• **Presenter Disclosure Information**

- **Presenter Name:** Ravi Nistala
- **Presentation Title:** Dpp4 Inhibition Substantially Reverses Ang II-mediated Inflammation And Kidney Injury Independent Of BP Reduction
- **Financial Disclosure:** Research support - Bristol Myers Squibb, Dialysis Clinics Inc.
- **Unlabeled/unapproved uses disclosure:** Investigational use of DPP4 inhibition in obesity and diabetic kidney disease
Dpp4 Inhibition Substantially Reverses Ang II-mediated Inflammation And Kidney Injury Independent Of BP Reduction

Ravi Nistala, M.D., M.S.
Division of Nephrology, Department of Medicine, University of Missouri School of Medicine, Columbia, MO, USA
What is DPP4

- Dipeptidyl peptidase 4 is a prolyl exopeptidase that cleaves after proline in second position
- H2N-Xaa-Pro-peptides
- Abundant expression in the epithelial cells of kidney, intestines, lymphocytes & macrophages
- Expression/activity is increased in obesity, kidney disease, diabetic nephropathy
- DPP4-/- mice
  - remain insulin sensitive
  - enhanced oral glucose tolerance
  - normal immune function

Rajagopalan et al Diabetes 2013; 62: 149-57
Nistala et al, Endocrinology 2014; 155: 2266-76
Marguet et al, PNAS 2000; 97: 6874-6879
RAAS is activated in obesity, DM and mediates HTN
- sympathetic activation
- adipokine secretion (AGT from adipose tissue)
- impairment of pressure natriuresis
- DPP4 activation/GLP1 degradation?
- Structural changes in kidney
- Hormones (insulin, leptin, NPY, adiponectin)
- Endothelial dysfunction

- Addition of high dose ACEi to DPP4 inhibition, may increase BP in a human study (Nancy Brown and colleagues)
- Addition of ARB to DPP4i in rodents has shown benefits to proteinuria (Hocher)
- However, it is not known if Ang II or RAAS components can regulate DPP4

Kotsis et al, Hypertens Res 2010; 33: 386–393
RAAS activates inflammation

- RAAS has been shown to increase inflammation via calcineurin (Coffmann lab, Th1, Th17 (Harrison lab), macrophages (MR KO) (Young lab), dendritic cells?, Tregs downregulation (Schiffrin & Muller labs)

Nataraj et al, J Clin Invest 1999; 104: 1693-701
Ang II activates DPP4 in proximal tubule cells

Comparison to control (Arbitrary units)

*†, † = p<0.05
Ang II activation of mTOR/S6K and ERK1/2 pathways are affected by DPP4 inhibition

And unpublished data
Hunyady et al Mol Endocrinol 2006 May;20(5):953-70
Hypothesis: DPP4 inhibition would mitigate Ang II-mediated increases in inflammation and kidney injury
Experimental design for Saxagliptin Rx

Table Phenotypic characteristics of study animals by group

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control + Saxagliptin</th>
<th>Ang II</th>
<th>Ang II + Saxagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g)</td>
<td>27.5 ± 0.8</td>
<td>27.2 ± 0.5</td>
<td>24.0 ± 0.5*</td>
<td>25.2 ± 0.9*</td>
</tr>
<tr>
<td>Heart Weight (mg)</td>
<td>118 ± 8</td>
<td>116 ± 3</td>
<td>148 ± 8</td>
<td>142 ± 9</td>
</tr>
<tr>
<td>HW/TL</td>
<td>67 ± 5</td>
<td>68 ± 1</td>
<td>87 ± 4*</td>
<td>83 ± 6*</td>
</tr>
<tr>
<td>Kidney Weight (mg)</td>
<td>334 ± 9</td>
<td>332 ± 9</td>
<td>291 ± 9*</td>
<td>303 ± 15*</td>
</tr>
<tr>
<td>Epididymal Fat Weight (mg)</td>
<td>332 ± 18</td>
<td>294 ± 18</td>
<td>165 ± 27*</td>
<td>206 ± 31*</td>
</tr>
<tr>
<td>Retroperitoneal Fat Weight (mg)</td>
<td>52 ± 7</td>
<td>50 ± 5</td>
<td>29 ± 5*</td>
<td>31 ± 9*</td>
</tr>
<tr>
<td>Gastrocnemius Muscle Complex</td>
<td>318 ± 6</td>
<td>331 ± 17</td>
<td>317 ± 11</td>
<td>344 ± 41</td>
</tr>
</tbody>
</table>

