EUReniOmics

Designing Strategies for Therapeutic Progress in Rare Kidney Diseases

Franz Schaefer

IRDiRC INTERNATIONAL RARE DISEASES RESEARCH CONSORTIUM

SEVENTH FRAMEWORK PROGRAMME

Dublin, 16 April 2013

Drug Repurposing:
The Humble Clinician‘s Perspective

Using approved drugs
in unapproved indications / populations:

Aren‘t we doing that all the time?

Used to be called „off-label use“ !

Some of the most efficient recent pharmacotherapies in nephrology are „re-purposed“ use:

ACE inhibitors / ARBs:

Hypertension ➔ Proteinuria, renal disease progression

Rituximab:

Lymphoma ➔ Auto-immune nephropathies, idiopathic nephrotic syndrome

Eculizumab:

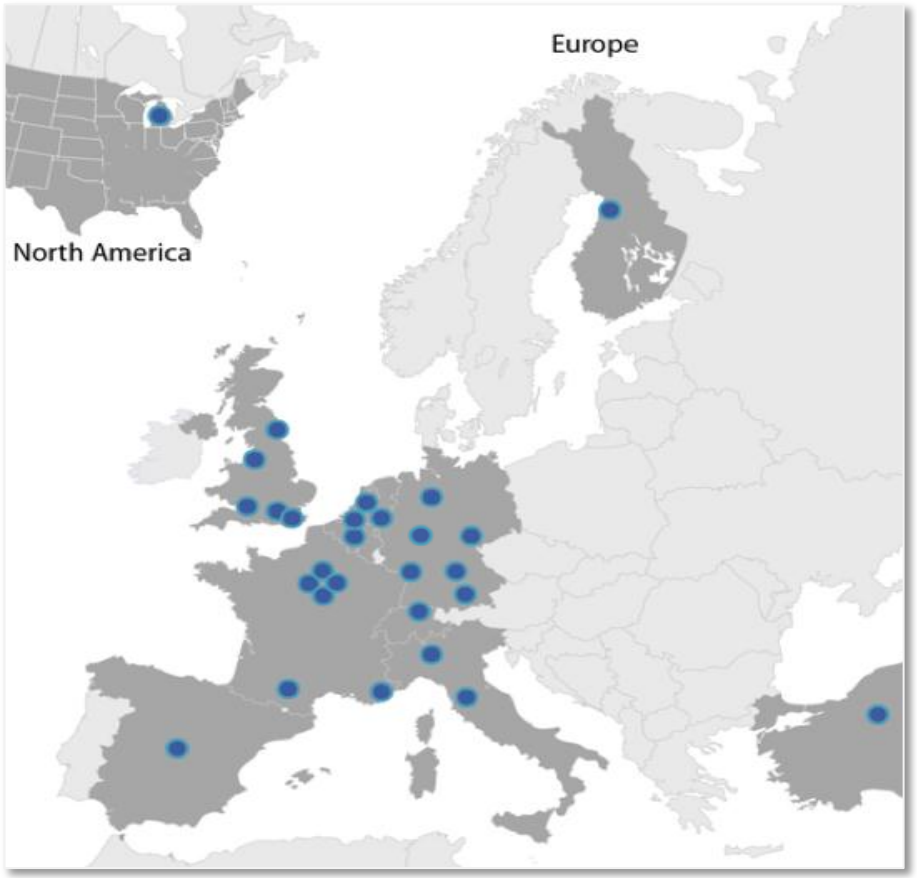
Paroxysmal nocturnal hemoglobinuria ➔ Atypical hemolytic uremic syndrome

Is there a Potential for Drug Repurposing in Rare Kidney Diseases ?

The easy way:
Apply known mode of action established for different indication

The smart way: Utilize known off-target effects of established drugs

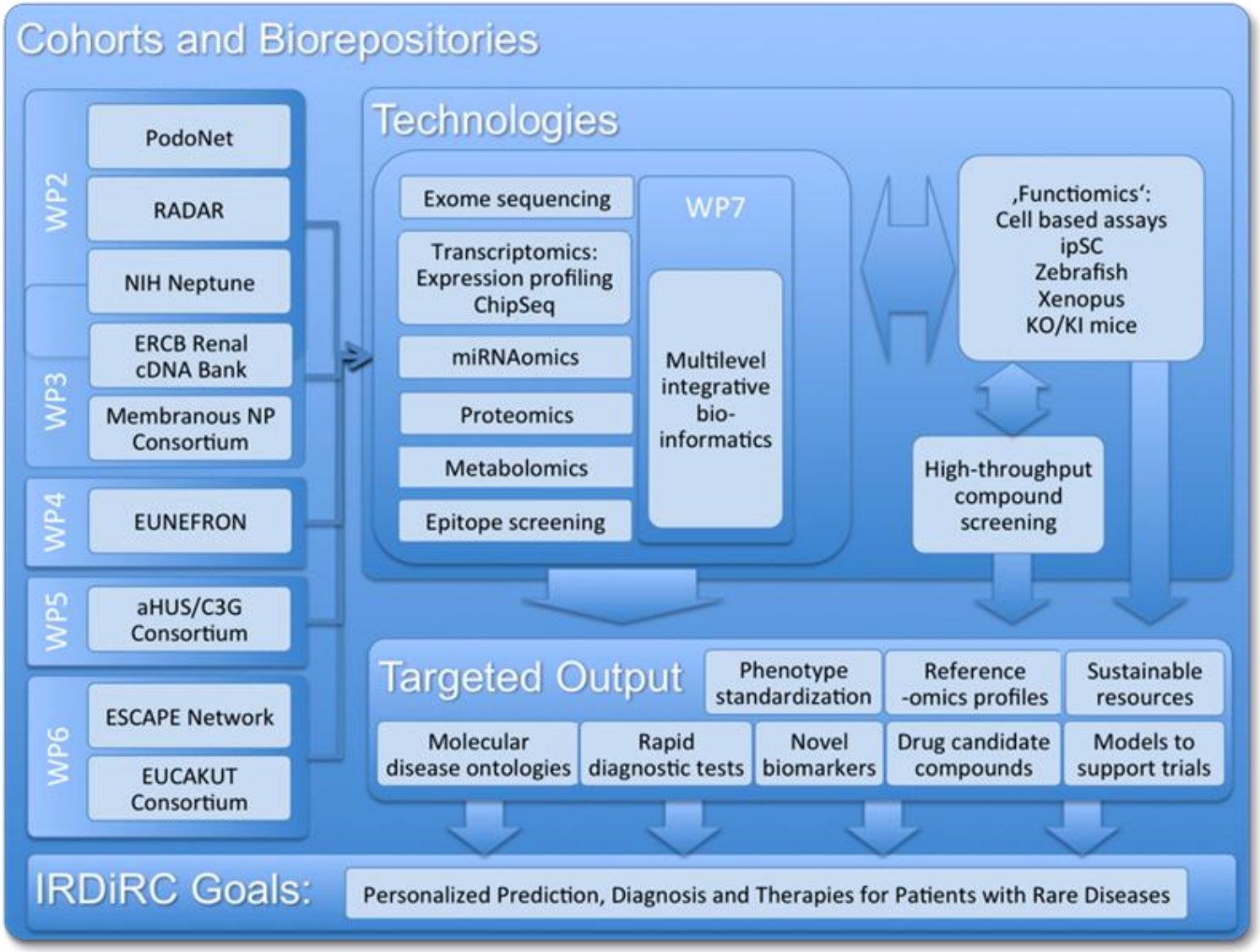
The hard way: High-throughput phenotype screening



