Novel experimental approaches for diabetic kidney disease

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OSPEDALE SAN RAFFAELE

Boston Children's Hospital

Disclosure

I have financial relationship with:

- Dompe
- Fate Therapeutics
- Enthera

And patents related to some of the science shown

Nuovi approcci 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
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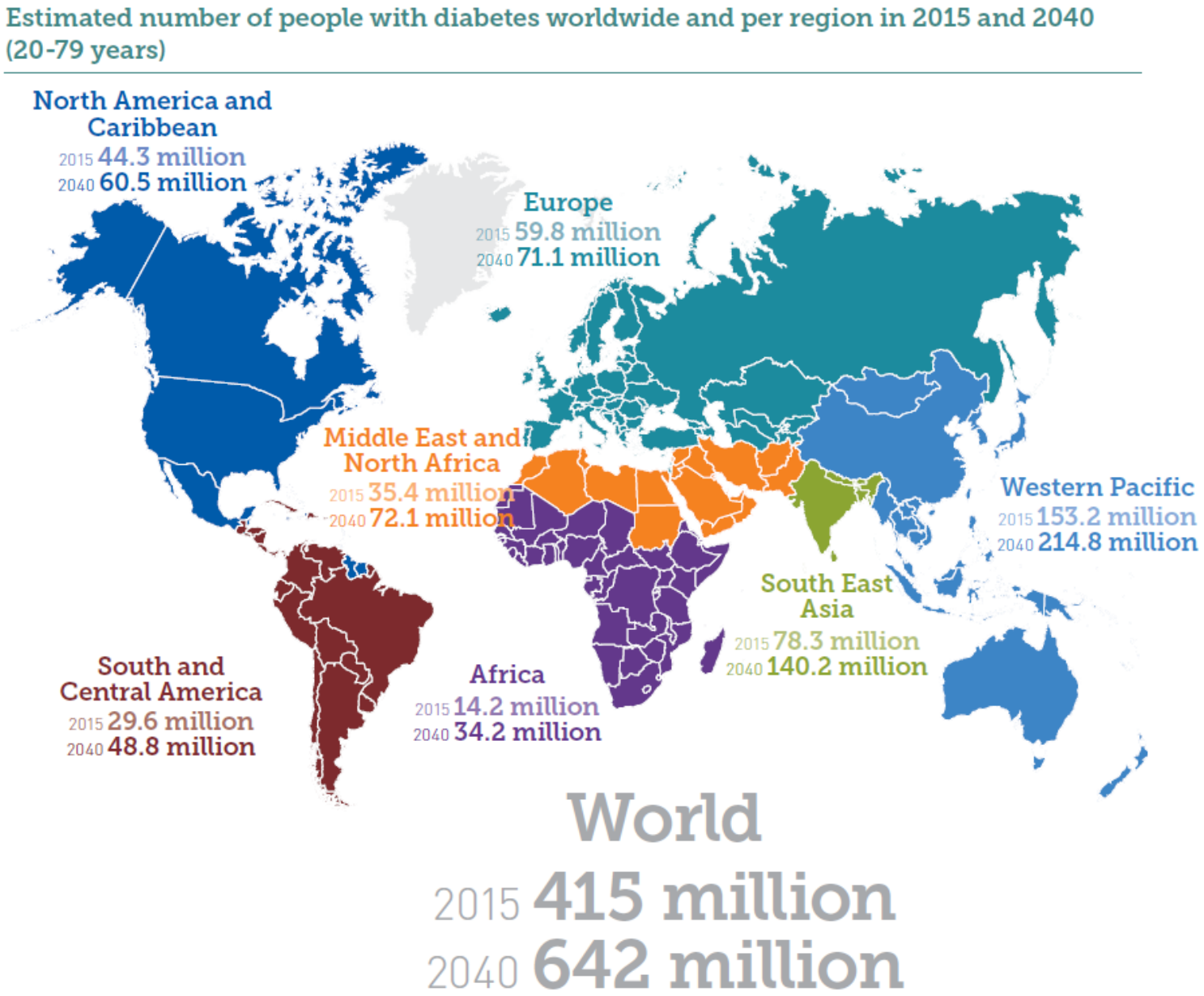
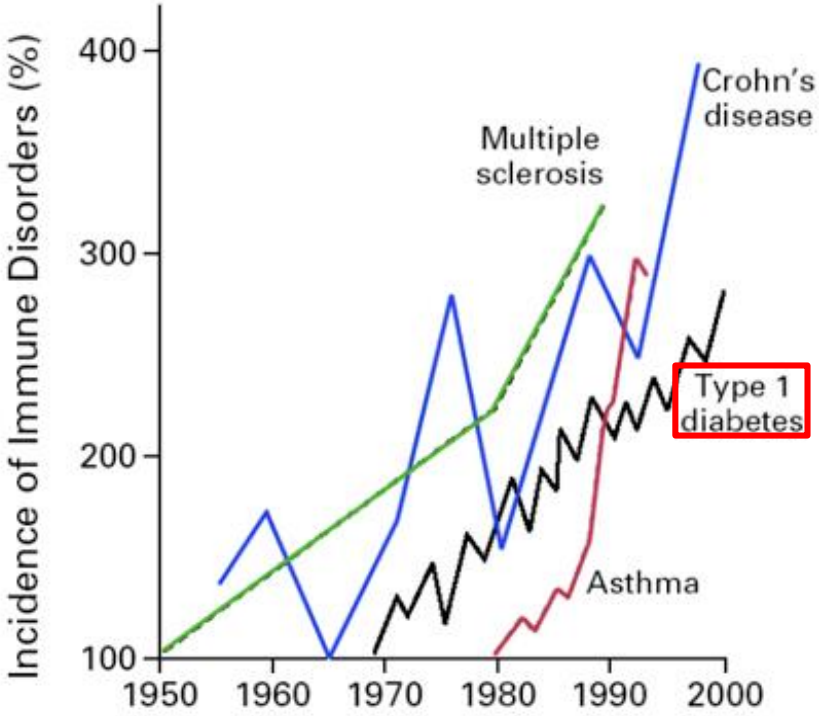
Diabetes: The New Millennium Pandemic



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

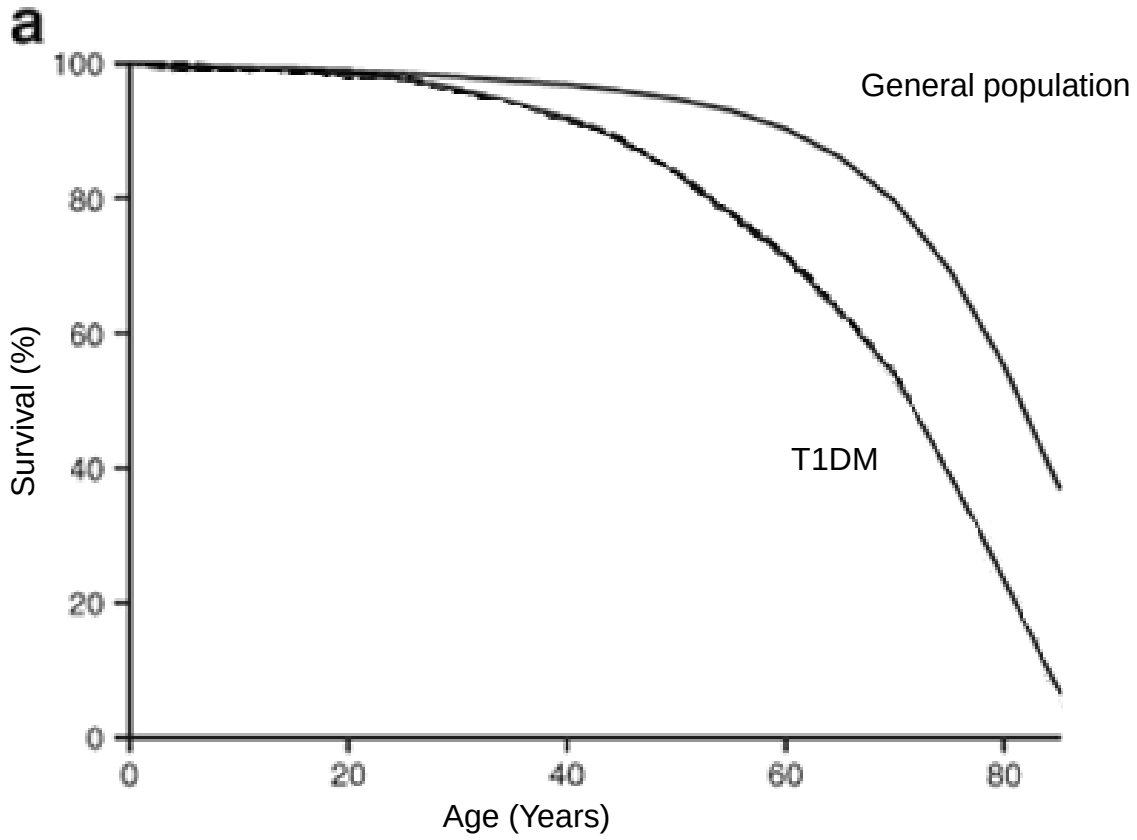
100% of Nigeria/Cile/Egitto GDP

Managing Diabetes

- Unfortunately only few patients reaches glycemic targets (HbA1c<7%)

Elisa Giani, Lori Laffel; Diabetes Technology & Therapeutics 2016
C S. Bloss et al; 2016 PeerJ

I pazienti con diabete perdono 10-13 anni di vita rispetto alla popolazione generale



L. Huo et al, Diabetologia 2016

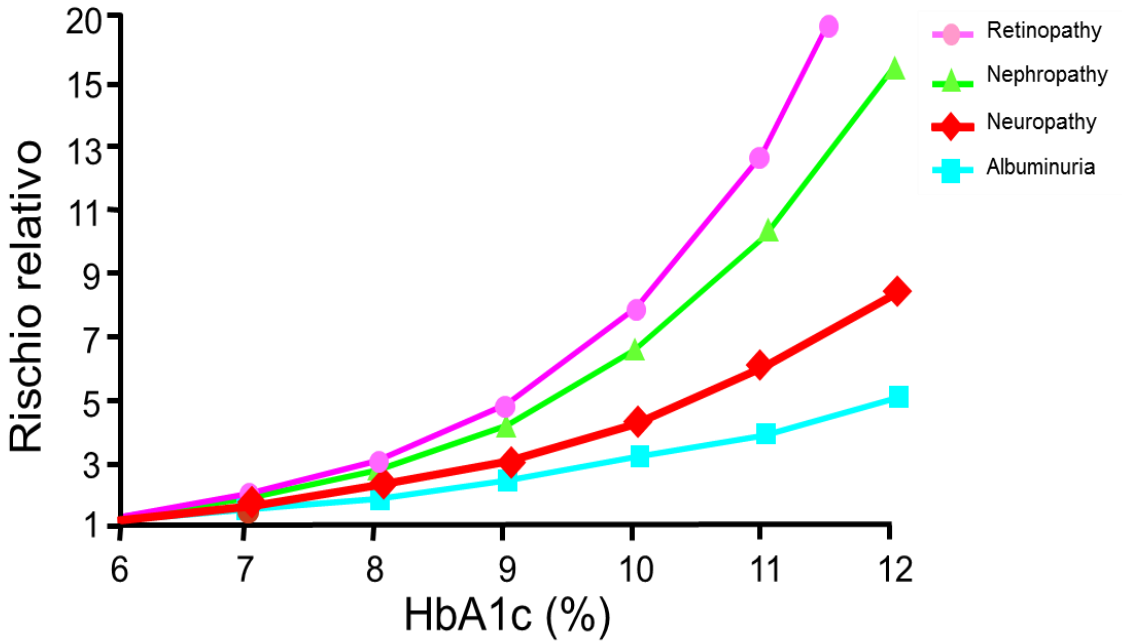
1998-2011 death rate in 34,000 T1D individuals

