Biomarkers for personalized treatment of diabetic kidney diseases (DKD).
An active field of research, but still far away from clinical practice.

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Ideal urinary and plasma biomarkers for DKD

- Identify patients who develop early and progresive CKD
- Monitor effects of treatment
- Be useful and convenient for clinical practice

Blood HbA1c and urinary albumin are good examples of "ideal" biomarkers for diabetes and DKD
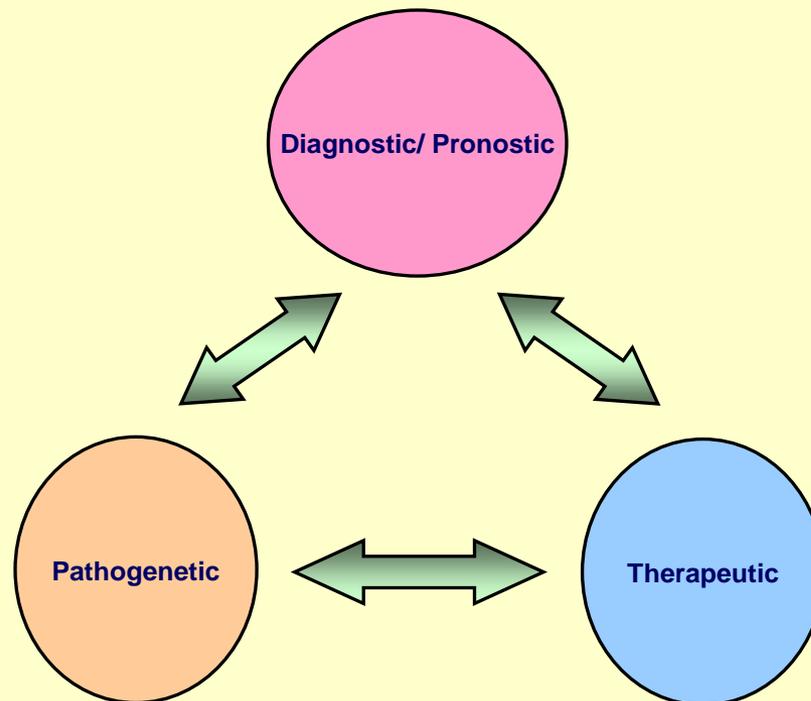
Searching for biomarkers in Diabetic Kidney Disease

Traditional approach

Investigating known physiological functions or pathways

- **Inflammatory and fibrogenic cytokines:**

  >Members of the TNF-alfa superfamily

  - **Soluble TNF receptors** 1 and 2
  - **TWEAK** (soluble TNF-like weak inducer of apoptosis)
  - **sFas/Fas ligand**

  >**TGFbeta1** and Connective Tissue Growth Factor **(CTGF)**

- **Glomerular and tubular epitheial cell injury**

  - **NGAL** (Neutrophil Gelatin-Associated Lipocalin)
  - **KIM1** (Kidney Injury Marker-1)

- **Matrix Molecules**

  - **type IV collagen**

TGF-beta 1 and CTGF in DKD

Urinary TGF-beta1 was more than fivefold higher in poorly controlled versus controlled diabetic Ethn Dis 2008 Spring;18:S2-54-9.

CTGF is a mediator of angiotensin II-induced fibrosis.

Rupérez M, et al . Circulation. 2003 23;108:1499-505.

Urinary CTGF excretion in patients with type 1 diabetes and nephropathy.

Gilbert RE et al . Diabetes Care. 2003 Sep;26(9):2632-6.

Fresolimumab (an anti-TGF beta antibody) and FG-3019 (an anti-CTGF antibody) in CKD

Biomarkers   of epithelial cell injury in   DKD

Podocyturia and   podocyte genes correlated well with albuminuria

Urinary tubular biomarkers in short-term type 2 diabetes mellitus patients: a cross-sectional study.

Fu WJ, et al Endocrine. 2012 ;41:82-8.