26 academic research groups
19 institutions in 11 countries
8 industry partners

UniversitätsKlinikum Heidelberg
Inserm
Institut national de la santé et de la recherche médicale
MARIO NEGRI
ISTITUTO DI RICERCHE FARMACOLOGICHE
Universität Zürich
University Medical Center Utrecht
HKI
ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS
UMC St Radboud
MANCHESTER 1824
The University of Manchester
KU LEUVEN
Walter Brendel Zentrum München
UNIVERSITY of OULU
CSIC
Newcastle University
University of BRISTOL
KIT
Karlsruher Institut für Technologie
UCL
UNIVERSITY OF MICHIGAN
mosaiques diagnostics
Multiplicom
Philogen
innovating targeting
Metabometrix
cbc
Comprehensive Biomarker Center
Philochem
innovating chemistry
genomatix
GABImi
A MILESTONE IN PROCESS MANAGEMENT

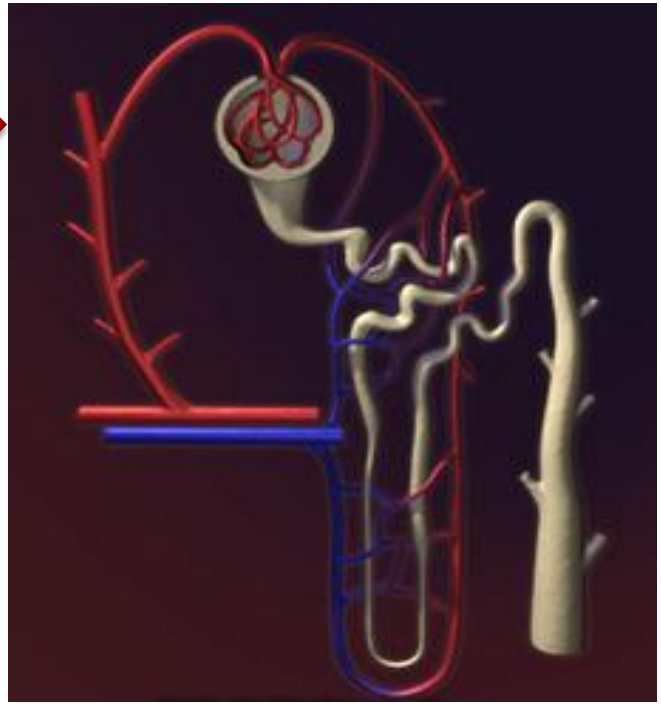
SEVENTH FRAMEWORK PROGRAMME


Cohorts and Biorepositories
WP2
PodoNet
RADAR
NIH Neptune
WP3
ERCB Renal cDNA Bank
Membranous NP Consortium
WP4
EUNEFRON
WP5
aHUS/C3G Consortium
WP6
ESCAPE Network
EUCAKUT Consortium
Technologies
Exome sequencing
Transcriptomics: Expression profiling ChipSeq
miRNAomics
Proteomics
Metabolomics
Epitope screening
WP7
Multilevel integrative bio-informatics
‚Functiomics': Cell based assays ipSC Zebrafish Xenopus KO/KI mice
High-throughput compound screening
Targeted Output
Phenotype standardization
Reference -omics profiles
Sustainable resources
Molecular disease ontologies
Rapid diagnostic tests
Novel biomarkers
Drug candidate compounds
Models to support trials
IRDiRC Goals:
Personalized Prediction, Diagnosis and Therapies for Patients with Rare Diseases