	Hazard ratios	
Mean HbA1c	**Death from any cause**	**Death from cardiovascular disease**
≤ 6.9%	2.36	2.92
7.0%-7.8%	2.38	3.39
7.9%-8.7%	3.11	4.44
8.8%-9.6%	3.65	5.35
≥ 9.7%	8.51	10.46

Mameli C/Fiorina P et al. WJD 2015
Lind M et al NEJM 2014

Diabetes Complications

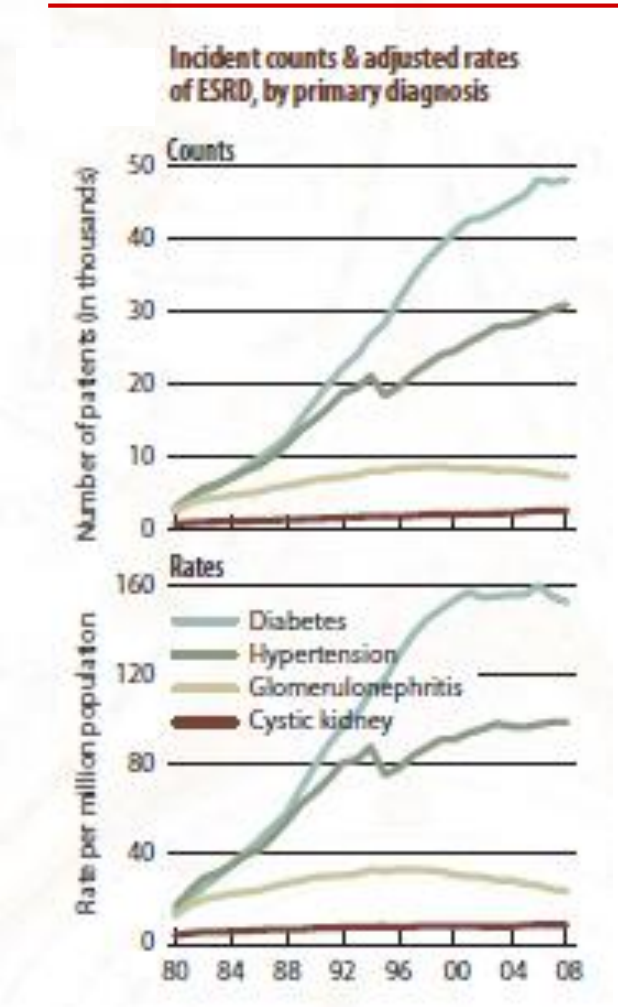
- Good glucose control prevents and delays complications



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



USRDS annual report, 2010

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.

Jones et al, Kidney International, 76, 2005
Rosolowsky, CJASN, 22, 2011
Finndiane Study, CJASN, 22, 2011

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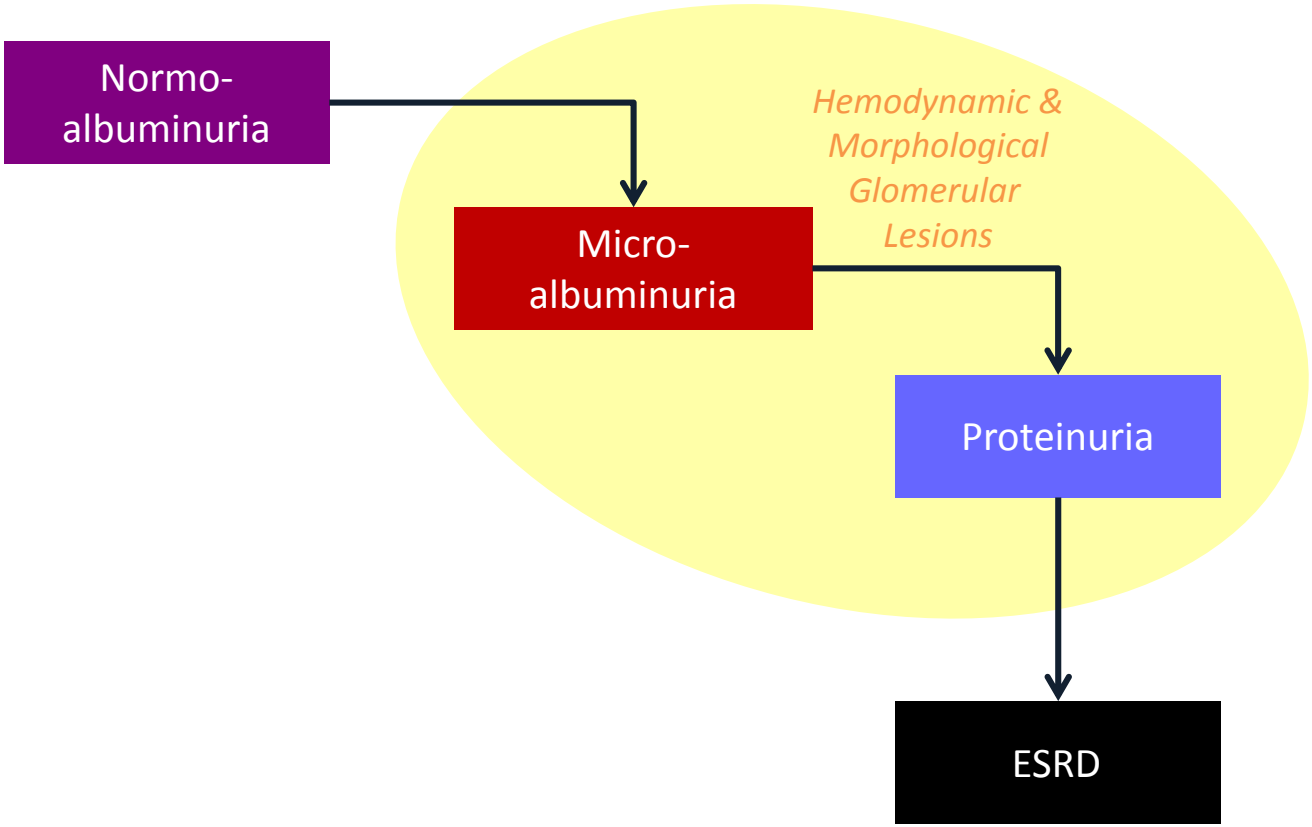
The 1990's Model of Nephropathy in T1D focused on urinary albumin abnormalities



The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JUNE 5, 2003 VOL. 348 NO. 23

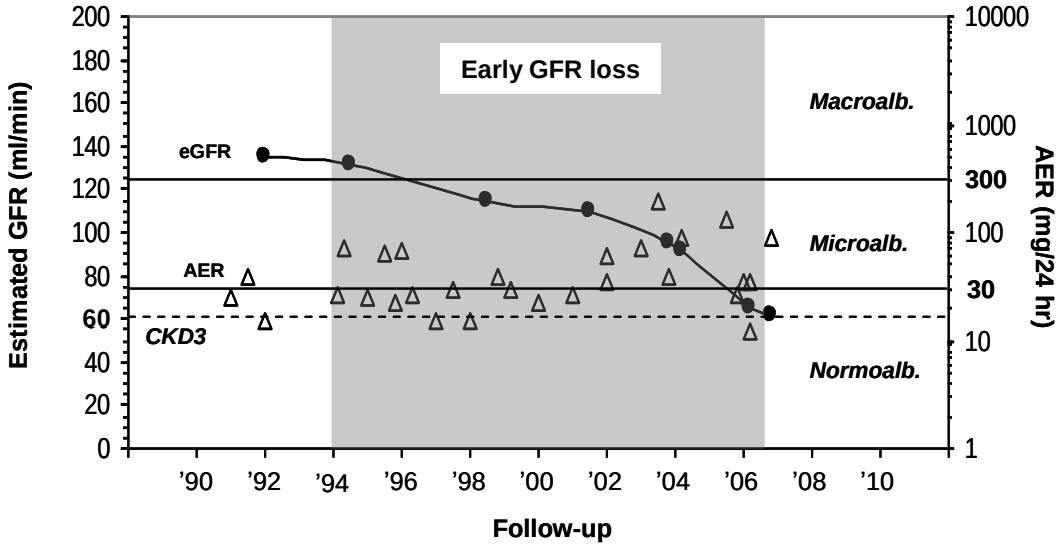
Regression of Microalbuminuria in Type 1 Diabetes

Bruce A. Perkins, M.D., M.P.H., Linda H. Ficociello, M.Sc., Kristen H. Silva, B.A., Dianne M. Finkelstein, Ph.D., James H. Warram, M.D., Sc.D., and Andrzej S. Krolewski, M.D., Ph.D.

Table 1. Nephropathy Status According to Two-Year Study Period.

