Novel experimental approaches for diabetic kidney disease

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Disclosure

I have financial relationship with:
- Dompe
- Fate Therapeutics
- Enthera

And patents related to some of the science shown
Nuovi approccii terapeutici per la nefropatia diabetica

1. Introduction
2. The new paradigm in DKD
3. Current strategies to prevent/delay DKD
4. Novel experimental strategies to prevent/delay DKD
5. Conclusions
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Diabetes: The New Millennium Pandemic

Estimated number of people with diabetes worldwide and per region in 2015 and 2040 (20-79 years)

North America and Caribbean
- 2015: 44.3 million
- 2040: 60.5 million

Europe
- 2015: 59.8 million
- 2040: 71.1 million

Middle East and North Africa
- 2015: 35.4 million
- 2040: 72.1 million

South and Central America
- 2015: 29.6 million
- 2040: 48.8 million

Western Pacific
- 2015: 153.2 million
- 2040: 214.8 million

South East Asia
- 2015: 78.3 million
- 2040: 140.2 million

Africa
- 2015: 14.2 million
- 2040: 34.2 million

World
- 2015: 415 million
- 2040: 642 million

www.IDF.org
Rising Incidence and Prevalence

- Steady rise in autoimmune diseases in developed countries
- 21% increase in T1D prevalence in youth from 2001 to 2009
- Incidence of childhood T1D increase ~3% annually

J.F. Bach; NEJM 2002
J.M. Lawrence, Diabetes 2014
Diabetes: The costs

Diabetes treatment will cost in 2035 about $245 billion a year in the U.S. alone

2% of USA GDP

11% of Italy GDP

100% of Nigeria/Cile/Egitto GDP
Managing Diabetes

• Unfortunately only few patients reaches glycemic targets (HbA1c<7%)
I pazienti con diabete perdono 10-13 anni di vita rispetto alla popolazione generale

L. Huo et al, Diabetologia 2016
1998-2011 death rate in 34,000 T1D individuals

<table>
<thead>
<tr>
<th>Mean HbA1c</th>
<th>Death from any cause</th>
<th>Death from cardiovascular disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 6.9%</td>
<td>2.36</td>
<td>2.92</td>
</tr>
<tr>
<td>7.0%-7.8%</td>
<td>2.38</td>
<td>3.39</td>
</tr>
<tr>
<td>7.9%-8.7%</td>
<td>3.11</td>
<td>4.44</td>
</tr>
<tr>
<td>8.8%-9.6%</td>
<td>3.65</td>
<td>5.35</td>
</tr>
<tr>
<td>≥ 9.7%</td>
<td>8.51</td>
<td>10.46</td>
</tr>
</tbody>
</table>
Diabetes Complications

- Good glucose control prevents and delays complications
Diabetes and kidney

- DN is the most serious complication that diabetic patients face and chronic renal failure is almost inevitable.

- Within 20 years more than 300 million people worldwide will have diabetes.

- 30% to 40% will develop DN.

- Diabetic kidney disease accounts for the majority of ESRD cases.
Diabetic nephropathy – global problem

Despite advances in albuminuria-targeting treatment:

ESRD incidence due to diabetes continued to rise over the last two decades

diabetes accounts for more than 40-55% prevalent End-Stage Renal Disease (ESRD) cases.

USRDS annual report, 2010

Jones et al, Kidney International, 76, 2005
Rosolowsky, CJASN, 22, 2011
Finndiane Study, CJASN, 22, 2011
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The 1990’s Model of Nephropathy in T1D focused on urinary albumin abnormalities

- Normo-albuminuria
- Micro-albuminuria
- Proteinuria
- Hemodynamic & Morphological Glomerular Lesions
- ESRD
Regression of Microalbuminurina in Type 1 Diabetes


<table>
<thead>
<tr>
<th>Status*</th>
<th>Initial Evaluation Period</th>
<th>1st Follow-up Period</th>
<th>2nd Follow-up Period</th>
<th>3rd Follow-up Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. (%)</td>
<td>no. (%)</td>
<td>no. (%)</td>
<td>no. (%)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>—</td>
<td>24 (7)</td>
<td>37 (13)</td>
<td>33 (15)</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>386 (100)</td>
<td>191 (54)</td>
<td>141 (49)</td>
<td>99 (45)</td>
</tr>
<tr>
<td>Normal albumin excretion</td>
<td>136 (39)</td>
<td>110 (38)</td>
<td>88 (40)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>386 (100)</td>
<td>351 (100) †</td>
<td>288 (100) †</td>
<td>220 (100) †</td>
</tr>
</tbody>
</table>
GFR Loss in the absence of proteinuria

Doria/Fiorina Sem Nephrol 2011
The 2010’s Model of Nephropathy in T1D focused on GFR abnormalities
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Current strategies to prevent kidney function loss in diabetes