Targeted Rare Kidney Disorders

WP2/3:
Nephrotic glomerulopathies

WP5:
Complement-mediated nephropathies



WP4:
Tubulopathies

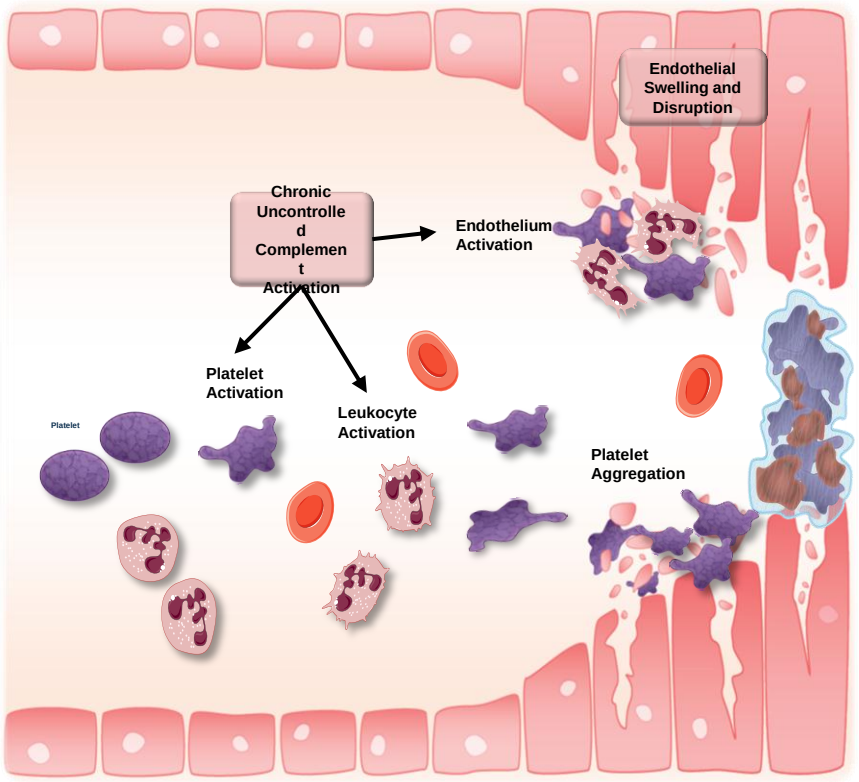
WP6: Kidney malformations

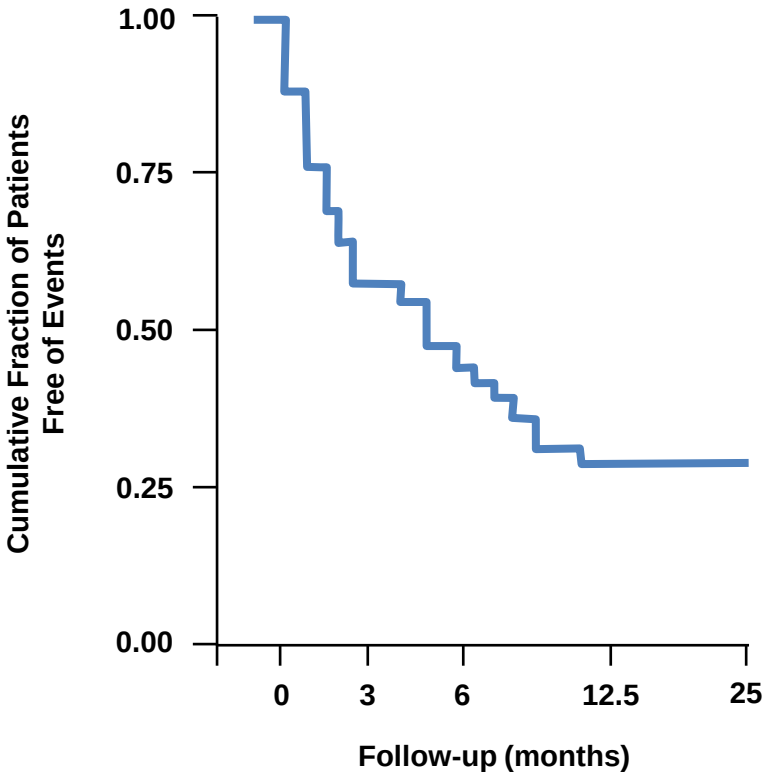
Is there a Potential for Drug Repurposing in Rare Kidney Diseases ?

The easy way:

Apply known mode of action established for different indication

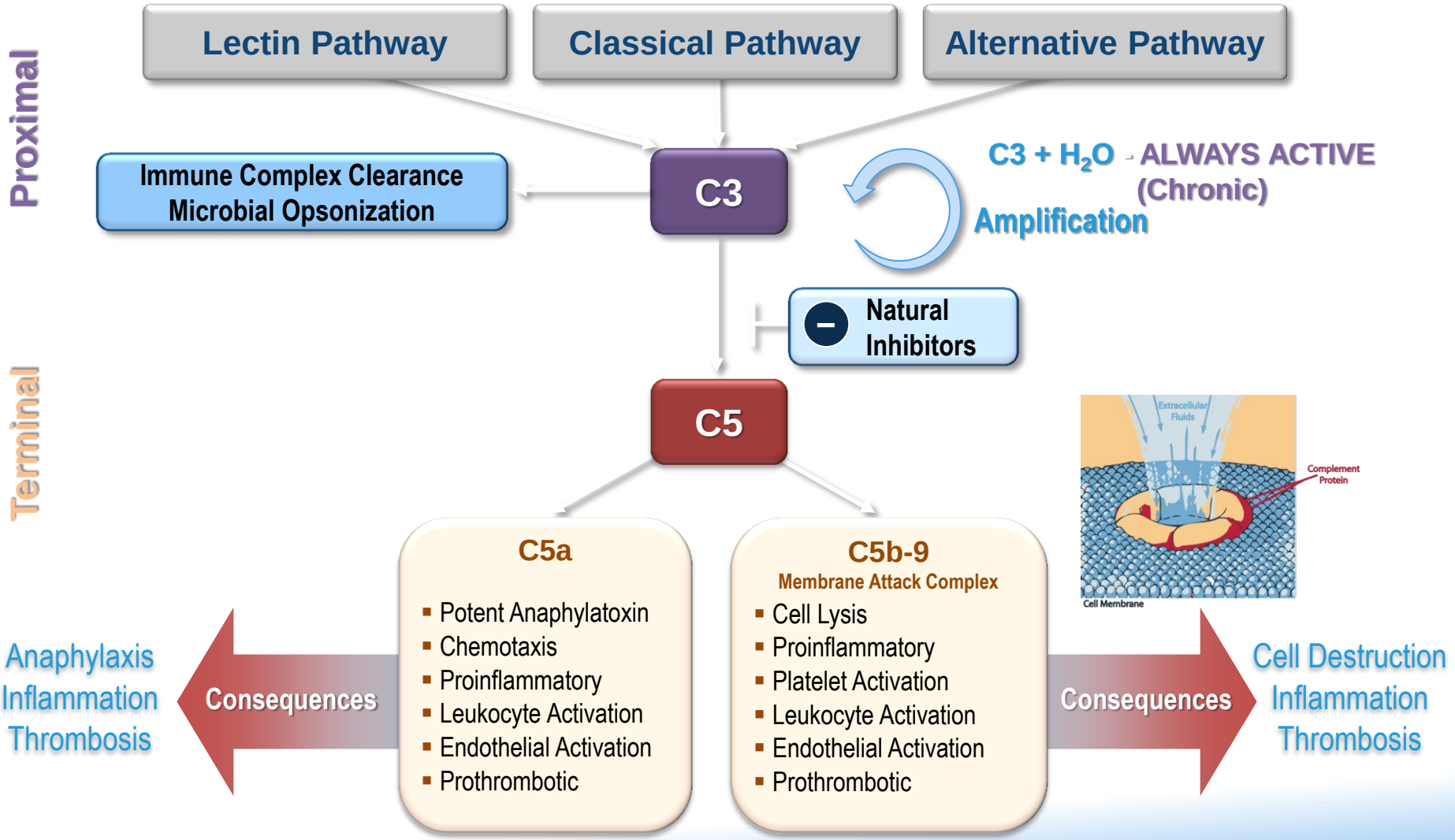
Atypical Hemolytic Uremic Syndrome: A Systemic Thrombotic Microangiopathy