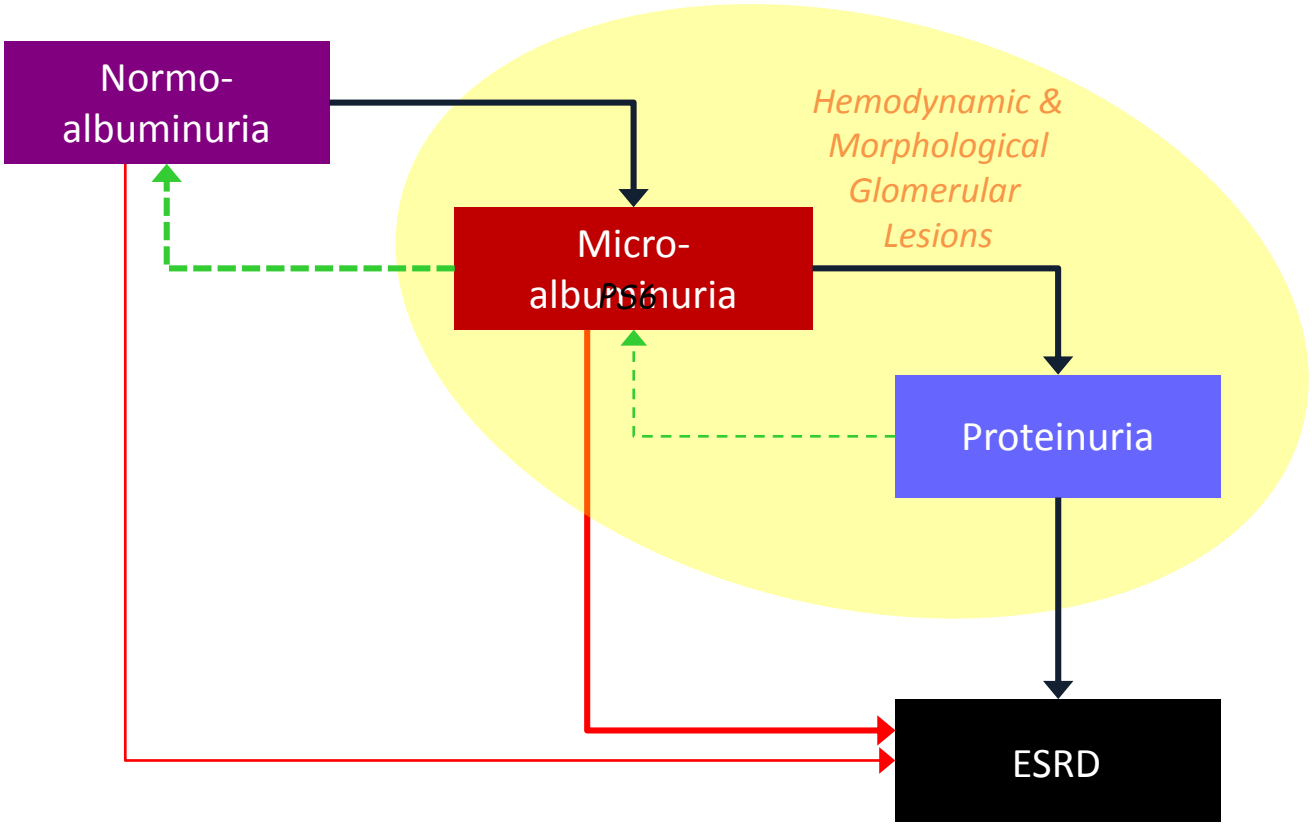
Status*	Initial Evaluation Period†	1st Follow-up Period	2nd Follow-up Period	3rd Follow-up Period
		no. (%)		
Proteinuria	—	24 (7)	37 (13)	33 (15)
Microalbuminuria	386 (100)	191 (54)	141 (49)	99 (45)
Normal albumin excretion	—	136 (39)	110 (38)	88 (40)
Total	386 (100)	351 (100)‡	288 (100)‡	220 (100)‡

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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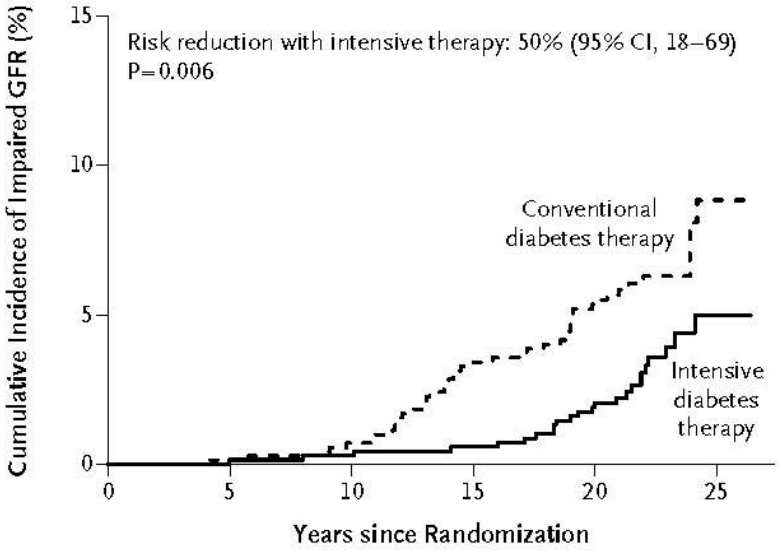
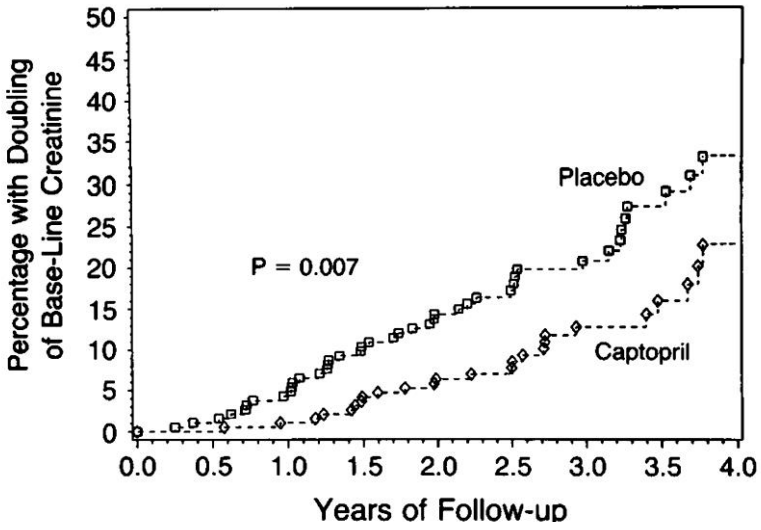
Current strategies to prevent kidney function loss on in diabetes

Intensive glycemic control



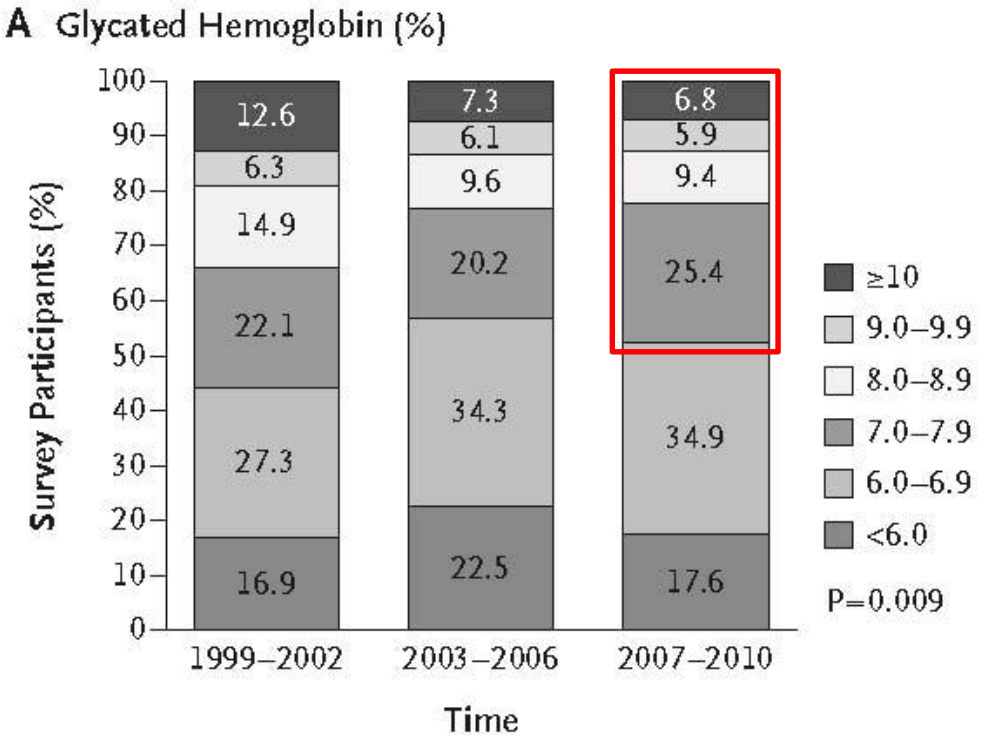
DCCT-EDIC, NEJM 2011

RAAS Inhibition



Lewis et al, NEJM 1993

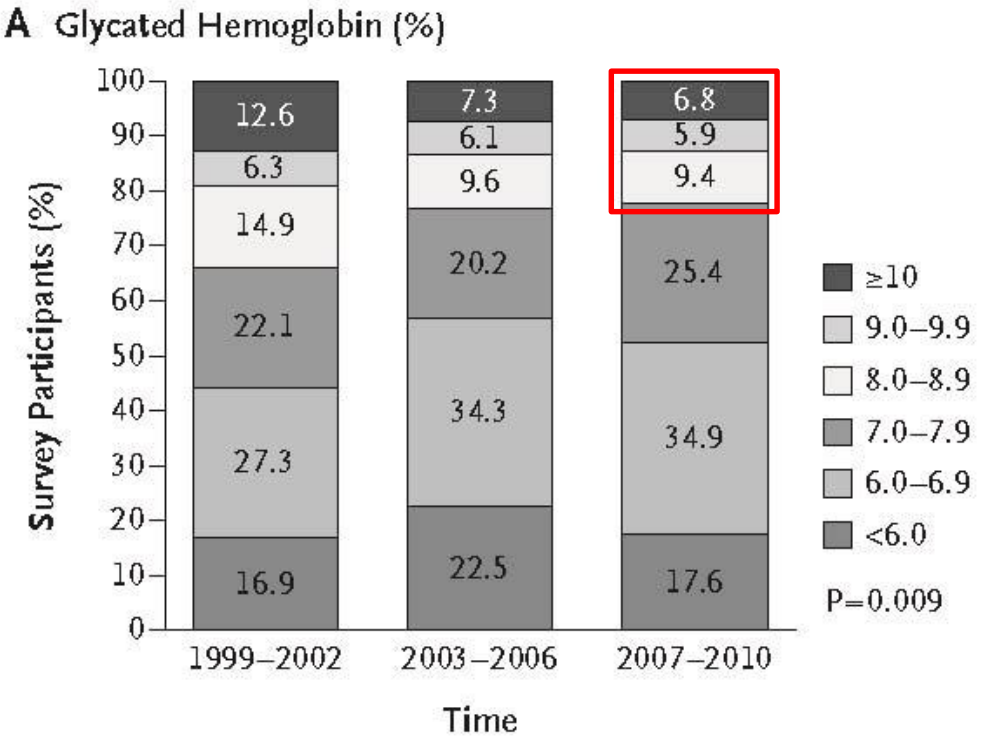
Persisting poor glycemic control in diabetes