Intensive glycemic control

RAAS Inhibition

Risk reduction with intensive therapy: 50% (95% CI, 18–69)
P = 0.006

DCCT-EDIC, NEJM 2011

Lewis et al, NEJM 1993
Persisting poor glycemic control in diabetes

48% have HbA1c ≥7%

Persisting poor glycemic control in diabetes

22% have HbA1c ≥8%

13% have HbA1c ≥9%

Limits of RAAS inhibitors in preventing kidney function loss in diabetes

GFR ≤50-60 ml/min

GFR >50-60 ml/min

From Lewis et al, NEJM 1993
High Risk of ESRD in Type 1 Diabetes: New Strategies Are Needed to Retard Progressive Renal Function Decline

Andrzej S. Krolewski, MD, PhD,* and Joseph V. Bonventre, MD, PhD†
Intervening early in the course of CKD

If we assume a baseline GFR of 80 ml/min and losing GFR at a constant rate of 4 ml/min/year:

- 12 years → double serum creatinine
- 17 years → ESRD

Early intervention to reduce by 50% decline (ESRD delay=20 yrs if GFR=90 ml/min)

Late intervention to reduce by 50% decline (ESRD delay=8 yrs if GFR<45 ml/min)

For young T1D difference between developing ESRD in their 70s as opposed to their 50s.
Lack of a good model for DKD

- Mutation of the leptin receptor
- Morbid obesity (3x normal weight)
- Hyperinsulinemia (50-fold increase)
- Hyperglycemia
- Proteinuria
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Experimental ongoing treatments

- **Drugs targeting metabolic pathways** (Allopurinol Joslin Trial)

- **Drug targeting inflammatory pathways**
  - (i) MMF inhibit macrophage depositon
  - (ii) MCP-1 inhibitor – Bindarit (NCT01109212)
  - (iii) Inhibitor of TGF-β production – pirfenidone - decrease matrix deposition in experimental models of kidney disease in small study but failed in large studies

- **Drug targeting immunological mechanisms** (immune related mechanism may be involved)
  - (i) Since T-cell depletion affects the development and the natural history of renal damage in animal models, anti-CD3 mAb or ATG may be suggested for worst cases

Doria/Fiorina P et al. Sem Nephrol 2012
Mesenchymal stem cells
MSCs are a rare, heterogeneous, stromal population of multipotent non-hematopoietic progenitor cells mainly located in the bone marrow, with extensive proliferation capacity that differentiate into mesenchymal lineages.
MSC characteristics

Minimum criteria are:
- Plastic adherence
- Tri-lineages (adipogenic/chondrogenic/osteogenic) differentiation
- Positive Surface markers: CD73/CD90/CD105
- Negative Surface markers: CD45/CD19/CD14

Dominici M et al. Cytotherapy. 2006
MSCs can be obtained from many tissues

- Bone Marrow (Sale 1983)
- Umbilical Cord blood (Erices 2000)
- Dental pulp (Gronthos 2000)
- Synovial membrane (De Bari 2001)
- Adipose tissues (Zuk 2002)
- Placenta (In’t Anker 2004)
- Skin (Shih 2005)
- Umbilical perivascular cells (Sarugaser 2005)
- Umbilical Cord Wharton’s Jelly (Nadri 2007)
- Amniotic fluid (Nadri 2007)
- Breast milk (Patki 2010)
MSC related FDA filings is growing

More than 70 application to FDA for clinical use of MSCs

- **Source:**
  - 2007 → 100% BM
  - 2014 → 55% BM

- **Origin:**
  - 2007 → 100% allogeneic
  - 2014 → 42% allogeneic

- **Differences:**
  - FBS (from 2% to 20%)
  - O2
  - Cryopreservation/Cell banking
  - Phenotyping, Proliferation ability
  - Distribution and Survival

Trialgov.com
## MSC immunomodulatory activities

<table>
<thead>
<tr>
<th>Model</th>
<th>Source of MSCs</th>
<th>MSC Treatment</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental autoimmune encephalomyelitis</td>
<td>Syngeneic</td>
<td>1x10^6 IV, twice</td>
<td>Ameliorated disease progression</td>
</tr>
<tr>
<td>Collagen-induced arthritis</td>
<td>Allogeneic</td>
<td>5x10^6 IP</td>
<td>Delayed and reduced severity of disease</td>
</tr>
<tr>
<td>Autoimmune enteropathy</td>
<td>BM-MSC</td>
<td>3x10^5 IP</td>
<td>Ameliorated disease progression</td>
</tr>
<tr>
<td>T1D</td>
<td>Allogeneic</td>
<td>5x10^5 IV once a week for 4 wk</td>
<td>Delayed onset of diabetes</td>
</tr>
<tr>
<td>Skin transplant</td>
<td>Allogeneic</td>
<td>20x10^6/kg, IV</td>
<td>Prolonged survival of skin graft</td>
</tr>
<tr>
<td>Heart transplant</td>
<td>Semiallogeneic</td>
<td>0.5x10^6 intraportal</td>
<td>Protected heart allograft</td>
</tr>
<tr>
<td>Islet transplant</td>
<td>Syngeneic</td>
<td>4x10^6 under kidney capsule</td>
<td>Prevented rejection of islet graft</td>
</tr>
</tbody>
</table>