Urinary NGAL and progression of diabetic nephropathy in type 1 diabetic patients in a four-year follow-up study.

Nielsen SE, et al Nephron Clin Pract. 2011;118:c130-5.

Tubular markers do not predict the decline in glomerular filtration rate in type 1 diabetic patients with overt nephropathy.

Nielsen SE, et al Kidney Int. 2011 ;79:1113-8.

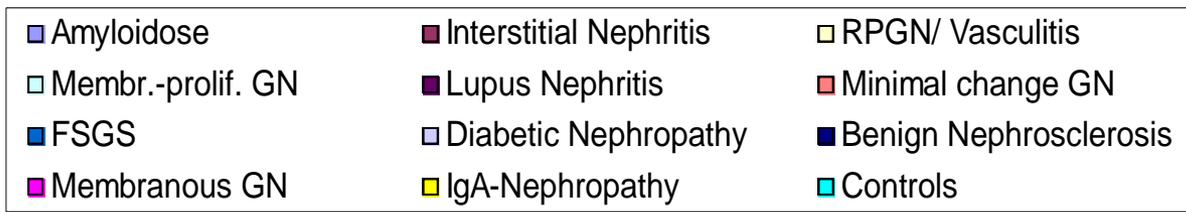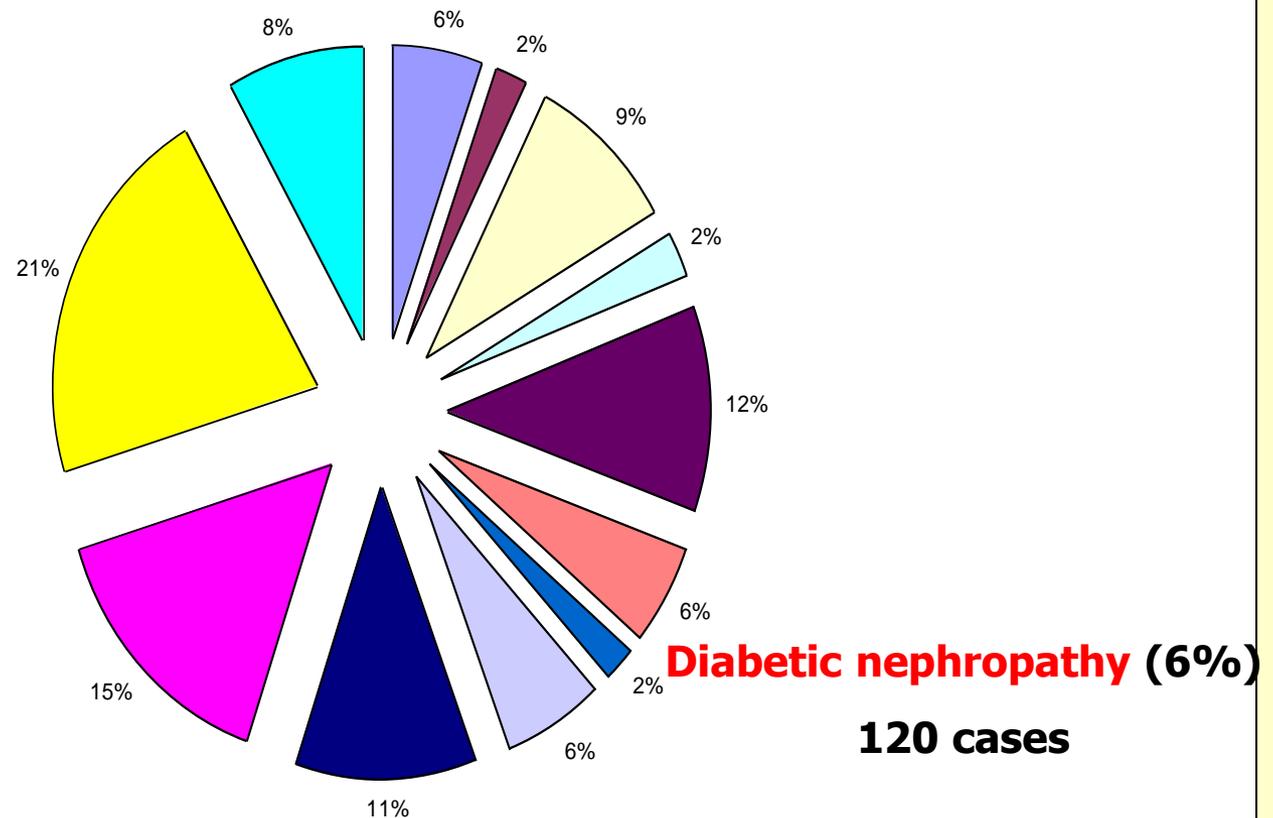# Searching for biomarkers in Diabetic Kidney Disease