48% have HbA1c ≥7%

Ali et al, *NEJM,* 2013

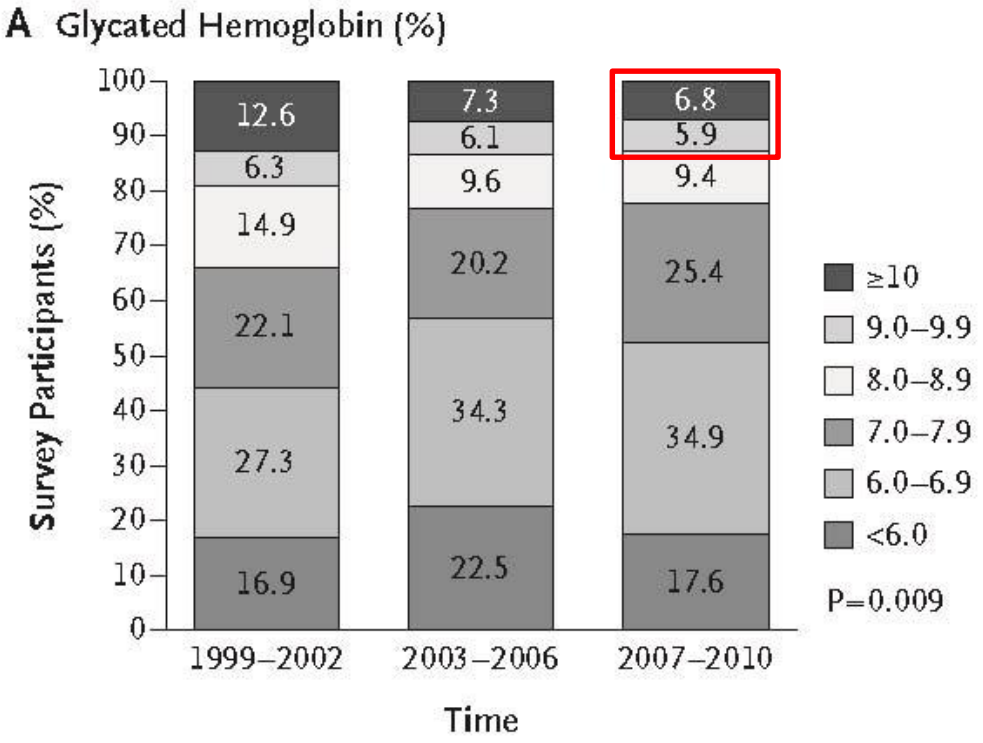
Persisting poor glycemic control in diabetes



22% have HbA1c ≥8%

Ali et al, *NEJM,* 2013

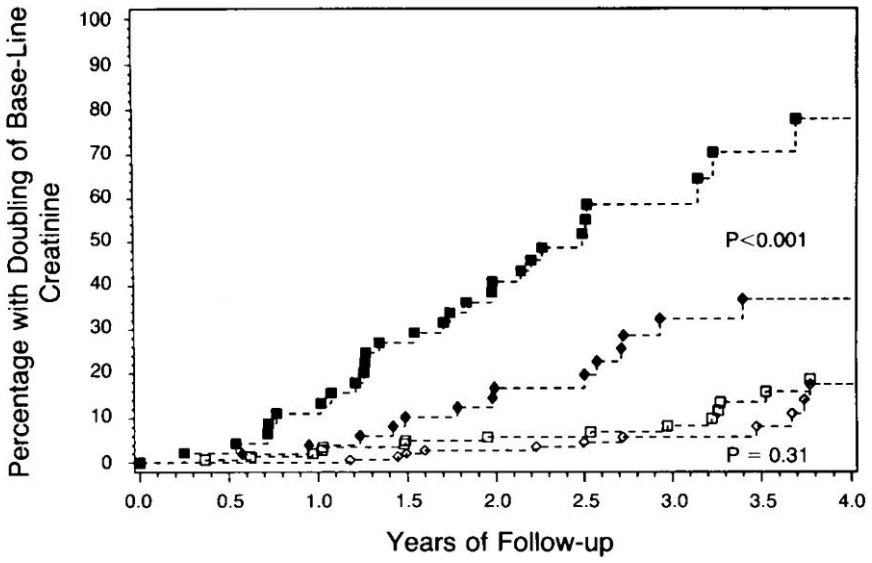
Persisting poor glycemic control in diabetes



13% have HbA1c ≥9%

Ali et al, *NEJM,* 2013

Limits of RAAS inhibitors in preventing kidney function loss in diabetes



Creatinine ⩾1.5 mg/dl									
■ Placebo	49	44	39	32	25	15	8	4	1
♦ Captopril	53	50	46	42	37	28	17	13	3
Creatinine <1.5 mg/dl									
□ Placebo	153	140	134	129	117	84	67	41	21
◇ Captopril	154	149	144	138	130	92	65	37	21

GFR ≤50-60 ml/min

GFR >50-60 ml/min

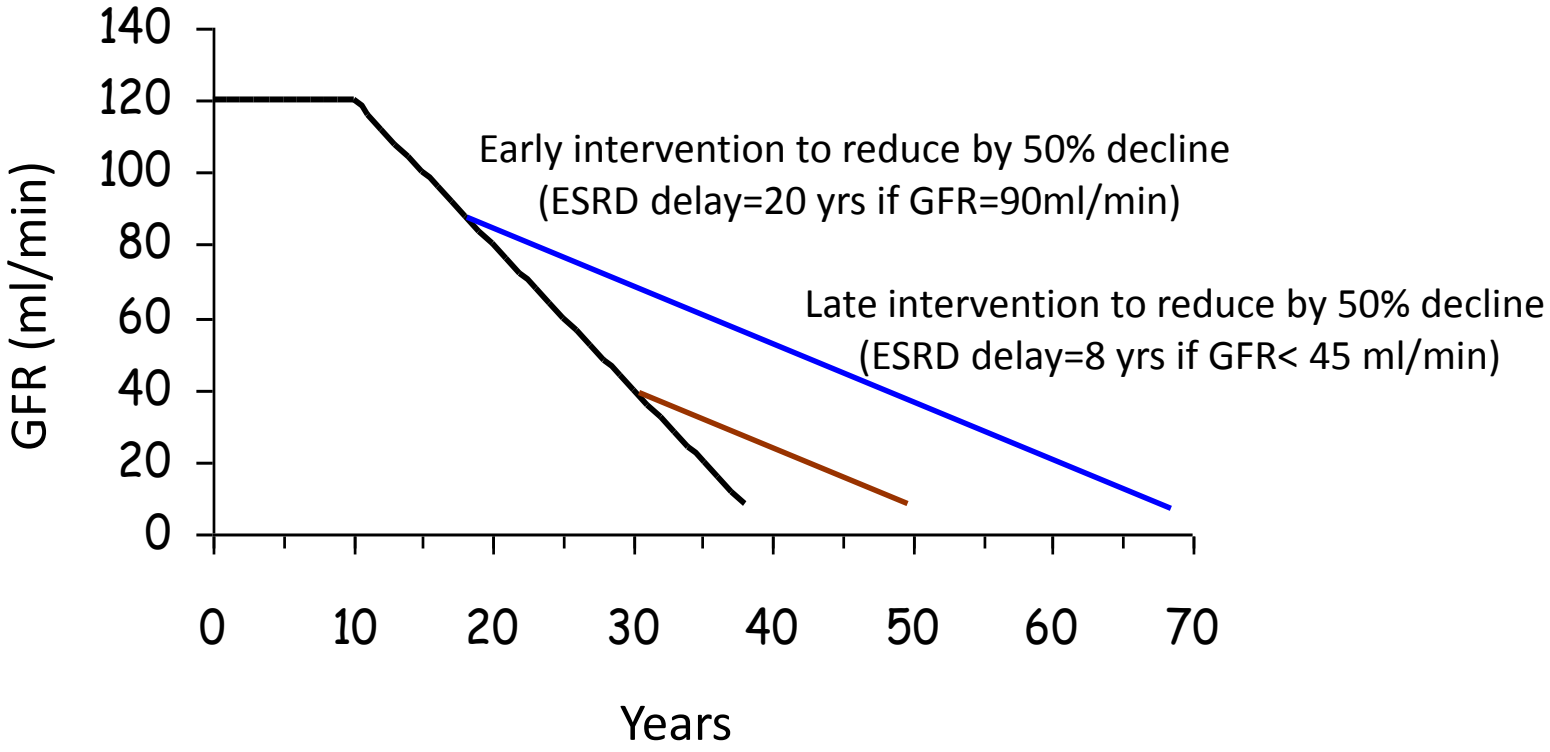
From Lewis et al, *NEJM* 1993

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



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

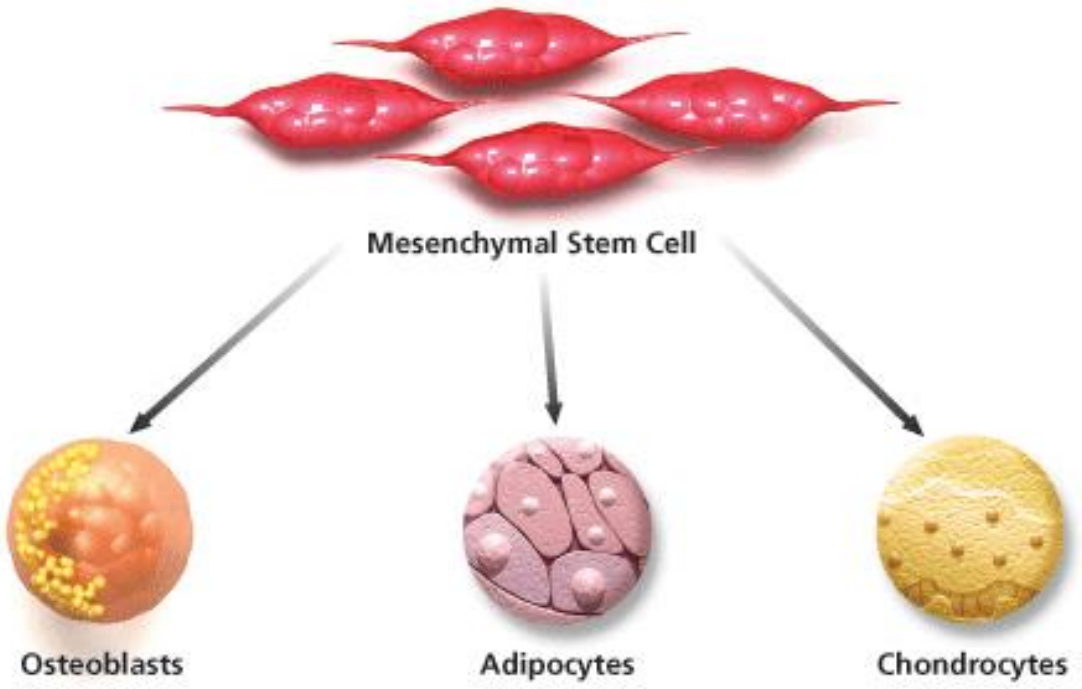
- Drug targeting immunological mechanisms (immune related mechanism may be involved)
- (i) Since T-cell depletion affects the development and the natural history of renal damage in animal models, anti-CD3 mAb or ATG may be suggested for worst cases