Values are mean ± SE, n=6/group, *p<0.05 versus control and control + saxagliptin. HW/TL, heart weight normalized to tibia length.
Saxagliptin is unable to suppress Ang II-mediated increases in BP and albuminuria

A

Systolic Blood Pressure

B

Proteinuria

C

Albuminuria (mg/mg Cr)

* = p<0.05
Saxagliptin suppresses DPP4 activity in the plasma & kidney

* = p<0.05
Ang II-induced mesangial widening is suppressed by Saxagliptin

TEM of kidney

PAS of kidney
Ang II-induced proximal tubule injury is only partially suppressed by Saxagliptin.
Saxagliptin increases nephrin protein expression in Ang II treated mice

* = p<0.05
CD26 in T-cell activation

- CD26 up-regulation correlates with disease activity in human autoimmune diseases such as rheumatoid arthritis and multiple sclerosis.
- CD26 has co-stimulatory T cell functions, through the direct interaction with adenosine deaminase (ADA) or caveolin, as well as regulatory functions.
- CD26 expression is increased on macrophages in visceral adipose tissue and partakes in T-cell co-stimulation.

Rajagopalan et al Diabetes 2013; 62: 149-57
DPP4 inhibition modulates inflammatory gene expression in the kidney

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>Ang II</th>
<th>Ang II+ Saxa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DPP4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>F4/80</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MCP1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD68</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD163</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RORγT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IFNγ</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T-bet</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>18S</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Macrophages**
- **Th17**
- **Th1**
Differential regulation of kidney cytokines on mouse 23-plex cytokine assay

* = p<0.05
Saxagliptin suppresses Ang II-mediated activation of CD26 expression on kidney mononuclear cells

* = p<0.05
Preliminary data on Ang II activation of memory type CD4 cells

<table>
<thead>
<tr>
<th></th>
<th>Control Kidney</th>
<th>Ang II Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ gated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorescence</td>
<td>FL1-H: CD4 FITC</td>
<td>FL1-H: CD4 FITC</td>
</tr>
<tr>
<td></td>
<td>FL3-H: CD8 PerCP</td>
<td>FL3-H: CD8 PerCP</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>FL2-H: CD44 PE</td>
<td>FL2-H: CD44 PE</td>
</tr>
<tr>
<td></td>
<td>FL4-H: CD62L APC</td>
<td>FL4-H: CD62L APC</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>FL1-H: CD4 FITC</td>
<td>FL1-H: CD4 FITC</td>
</tr>
<tr>
<td></td>
<td>FL3-H: CD8 PerCP</td>
<td>FL3-H: CD8 PerCP</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>FL2-H: CD44 PE</td>
<td>FL2-H: CD44 PE</td>
</tr>
<tr>
<td></td>
<td>FL4-H: CD62L APC</td>
<td>FL4-H: CD62L APC</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>FL1-H: CD4 FITC</td>
<td>FL1-H: CD4 FITC</td>
</tr>
<tr>
<td></td>
<td>FL3-H: CD8 PerCP</td>
<td>FL3-H: CD8 PerCP</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>FL2-H: CD44 PE</td>
<td>FL2-H: CD44 PE</td>
</tr>
<tr>
<td></td>
<td>FL4-H: CD62L APC</td>
<td>FL4-H: CD62L APC</td>
</tr>
</tbody>
</table>

CD4+ gated and CD8+ gated plots showing the expressions of CD8 and CD44 in Control and Ang II Kidney conditions.
Preliminary data on Ang II reduction of FoxP3+ CD4+ cells

Blood

Kidney

Con

Ang II

FoxP3

CD4

1.77 0.14

96.9 100 101 102 103 104

1.18

0.093

9.09 1.01

96.9 100 101 102 103 104

1.75 0.093

13.9 1.15

84.6 0.38

96.2 1.91

89.7 0.19

0.19
Saxagliptin reverses moderate dose Ang II-mediated proteinuria

* = p<0.05
Acknowledgements

University of Missouri

Endocrinology/Medicine
  James R. Sowers MD
  Annayya Aroor MD PhD
  Javad Habibi PhD
  MR Hayden MD
  Mona Garro MLS
  Dongqin Chen BS
  Cassandra Smith PhD
  William Stocker MD

Nephrology/Medicine
  Adam Whaley-Connell DO MSPH
  Alex Meuth BS

Microbiology, Immunology and Surgery
  Susan McKarns PhD

Harry S Truman VA Medical Center
  Shawn Bender PhD

RADIL/IDEXX
  Cynthia Besch-Williford PhD
  Jill Hansen MS

Past Mentor
  Curt Sigmund PhD