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

* Cadnapaphornchai CJASN 2014;9:889
Liraglutide: Renal Outcomes


GLP-1ra
Canagliflozin: Renal Outcomes

A Hospitalization for Heart Failure

B Death from Any Cause

C Progression of Albuminuria

D Composite of 40% Reduction in eGFR, Requirement for Renal-Replacement Therapy, or Death from Renal Causes

No. at Risk
Placebo 4347 4267 4198 4123 3011 1667 1274 1256 1236 1210 1180 1158 829 233
Canagliflozin 5795 5732 5653 5564 4437 3059 2643 2610 2572 2540 2498 2451 1782 490

No. at Risk
Placebo 4347 4316 4279 4236 3119 1759 1356 1344 1328 1310 1292 1280 924 258
Canagliflozin 5795 5768 5733 5679 4576 3182 2761 2736 2710 2687 2651 2613 1904 532

No. at Risk
Placebo 3819 3473 3096 2700 1690 877 724 652 626 565 548 485 303 67
Canagliflozin 5196 4791 4475 4027 2968 1951 1730 1593 1528 1408 1354 1213 775 185

No. at Risk
Placebo 4347 4287 4227 4151 3029 1674 1274 1253 1229 1202 1173 1148 819 229
Canagliflozin 5795 5737 5664 5578 4454 3071 2654 2623 2576 2542 2495 2450 1781 493

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**Pirfenidone**: study withdrawn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

N=416 DB, randomised phase 2

Voelker J et al. JASN 2017;28:953-962
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>Target(s)</th>
<th>Disease</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 11, 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
Both TCF & Foxo bind to the Armadillo repeats 1-7 of β-catenin.

Inhibition of β-catenin/TCF should increase β-catenin/Foxo binding.

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

UUO (U)

UUO + rhTGFβ1 (U+T)

UUO + rhTGFβ1 + ICG (U+T +I)

UUO model

Duracell red spot

U

U+T

U+T+I
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
**β-catenin/Foxo1/TCF in human diabetic nephropathy & kidney transplant**

Proximity ligation assay

**Diabetic Nephropathy**
- $r = -0.8223$
- $P < 0.01$

**Transplant kidney**
- $r = -0.7611$
- $P < 0.01$

**Fibrosis score**
- $β$-catenin/TCF
  - Diabetic Nephropathy: $r = 0.9223$
  - Transplant kidney: $r = 0.7643$
  - $P < 0.01$
Renal β-catenin/Foxo1/TCF in human CKD

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

*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)
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

IF staining of E-cadherin / α-SMA

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

48 h

WT

KO FoxO1

KO TCF1

Control  TGF-β  TGF-β + ICG-001  ICG-001

Percentage of wound closure vs. Time (h)
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 (*in vitro*)

Which macrophages are pro-fibrotic?

<table>
<thead>
<tr>
<th>Macrophage Type</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td><em>Proinflammatory (M1)</em></td>
<td>NO</td>
</tr>
<tr>
<td><em>Suppressor (M2c)</em></td>
<td>NO</td>
</tr>
<tr>
<td><em>Wound-healing (M2a)</em></td>
<td>YES*</td>
</tr>
<tr>
<td><em>Fibrinolytic</em></td>
<td>NO</td>
</tr>
</tbody>
</table>

*but our studies show net effect *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
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*

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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 FIBROSION

**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