Doria/Fiorina P et al. Sem Nephrol 2012

Mesenchymal stem cells

MSCs definition

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



Abdi R/Fiorina P et al. Diabetes 2008

MSC characteristics

Minimum criteria are:

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

Dominici M et al. Cytotherapy. 2006
Abdi R/Fiorina P et al. Diabetes 2008

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

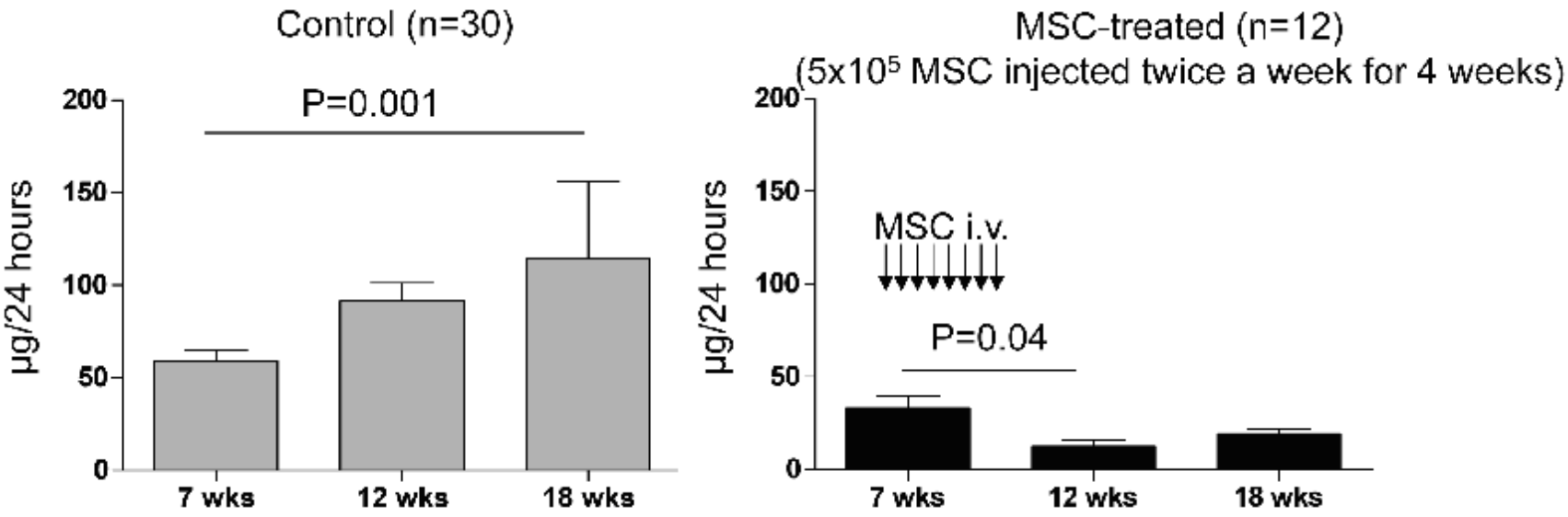
Mendicino M et al. Cell Stem Cells. 2014

MSC immunomodulatory activities

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

Ding, Transplantation, 2010

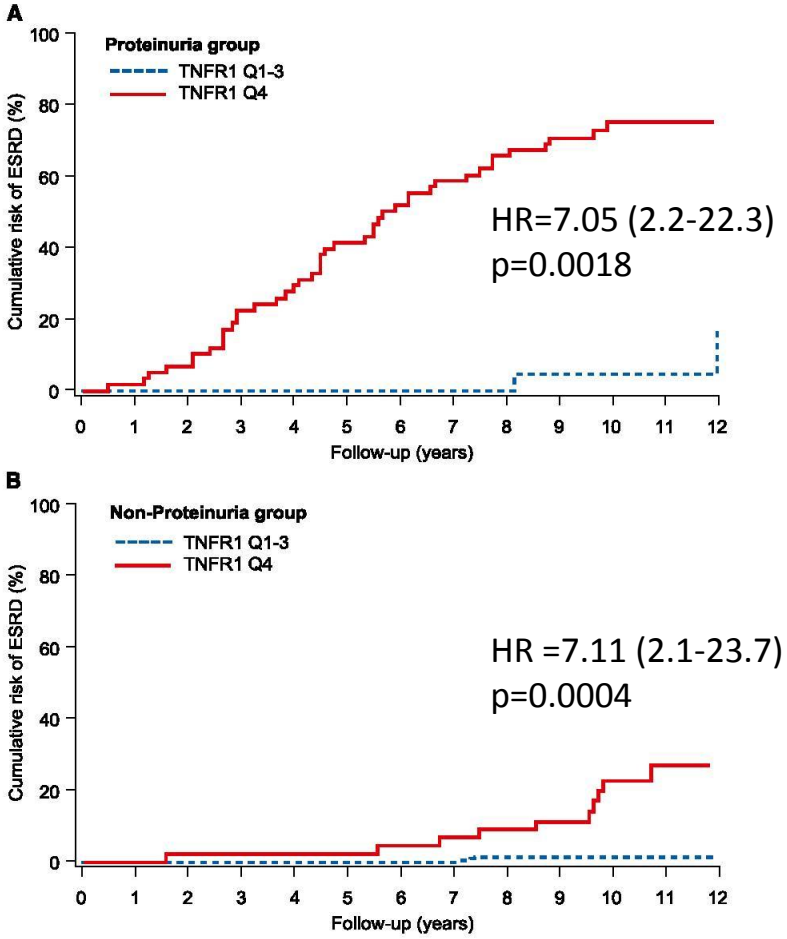
MSCs prevent UAE increase



D'Addio/Fiorina
Acta Diabetologica 2014

Targeting sTNFR1

Serum levels of TNFR1 are associated with increased risk of ESRD in T1D/T2D



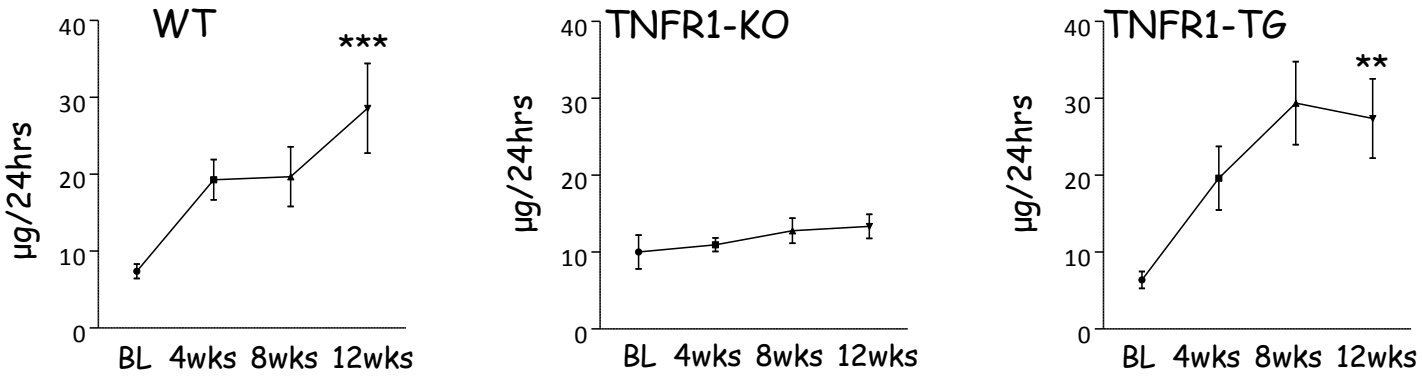
Niewczas et al, *JASN* 2012

Murine models

TNFR1 KO: Mice homozygous for the Tnfrsf1^{atm1Imx} 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