Emerging technologies

Allow the systematic, unbiased characterization of variations

in genes,RNA, proteins and metabolites associated with diseases.

- **Transcriptomic analyses of human kidney biopsies**

- **Proteomic and peptidomic analyses**

- **MicroRNA profiling**

The transcriptomic search of biomarkers
European renal DNA bank (2006)

More than 2000 human kidney biopsies

Diabetic nephropathy (6%)

120 cases

■ Amyloidose	■ Interstitial Nephritis	□ RPGN/ Vasculitis
□ Membr.-prolif. GN	■ Lupus Nephritis	■ Minimal change GN
■ FSGS	■ Diabetic Nephropathy	■ Benign Nephrosclerosis
■ Membranous GN	□ IgA-Nephropathy	□ Controls

Some biomarkers identified by transcriptomic in the diabetic kidneys

347 inflammatory /apoptotic genes were detected in progresive DKD

The death ligand TRAIL in diabetic nephropathy.

Lorz C et al , **J Am Soc Nephrol.** 2008 ;19:904-14

The MIF receptor CD74 in diabetic podocyte injury.

Sanchez Niño et al . **J Am Soc Nephrol** 2009 :353-62

## BASP1 promotes apoptosis in diabetic nephropathy

Sanchez-Niño et al **J Am Soc Nephrol** 21: 610-621 2010

Differentially expressed transcripts in DKD tubulointerstitium compared with control samples.

They identified 1,700 differentially expressed probesets in glomeruli and 1,831 in tubuli

This study has cataloged gene-expression regulation and identified multiple novel genes and pathways that could serve as biomarkers