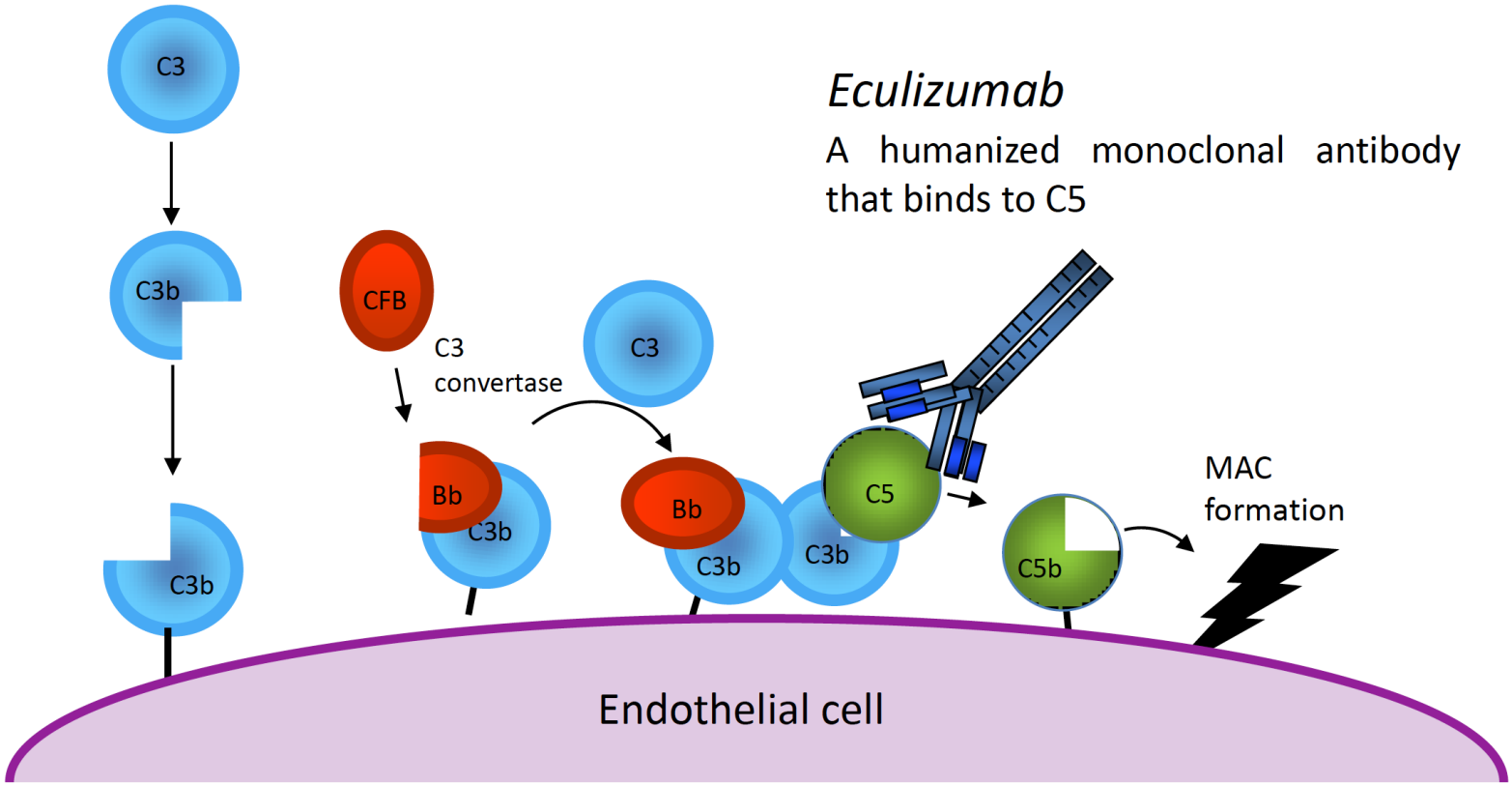


Modified from Caprioli et al. 2006. CFH Mutation Depicted

Dysfunctional Complement Regulation in aHUS



Eculizumab binds to C5 and inhibits membrane attack complex formation

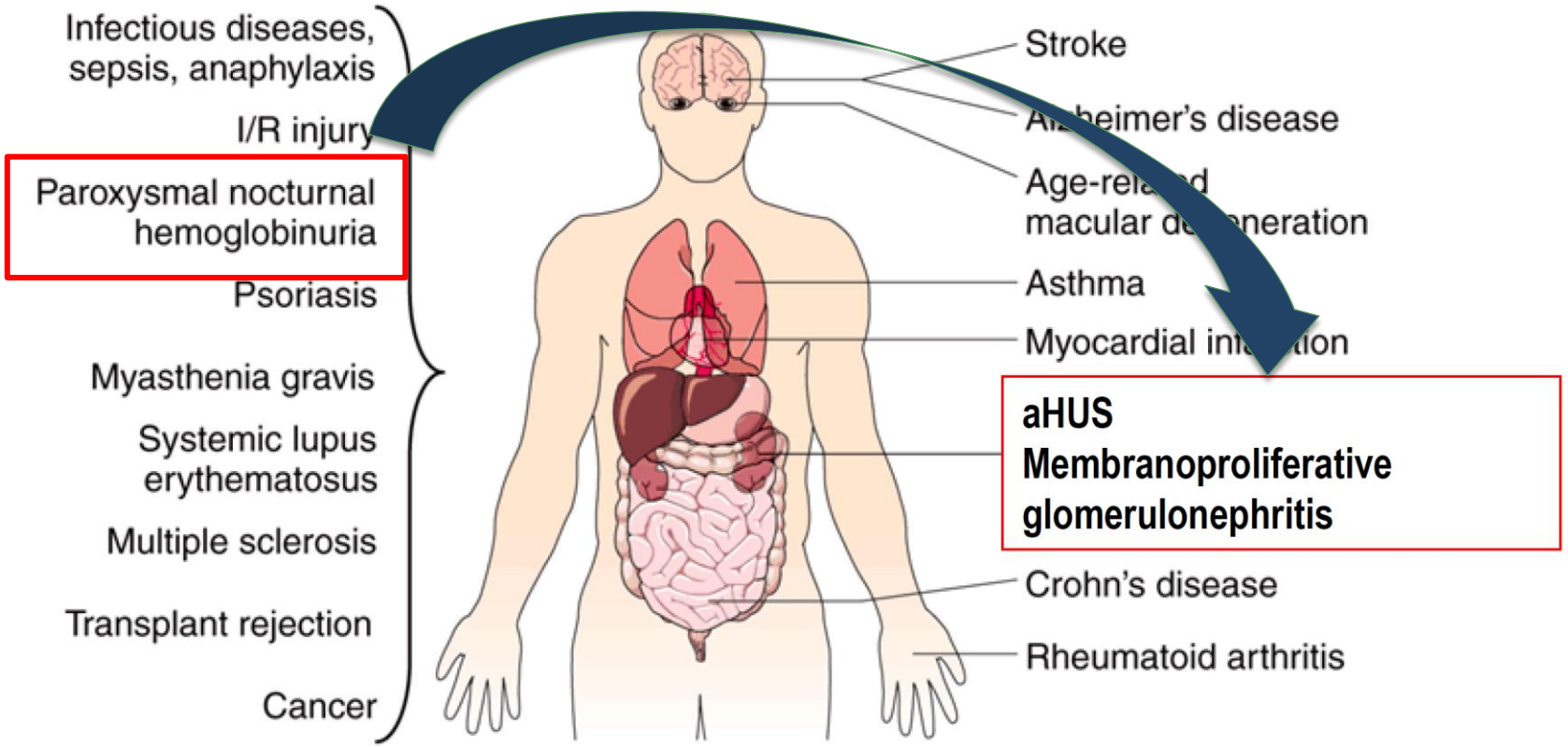


Eculizumab

A humanized monoclonal antibody that binds to C5

Eculizumab ‚Repurposing‘

DISEASES ASSOCIATED WITH COMPLEMENT ACTIVATION



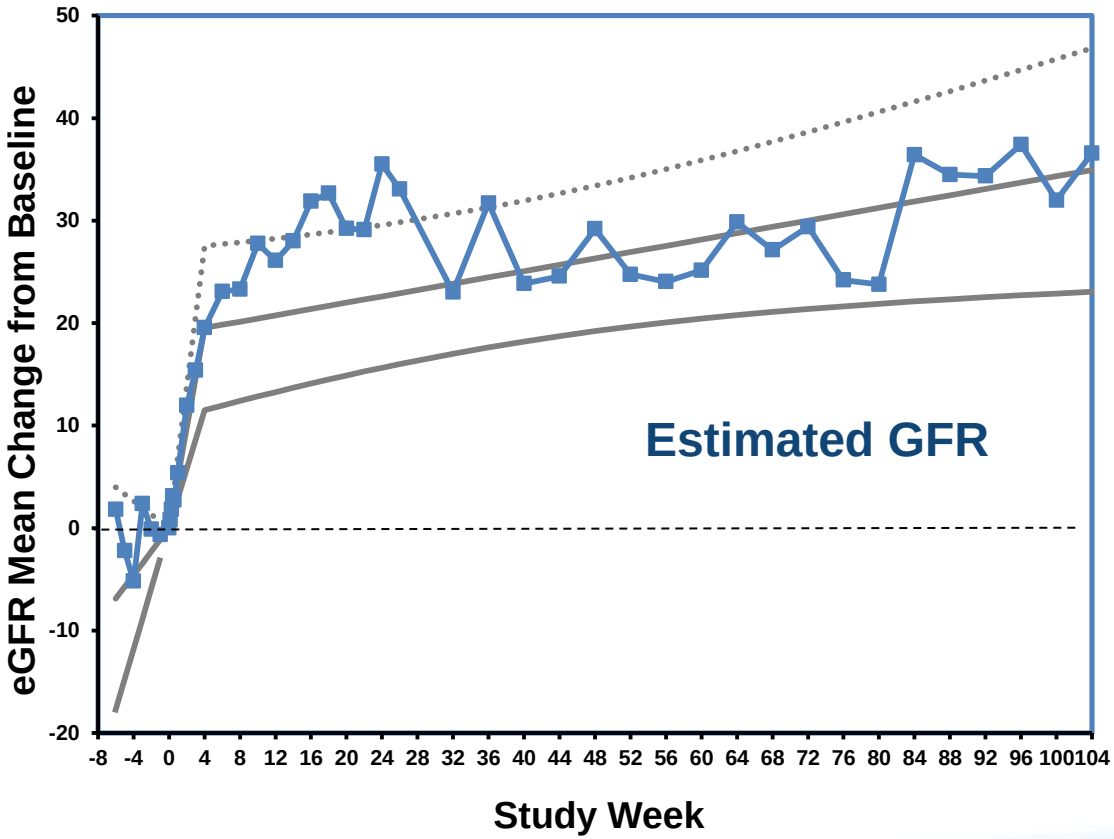
Complete Disease Control by Eculizumab in atypical HUS

2-yr treatment results:

Complete remission, in more than 90% of patients

Impressive recovery of renal function

So far no significant side effects



Eculizumab Repurposing to be continued...

The NEW ENGLAND JOURNAL of MEDICINE N ENGL J MED 366;12 NEJM.ORG MARCH 22, 2012

Eculizumab in a Patient with Dense-Deposit Disease	Daina, Noris, Remuzzi