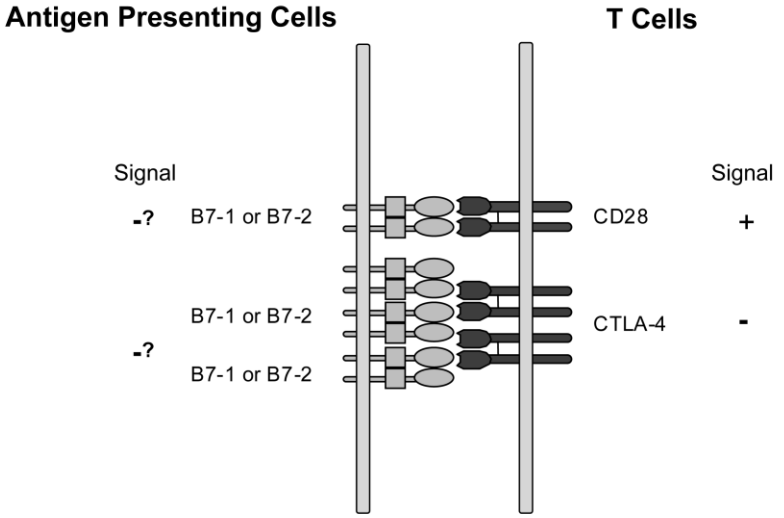


Bassi R/Niewczas M/Fiorina P In preparation

Targeting B7-1

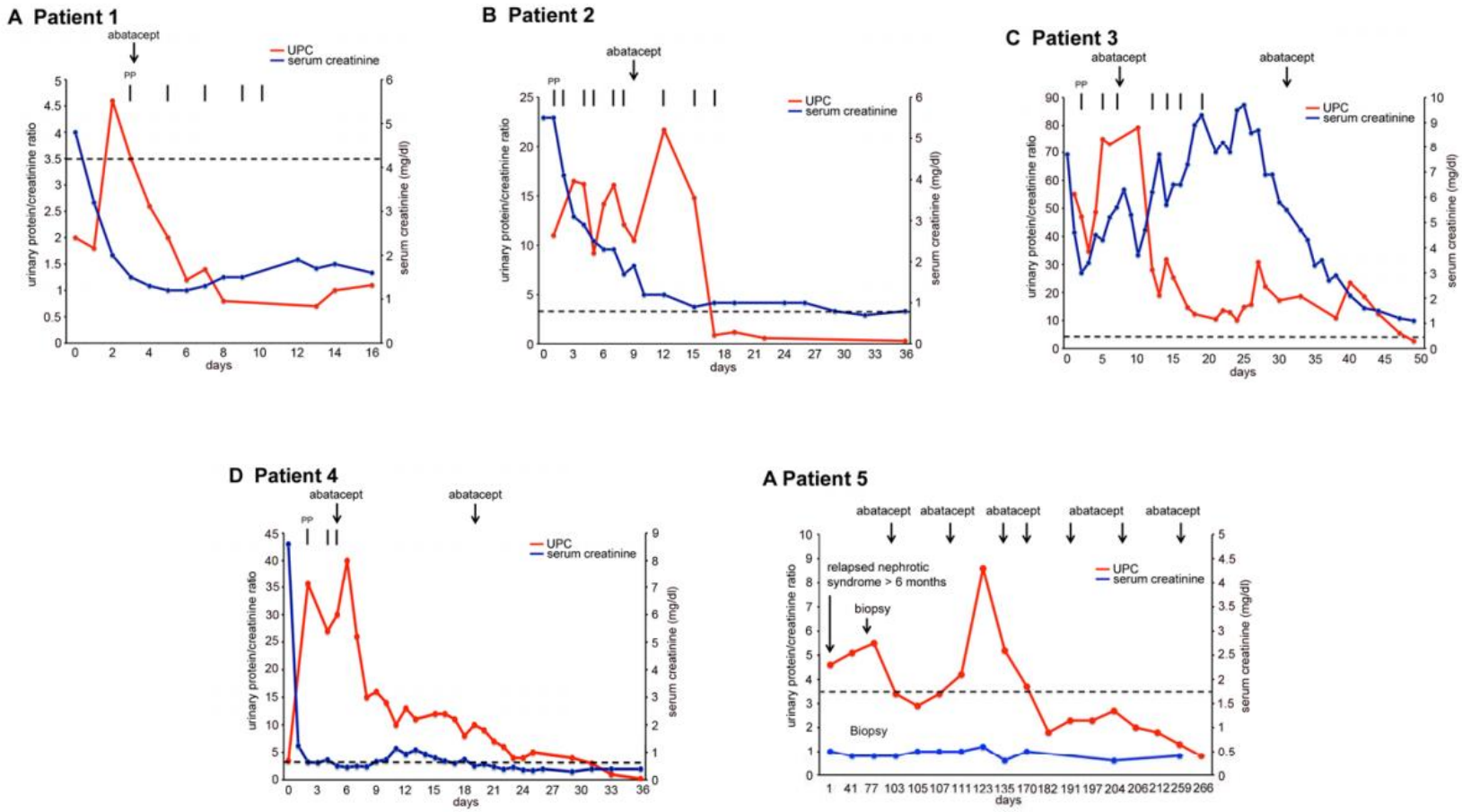
B7-1 an immune related molecule

- 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)



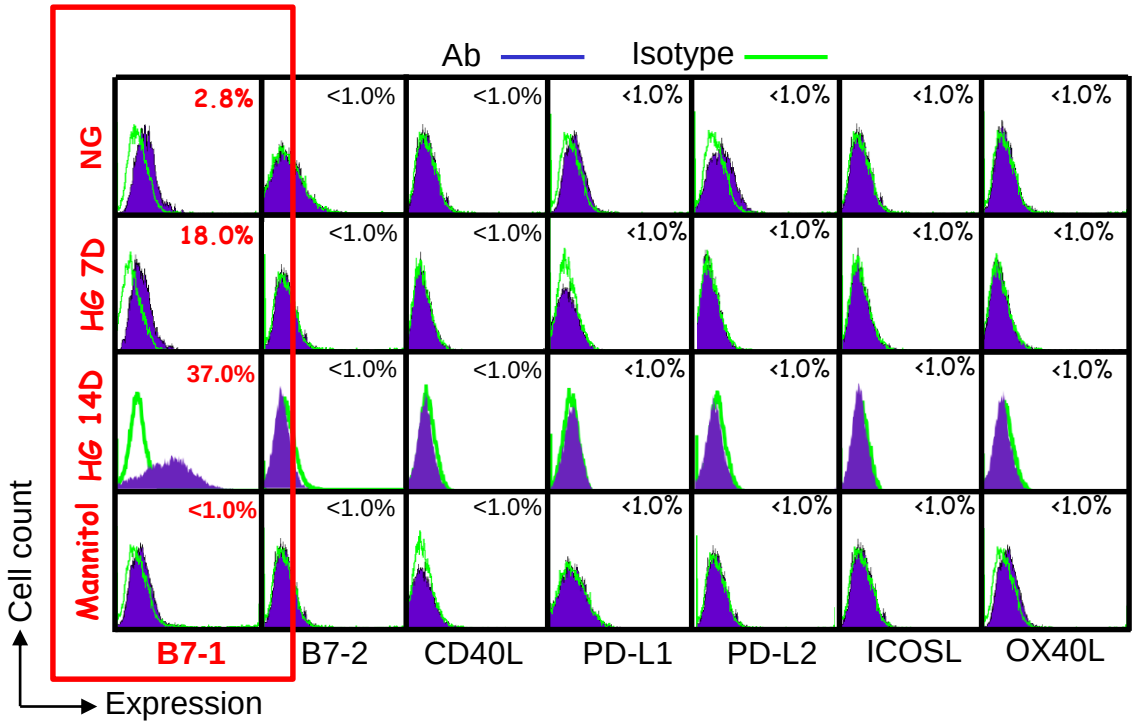
Sayegh MH et al. NEJM 1998
Reiser J et al. JCI 2004
Doria A/Fiorina P et al. Semin Nephrol 2012

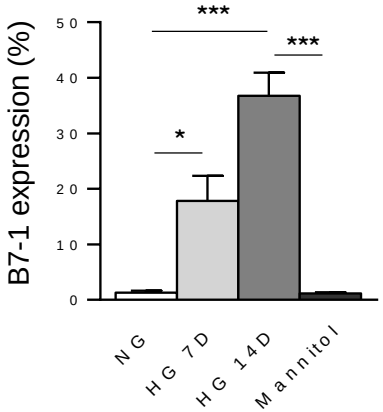
CTLA4-Ig reverted proteinuria in B7-1 positive proteinuric FSGS



CC Yu et al. NEJM 2014

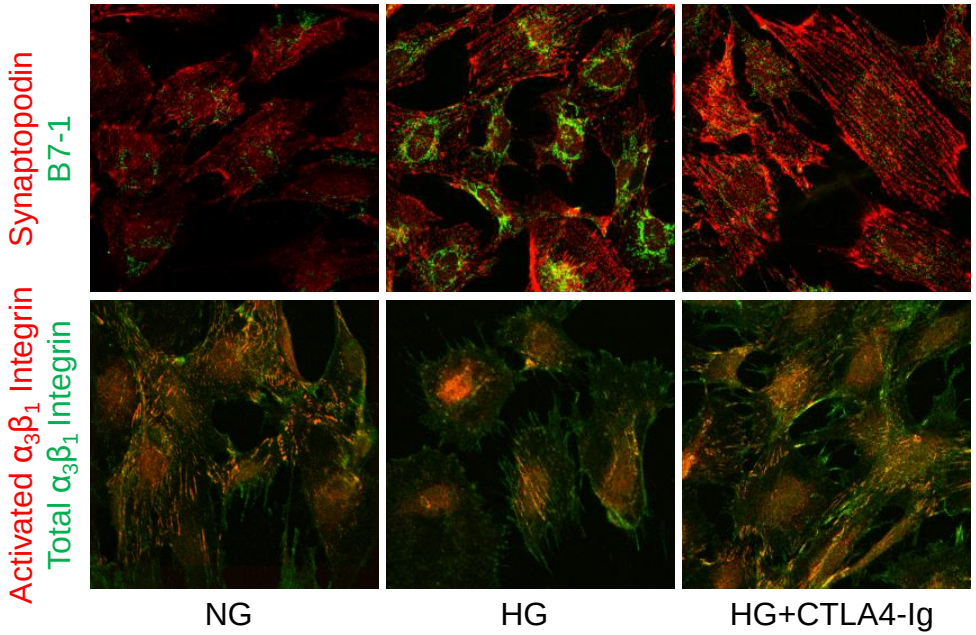
B7-1 is upregulated in podocytes in high glucose *in vitro*

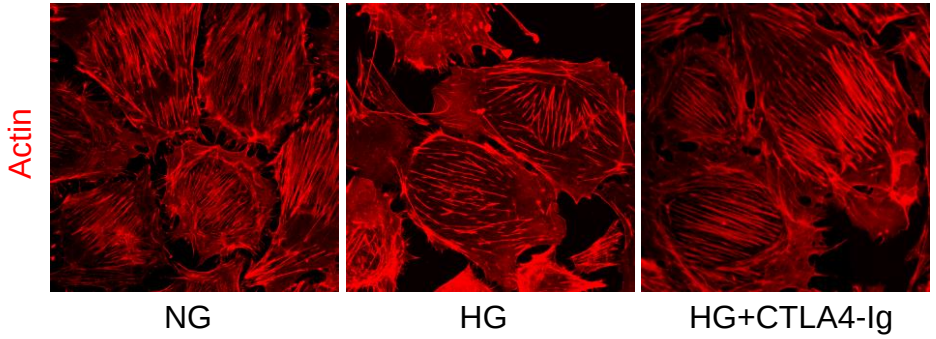


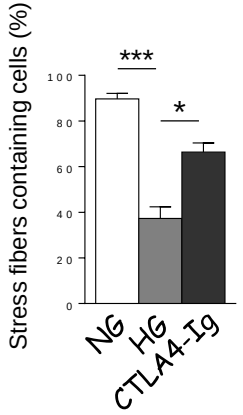


Fiorina P et al. JASN *2014*

CTLA4-Ig protects podocytes from high glucose-induced cytoskeleton injuries *in vitro*

