF-β TGF-β + ICG-001 ICG-001

Percentage of wound closure

Time (h)

WT
Foxo1 KO TCF1 KO
Therapeutic targeting β-catenin/Foxo by inhibition of β-catenin/TCF

*reduces*

fibrosis (kidney, lung, liver)

infiltration of lymphocytes & macrophages, (Treg-dependent)

*increases*

non-fibrotic wound healing
TARGETING INFLAMMATION

DNA VACCINATION
chemokines/receptors: CCL2, CCL5, CX3CR1
costimulatory molecules: CD40

INHIBITING EFFECTOR CELLS

REGULATORY CELLS
(mesenchymal stem cells)
protective macrophages: M2a, M2c, Mreg
tolerogenic dendritic cells
regulatory lymphocytes
regulatory innate lymphoid cells
anti-inflammatory macrophages may be profibrotic \textit{(in vitro)}

Which macrophages are pro-fibrotic?

- Proinflammatory (M1) \hspace{1cm} \text{NO}
- Suppressor (M2c) \hspace{1cm} \text{NO}
- Wound-healing (M2a) \hspace{1cm} \text{YES*}
- Fibrinolytic \hspace{1cm} \text{NO}

*but our studies show net effect \textit{in vivo} is anti-fibrotic
M2a or M2c in Adriamycin nephropathy

M2c > M2a: proteinuria, tubular cell atrophy, interstitial CD4 infiltration
Biopsy transcriptome expression profiling to identify kidney transplants at risk of chronic injury

O’Connell PJ et al. Lancet 2016;388:983-93
 IDENTIFYING PATHOGENIC GENE PATHWAYS IN RENAL TRANSPLANT FIBROSIS

identify kidney transplants at risk of chronic injury

*Biopsy transcriptome expression profiling*

evaluate profibrotic potential of genes predictive of progressive graft fibrosis

*deletional mutations in cell lines*
*deletional mutations in zebrafish*

determine the importance of HIF-1α and Wnt/β-catenin in progressive graft fibrosis

*conditional knockout*

O’Connell PJ, Grey S, Harris D, Zheng G, Yi S.
Renal MRI for function & severity of fibrosis

Morrell GR et al. JASN 2017;28:2564-2570

diffusion-weighted MRI
blood oxygen level–dependent MRI
MR elastography
susceptibility imaging

show promise
but currently limited accuracy & practicality
→ further development
MR elastography: heterogeneous kidney stiffness

Kirpalani A et al. CJASN 2017
MR elastography stiffness scores may predict future changes in kidney allograft function

Kirpalani A et al. CJASN 2017
TARGET FIBROSIS

INHIBIT $\beta$-catenin/TCF

STIMULATE $\beta$-catenin/Foxo

evaluate profibrotic potential of genes predictive of progressive fibrosis

TARGET INFLAMMATION

DNA VACCINATION

INHIBIT EFFECTOR CELLS

REGULATORY CELLS
  MSCs, Mφ, DCs, Tregs, ILCs