Eculizumab for the Treatment of Dense-Deposit Disease	Vivarelli, Pasini, Emma
Eculizumab and Refractory Membranoproliferative Glomerulonephritis	Radhakrishnan, et al

Clin J Am Soc Nephrol 7: 748–756, 2012.

Eculizumab for Dense Deposit Disease and C3 Glomerulonephritis — Bomback, et al

"Clinical and histopathological data suggest a response to Eculizumab in some but not all subjects with dense deposit disease/C3 glomerulonephritis"

But:

- Annual Eculizumab treatment costs: up to 400,000 € per year!
  - Life-long infusion therapy required

-> Search for alternative complement inhibitors

EURenOmics WP5:

- Establish ex vivo & animal models of complement disorders

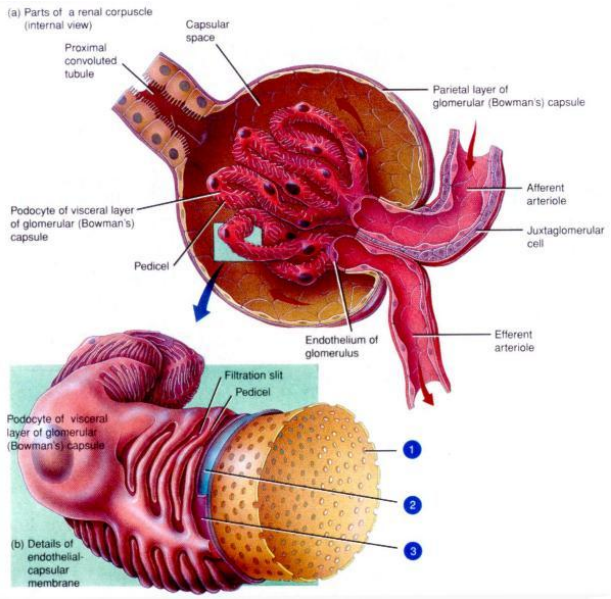
- Test small molecular compounds modifiying complement/regulator activity

Is there a Potential for Drug Repurposing in Rare Kidney Diseases ?