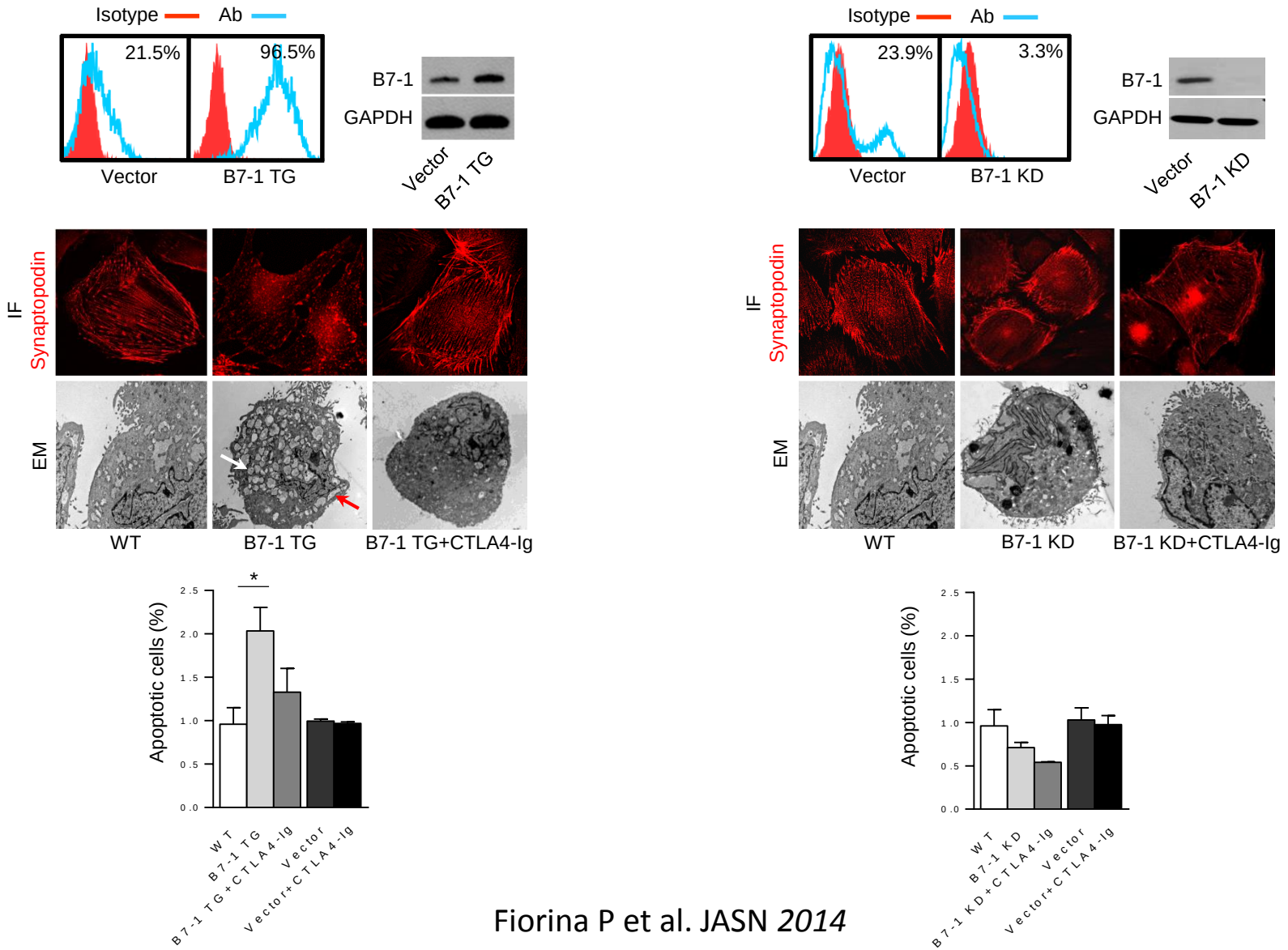






Fiorina P et al.
JASN *2014*

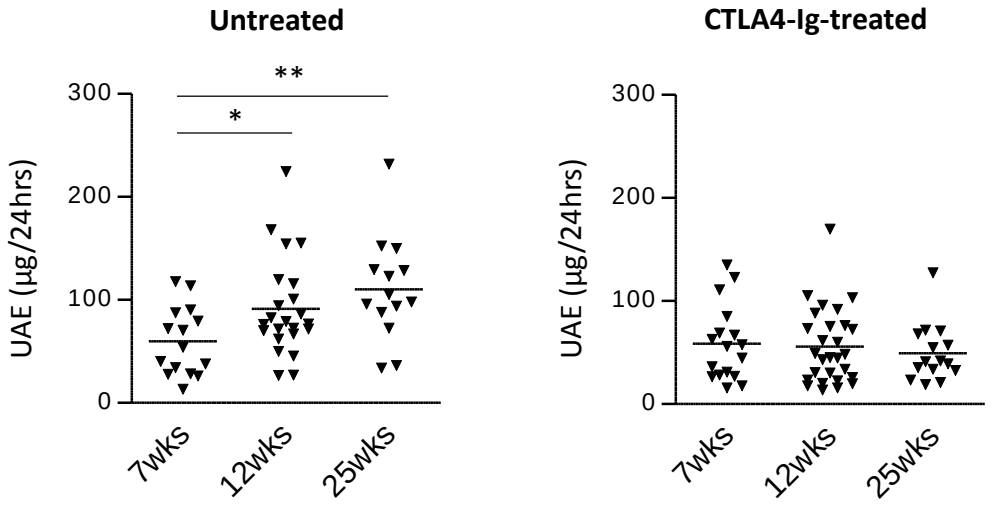
B7-1 overexpression/knock down impact on podocyte morphology and survival



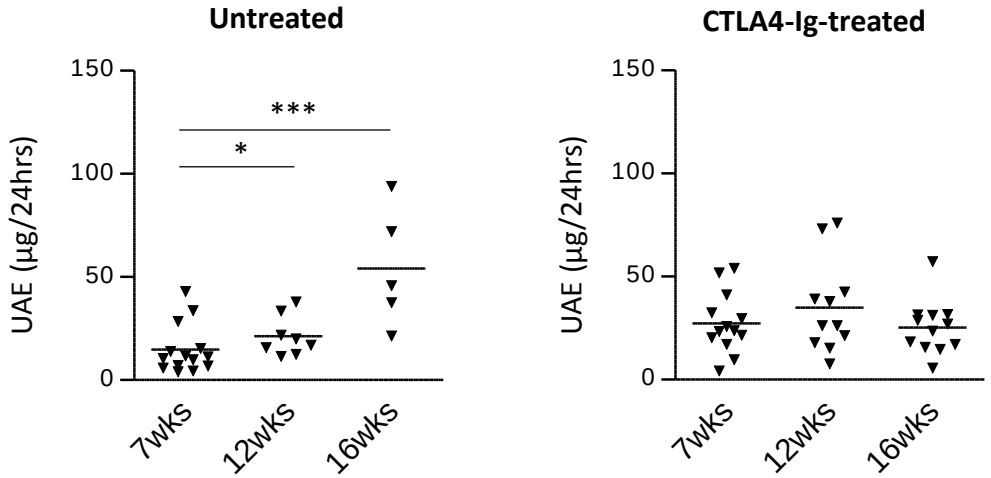
Fiorina P et al. JASN *2014*

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

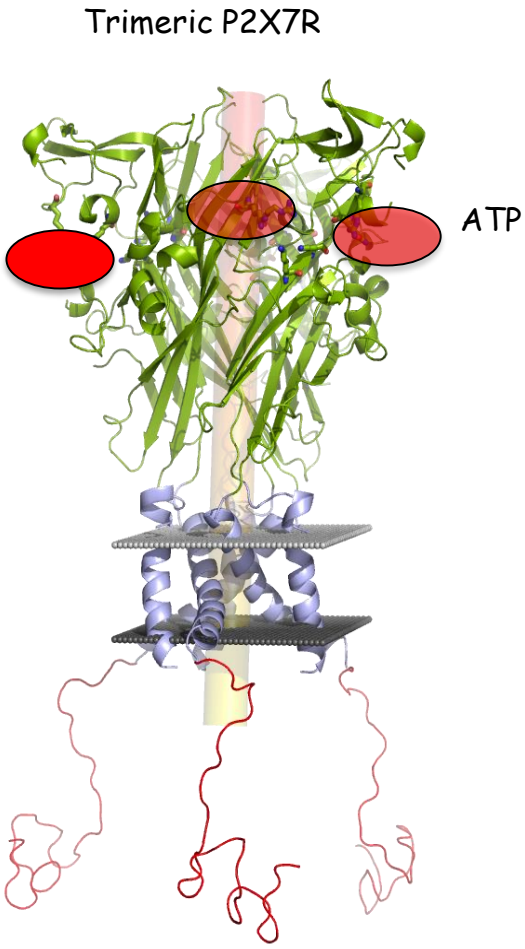
Fiorina P et al.
JASN *2014*

Targeting eATP/P2X7R signaling

ATP

Adenosine 5'-triphosphate (ATP) is released by necrotic cells or secreted by activated cells into the extracellular space.

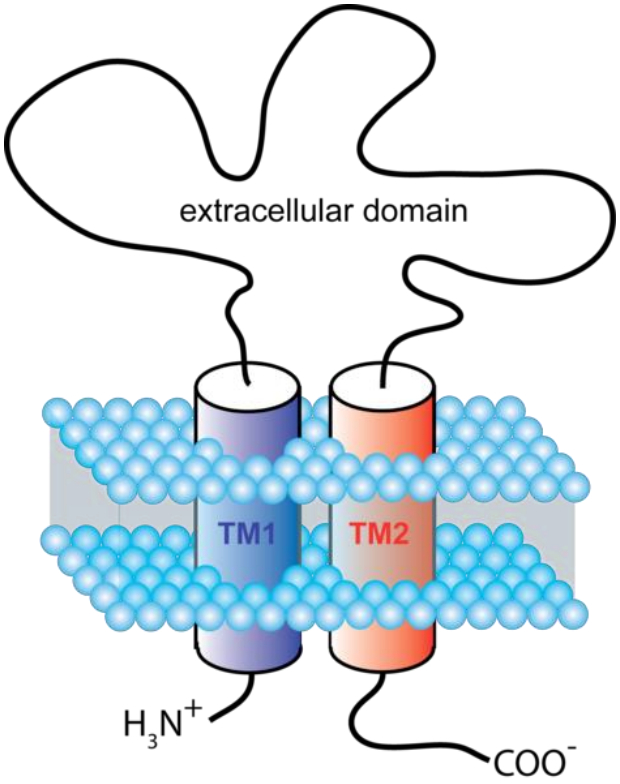
P2X7R is a homotrimeric cation-permeable ligand-gated ion-channel that senses ATP and is expressed by leukocytes and allows the entrance of calcium into the cells



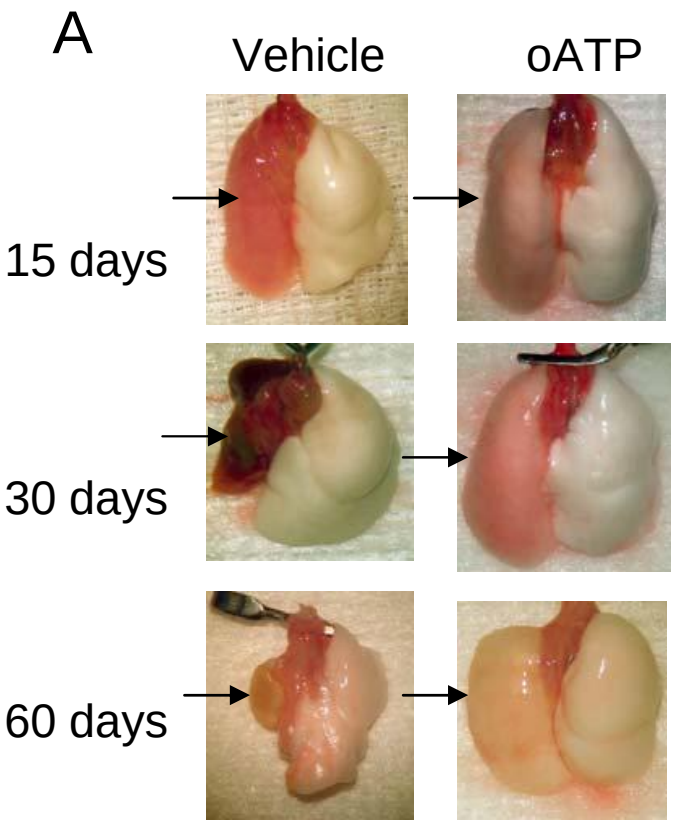
Vergani A/Fiorina P. Am J Transplant 2014