Ding, Transplantation, 2010
MSCs prevent UAE increase

Control (n=30)
P=0.001

MSC-treated (n=12)
(5x10^5 MSC injected twice a week for 4 weeks)

D’Addio/Fiorina
Acta Diabetologica 2014
Targeting sTNFR1
Serum levels of TNFR1 are associated with increased risk of ESRD in T1D/T2D

HR = 7.05 (2.2-22.3)  
*p* = 0.0018

HR = 7.11 (2.1-23.7)  
*p* = 0.0004

Niewczas et al, JASN 2012
Murine models

**TNFR1 KO**: Mice homozygous for the Tnfrsf1^{atm1lmx} targeted mutation

**TNFR1 Tg**: Mice bearing a transgene coding for a chimeric fusion TNFR1-FcIgG3 protein and placed under the control of the AAT promoter responsible for its liver expression.
Genetic deletion of sTNFR1 prevent UAE increase
Targeting B7-1
B7-1 an immune related molecule

- B7-1 is a co-stimulatory molecule on antigen presenting cells
- B7-1 is the ligand for CD28 and CTLA4 (expressed on T-cells)
- B7-1 can be targeted with CTLA4-Ig (available for clinical use)

Sayegh MH et al. NEJM 1998
Reiser J et al. JCI 2004
Doria A/Fiorina P et al. Semin Nephrol 2012
CTLA4-Ig reverted proteinuria in B7-1 positive proteinuric FSGS

CC Yu et al. NEJM 2014
B7-1 is upregulated in podocytes in high glucose *in vitro*

Fiorina P et al. JASN 2014
CTL4-Ang Ig protects podocytes from high glucose-induced cytoskeleton injuries in vitro

Fiorina P et al. JASN 2014
B7-1 overexpression/knock down impact on podocyte morphology and survival

Fiorina P et al. JASN 2014
CTLA4-Ig prevented the rise in urinary albumin excretion (UAE) *in vivo*

**db/db**

**STZ-C57BL/6**

*Induction: 500μg D0*

*Maintenance: 250μg D2, D4, D6, D8, D10 and then twice a week*

Fiorina P et al.  
*JASN 2014*
Targeting eATP/P2X7R signaling
Adenosine 5'-triphosphate (ATP) is released by necrotic cells or secreted by activated cells into the extracellular space.

P2X7R is a homotrimeric cation-permeable ligand-gated ion-channel that senses ATP and is expressed by leukocytes and allows the entrance of calcium into the cells.

Vergani A/Fiorina P. Am J Transplant 2014
**P2X receptors**

P2X receptors (P2X1-P2X7 or P2XsR) are a family of cation-permeable ligand gated ion channels that open in response to the binding of extracellular adenosine 5'-triphosphate (ATP)
Targeting ATP/P2X7R axis prevents lung fibrosis

Figure 1

Vehicle oATP

15 days
30 days
60 days

Vehicle oATP

15 days
30 days
60 days

Vehicle

oATP

15 days
30 days
60 days

Kaifeng/Fiorina et al. Am J Respir Cell Mol Biol 2014
Preclinical targeting of eATP signaling in Diabetic Nephropathy

<table>
<thead>
<tr>
<th>P2X&lt;sub&gt;7&lt;/sub&gt;</th>
<th>Brilliant Blue G</th>
<th>Causes a reduction in arterial blood pressure and a decrease in renal vascular resistance in the Fischer rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
<td>A-438079</td>
<td>Diminishes renal fibroblast death</td>
</tr>
<tr>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;</td>
<td>P2X&lt;sub&gt;7&lt;/sub&gt;R silencing</td>
<td>In murine podocytes attenuates upregulated expression of NLRP3, pro-caspase 1 and release of IL-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diminishes renal fibroblast death</td>
</tr>
</tbody>
</table>
P2X7R expression in podocytes is increased by serum from individuals with T2D and DKD

Bassi R/Forina P In preparation
P2X7R expression in podocytes is increased by high glucose challenge

Bassi R/Forina P In preparation
Building a novel biologic sP2X7R-Ig
Building a novel biologic
sP2X7R-Ig
Summary and Conclusions

• Despite improvements in glycemic and blood pressure control, the incidence of ESRD in diabetes is not declining.

• Epidemiology can help us develop new therapies that are effective early in the natural history of GFR loss when renal damage is most likely to be reversible and interventions can yield the longest delay of ESRD

• Novel drugs will be tested soon
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American Heart Association (AHA)

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