The smart way:

Utilize known off-target effects of established drugs

Nephrotic Syndrome: A Podocytopathy



Steroid-sensitive NS

85%

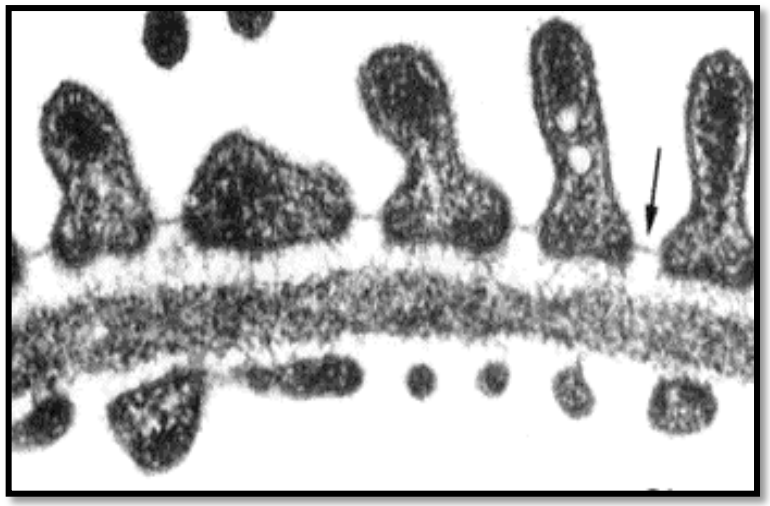
Steroid-resistant NS

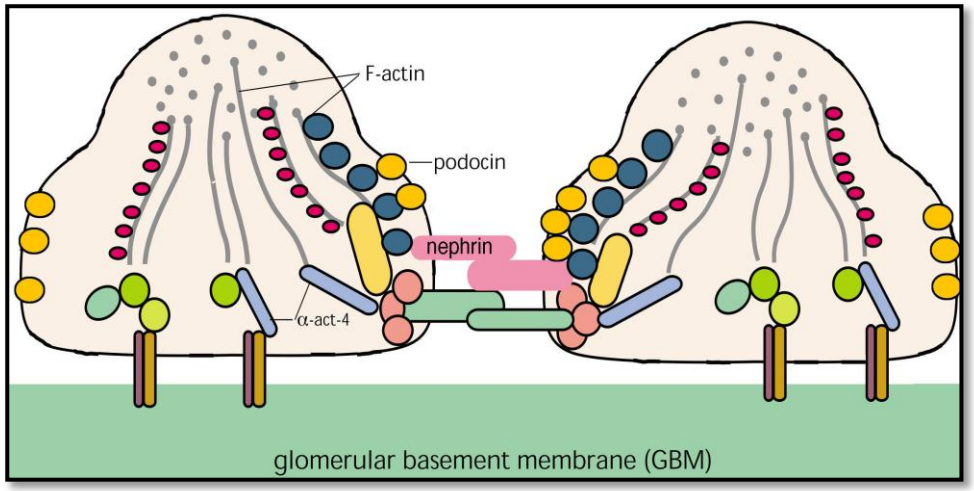
15%

CNI sensitivity
Post-tx recurrence

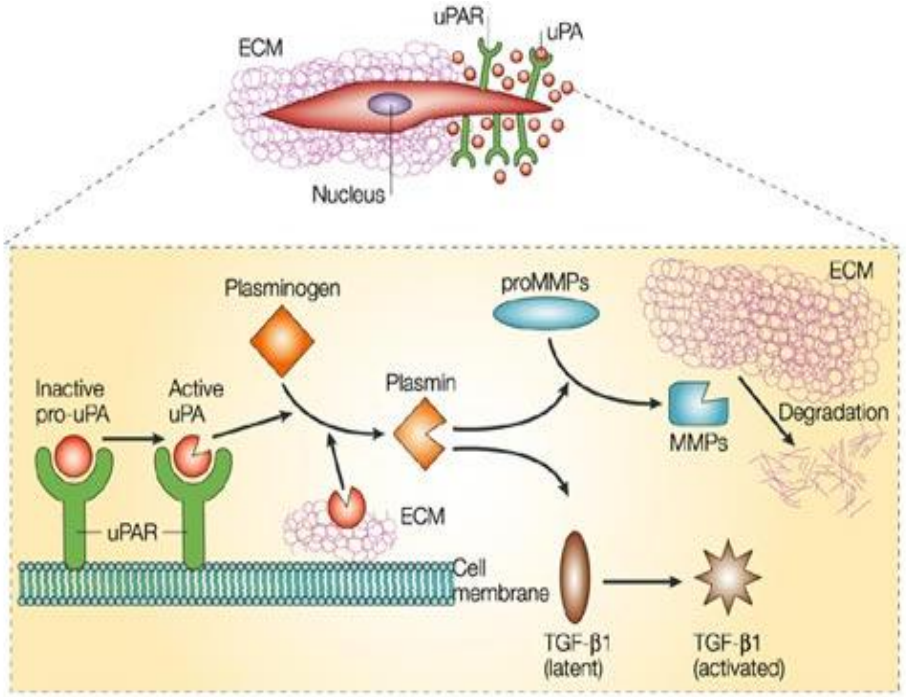
T cell disorder
Circulating
'permeability' factor

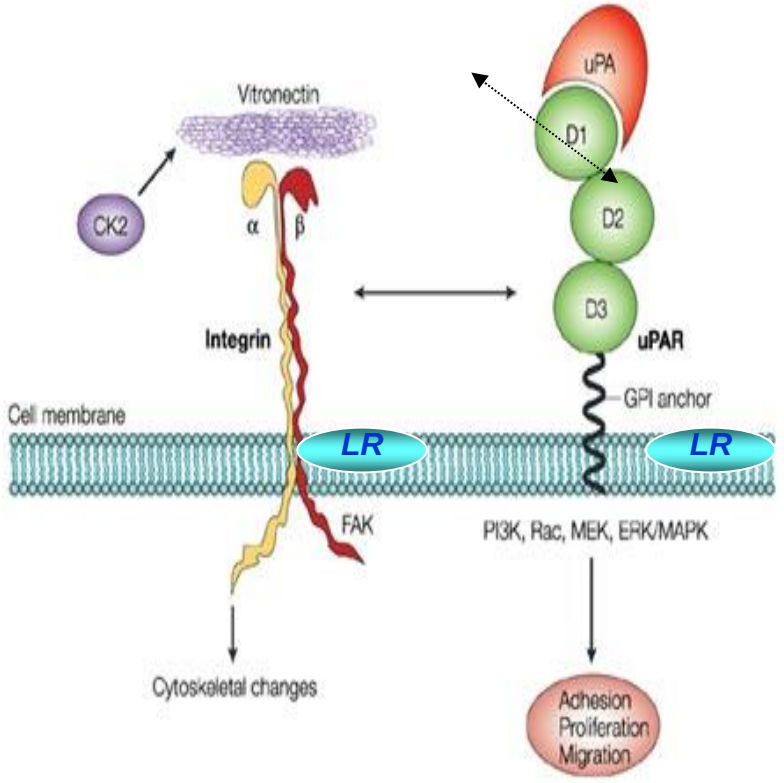
Structural anomalies of glomerular filtration barrier