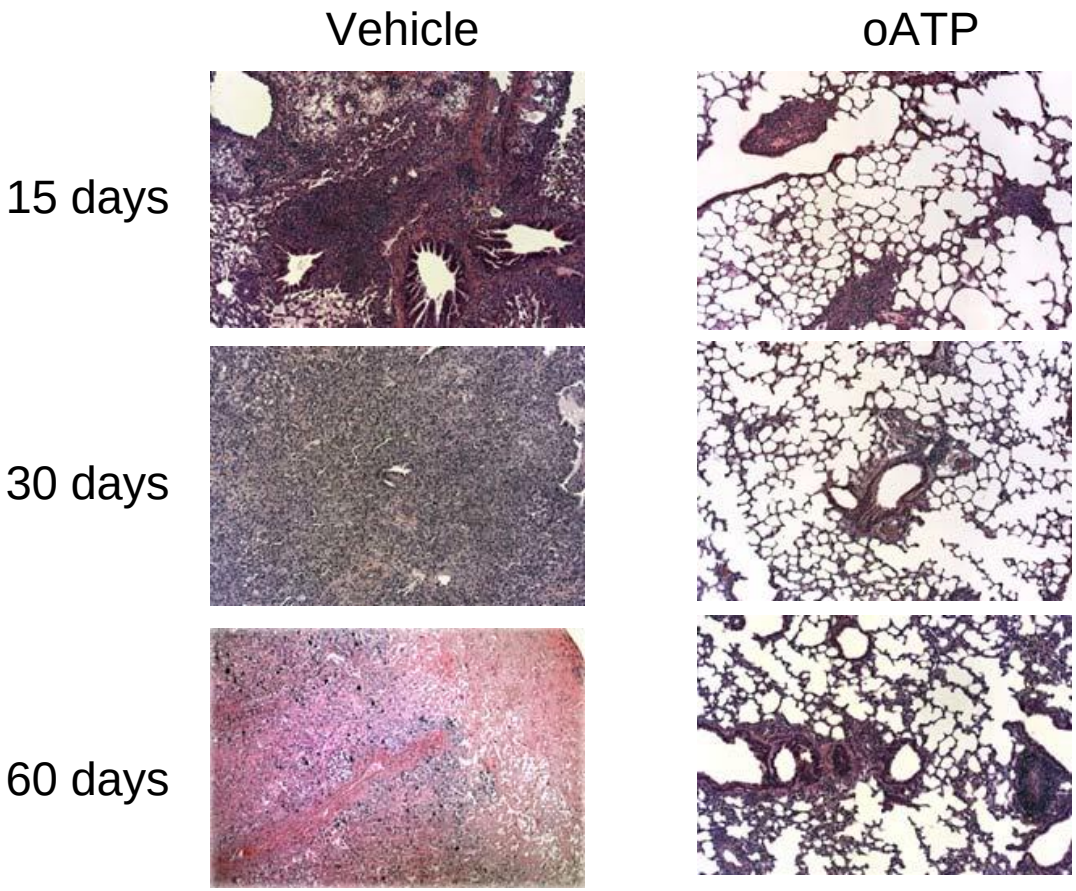
P2X receptors

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





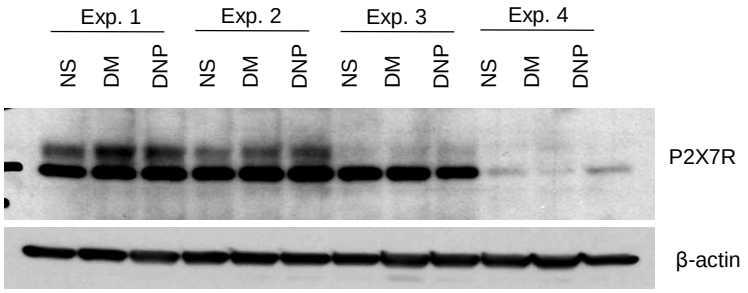
Kaifeng/Fiorina et al. Am J Respir Cell Mol Biol 2014

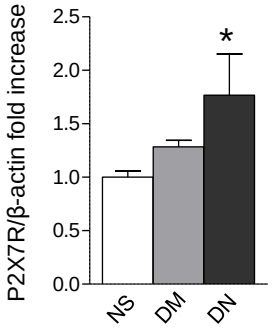
Preclinical targeting of eATP signaling in Diabetic Nephropathy

$P2X_7$	Brillant Blue G	Causes a reduction in arterial blood pressure and a decrease in renal vascular resistance in the Fischer rat
$P2X_7$	A-438079	Diminishes renal fibroblast death
$P2X_7$	$P2X_7R$ silencing	In murine podocytes attenuates upregulated expression of NLRP3, pro-caspase 1 and release of IL-18 Diminishes renal fibroblast death

Solini A/Fiorina P. JASN 2015

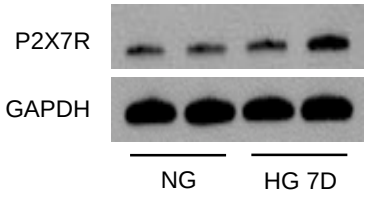
P2X7R expression in podocytes is increased by serum from individuals with T2D and DKD

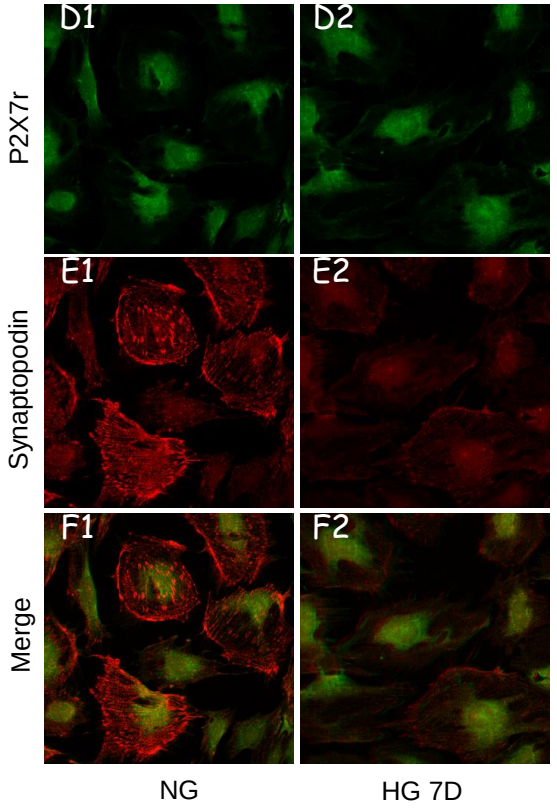




Bassi R/Forina P In preparation

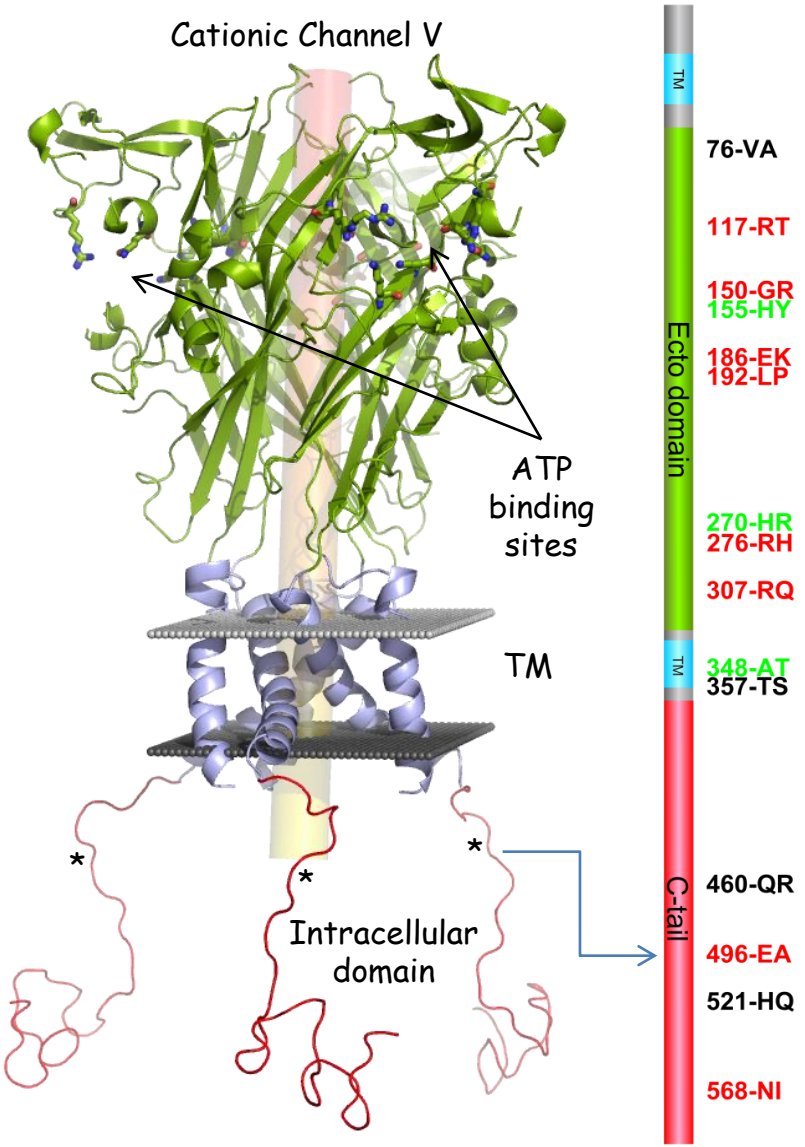
P2X7R expression in podocytes is increased by high glucose challenge



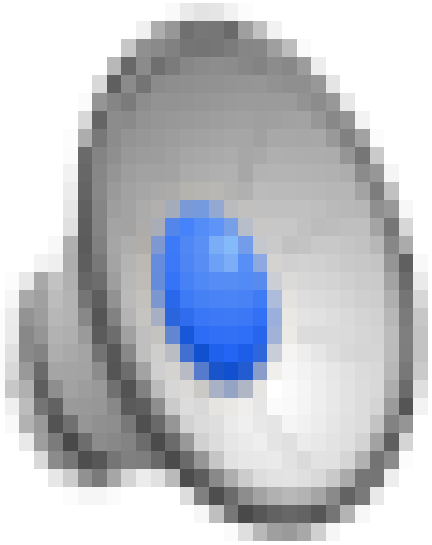


Bassi R/Forina P In preparation

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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