Diabetes 60:2354–2369, 2011
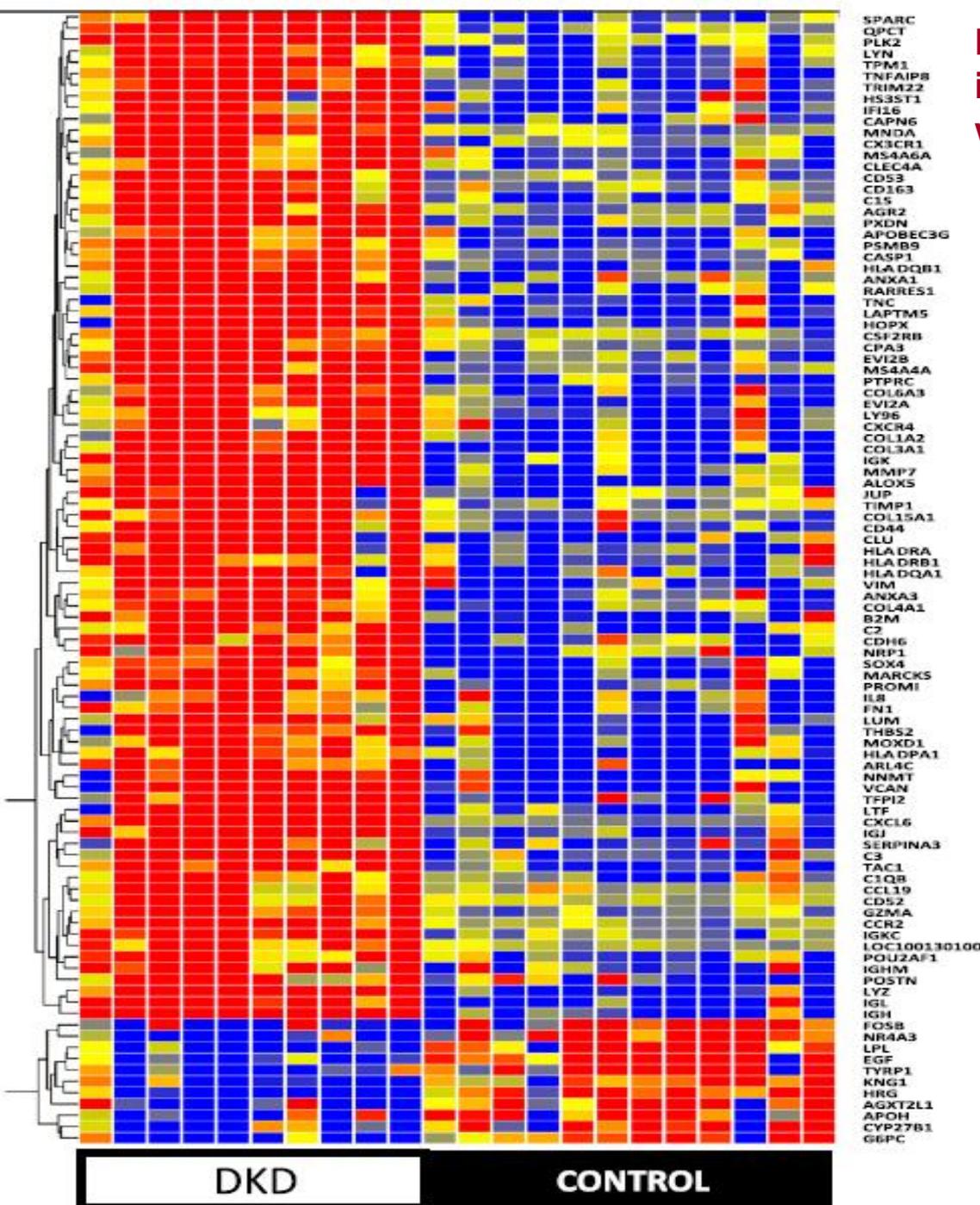
Proteomic Strategies in the Search of New Biomarkers in Atherothrombosis

José Tuñón, MD,*‡ José Luis Martín-Ventura, PhD,†‡ Luis Miguel Blanco-Colio, PhD,†‡ Óscar Lorenzo, PhD,†‡ Juan Antonio López, PhD,§ Jesús Egido, MD†‡

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- **HSP (Heat Shock Protein) 27 (Circulation , 2004)**

- **Fas/Fas Ligand (J Am Coll Cardiol , 2007)**

- **TWEAK (Tumor necrosis factor-like weak inducer of apoptosis) (Arterioscler Thromb Vasc Biol, 2007).**

- **CD74 Receptor (Cardiovascular Res, 2009)**

- **Thioredoxin (Atherosclerosis , 2010)**

Proteomics /peptidomics in DKD

A panel of four novel protein biomarkers , **Tamm-Horsfall glycoprotein , progranulin, clusterin, and α-1 acid glycoprotein** **predicts the development of microalbuminuria and renal function decline in type 1 diabetes.**

Schlatzer D, et al Diabetes Care. 2012 ;35:549-55.

Urinary fragments of collagen and alpha -2- HS-Glycoprotein predicts worsening of albuminuria in type 2 diabetes mellitus.

Roscioni SS, et al Diabetologia. 2013;56:259-67.

High urinary sulphate excretion was associated with slower eGFR decline in type 1 diabetes nephropathy .