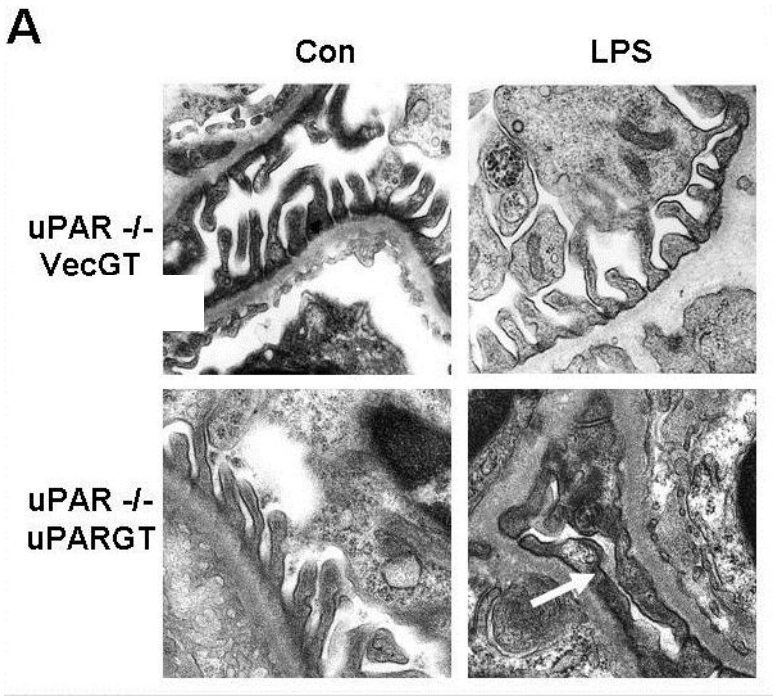
Urokinase plasminogen activator receptor (uPAR): Modifying Matrix Composition and Cell Motility

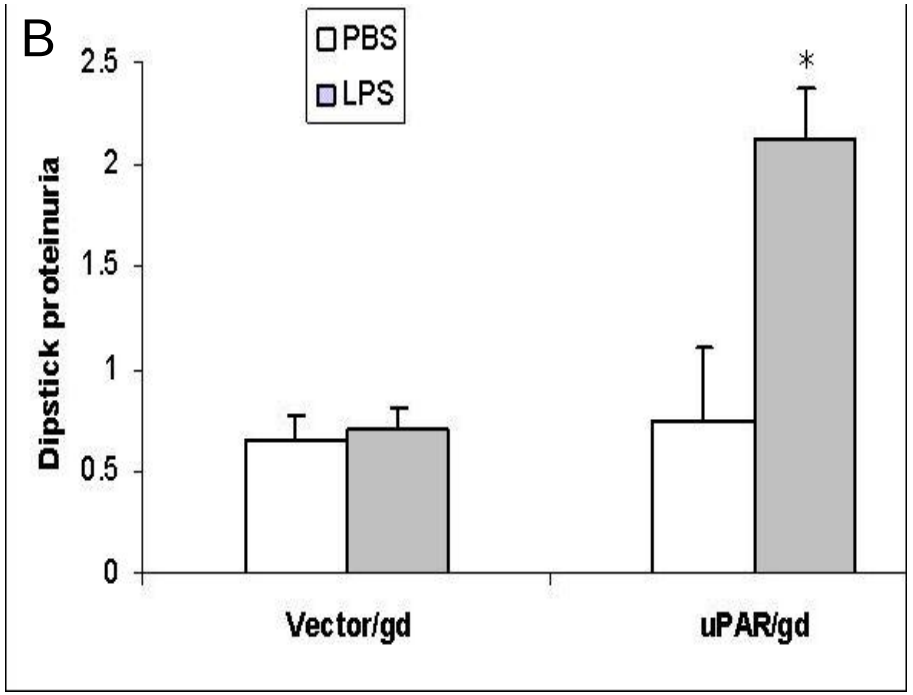




Blasi F& Carmeliet P. Nat Rev Mol Cell Biol 2002

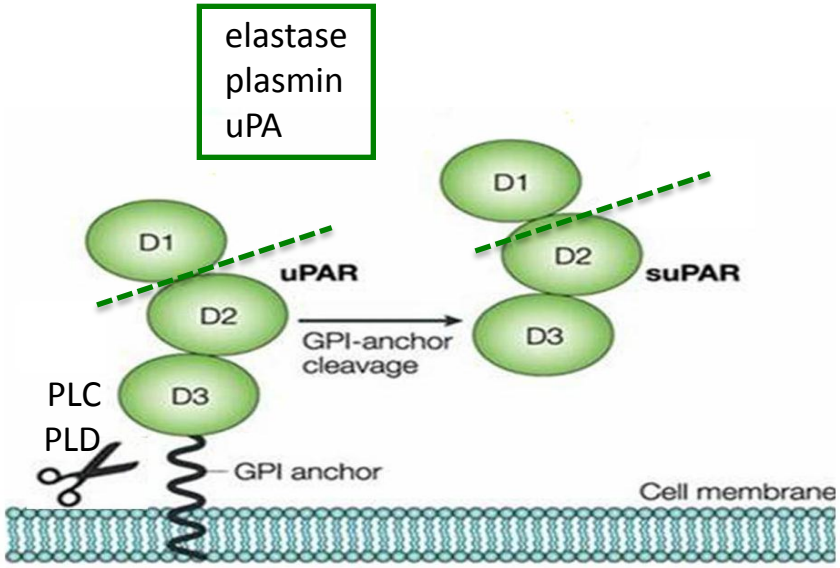
Podocyte uPAR is required for FP effacement and proteinuria