Andrésdóttir G et al Diabet Med 2013 . Jan 16.
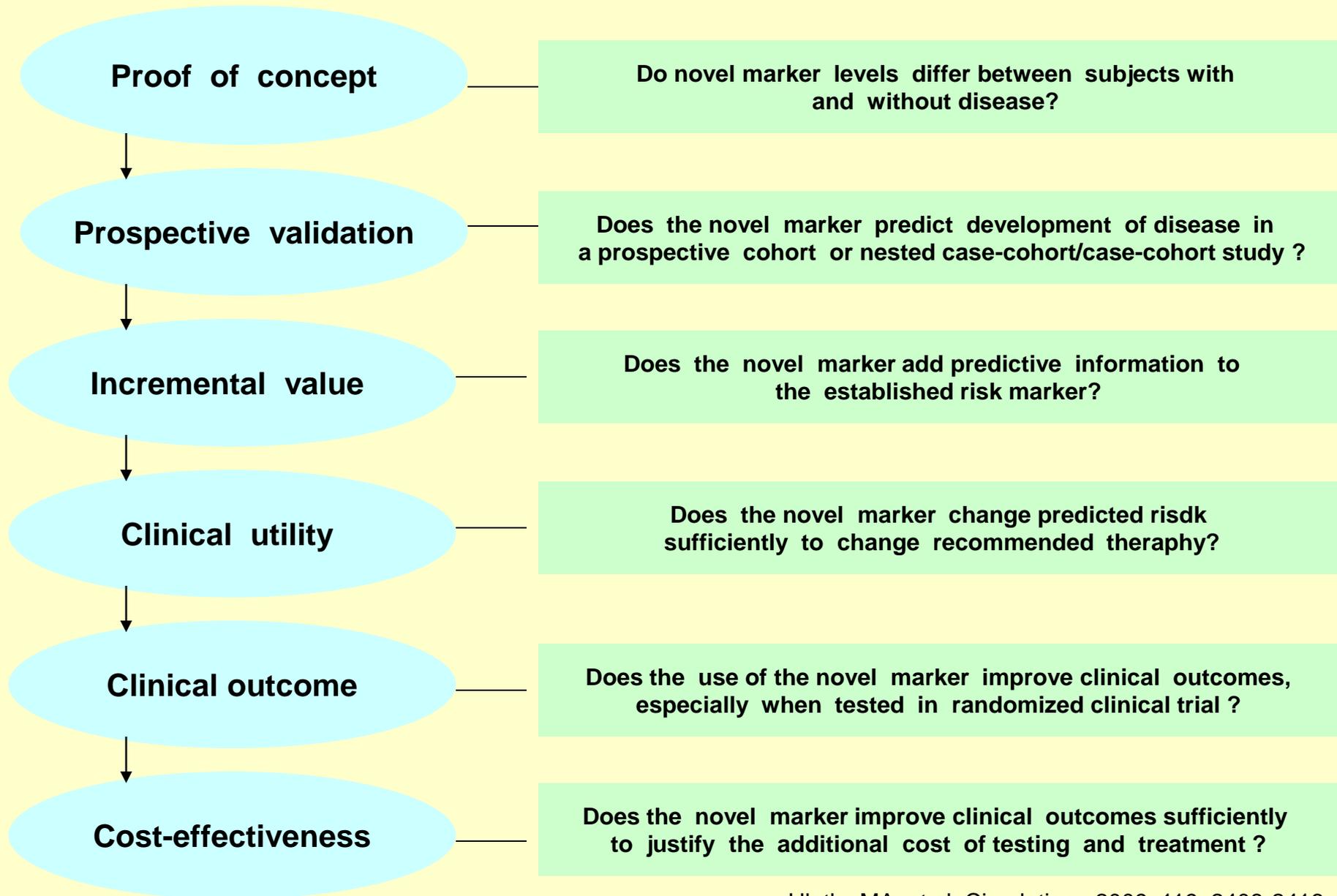
MicroRNA and DKD

A total of 27 microRNAs (related to growth and renal fibrosis) were found in the urine at significantly different levels in different stages of untreated nephropathy.

Argyropoulos C, et al PLoS One. 2013;8:e54662.

Towards microRNA-based therapeutics for diabetic nephropathy.

Alvarez ML,et al Diabetologia. 2013 ;56:444-56.

The long road from finding targets to clinical use

Proof of concept — Do novel marker levels differ between subjects with and without disease?

Prospective validation — Does the novel marker predict development of disease in a prospective cohort or nested case-cohort/case-cohort study ?

Incremental value — Does the novel marker add predictive information to the established risk marker?

Clinical utility — Does the novel marker change predicted risdk sufficiently to change recommended theraphy?

Clinical outcome — Does the use of the novel marker improve clinical outcomes, especially when tested in randomized clinical trial ?

Cost-effectiveness — Does the novel marker improve clinical outcomes sufficiently to justify the additional cost of testing and treatment ?

Hlatky MA et al Circulation; 2009: 119; 2408-2416

Summary

- **The search for novel biomarkers of Diabetic Kidney Disease is challenging**

- **Most studies generally include**
  - **limited number of patients**
  - **short follow-up**
  - **absence of hard-end points**
  - **concerns in statistical interpretation**

- **Future studies will need to examine how potential novel biomarkers   perform in well designed prospective interventional clinical trials and natural studies**

Despite numerous studies, no biomarker has surpassed albuminuria as the gold standad marker of Diabetic Kidney Disease so far.

'Nobody's Perfect': Billy Wilder's Some Like It Hot
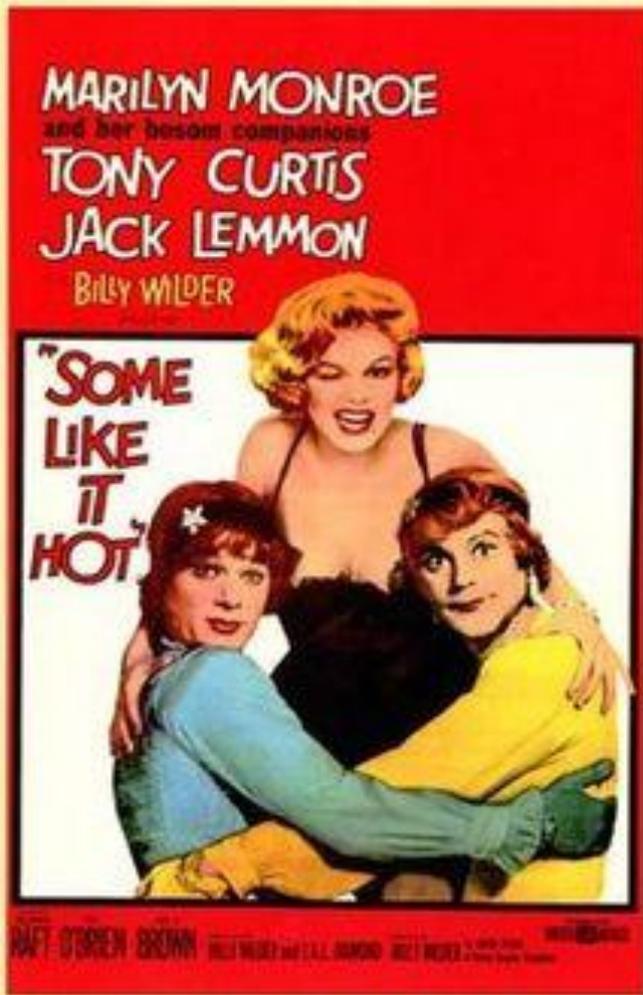
Jerry: Ohh... It is about the gold standard for diagnosis and progression of Diabetic Kidney Disease

Osgood: No biomarker is perfect!

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Autónoma University. Madrid.