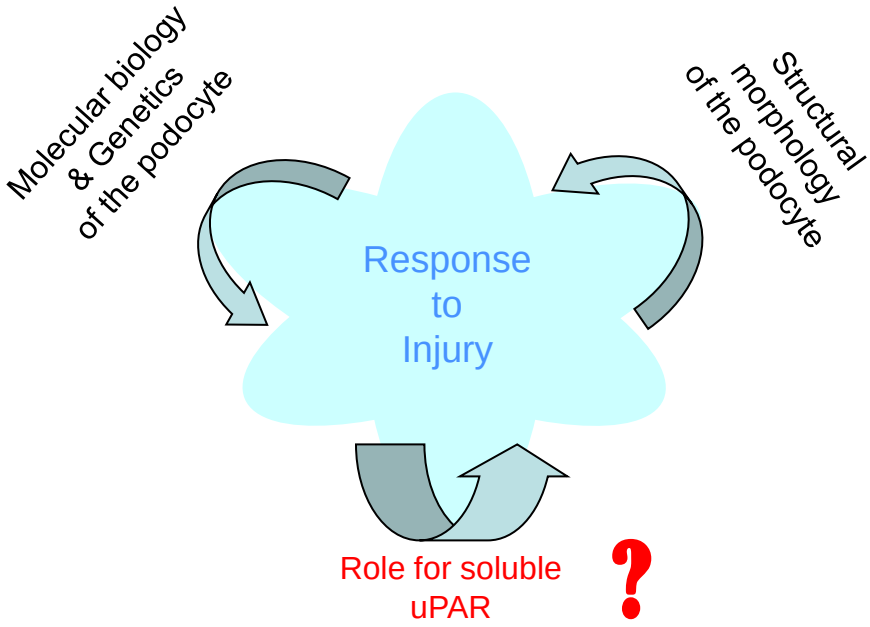


Wei, C. et al. Nat Med 2008

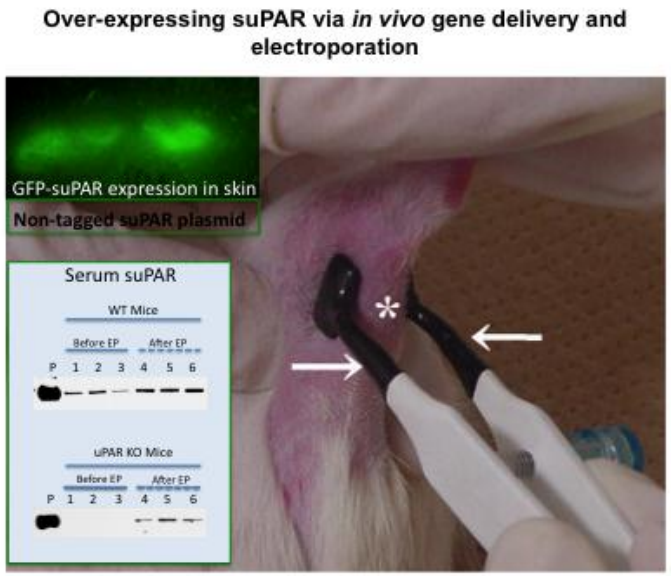
soluble uPAR (suPAR): A circulating glomerular disease factor?

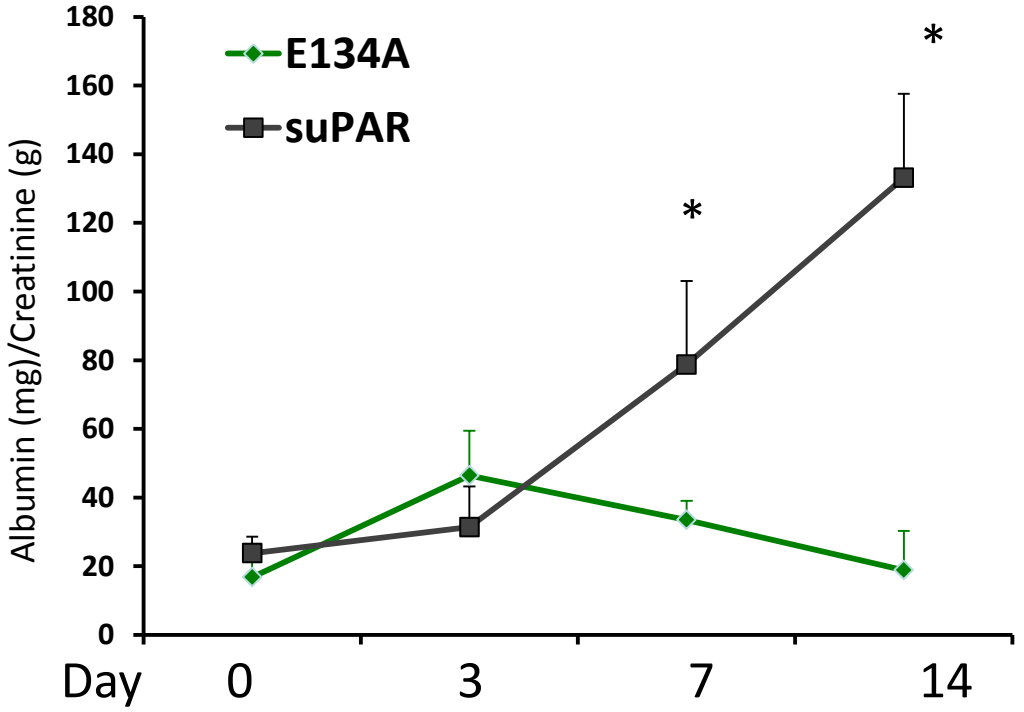


Blasi F& Carmeliet P. Nat Rev Mol Cell Biol 2002



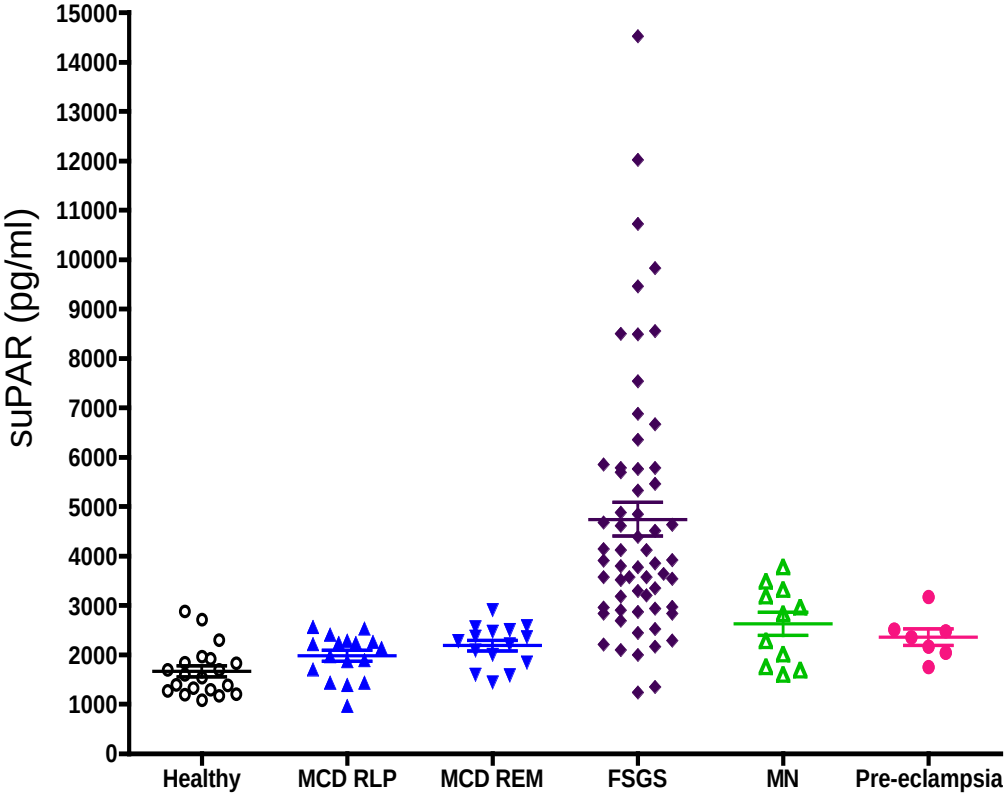
Elevated soluble uPAR causes albuminuria in mice

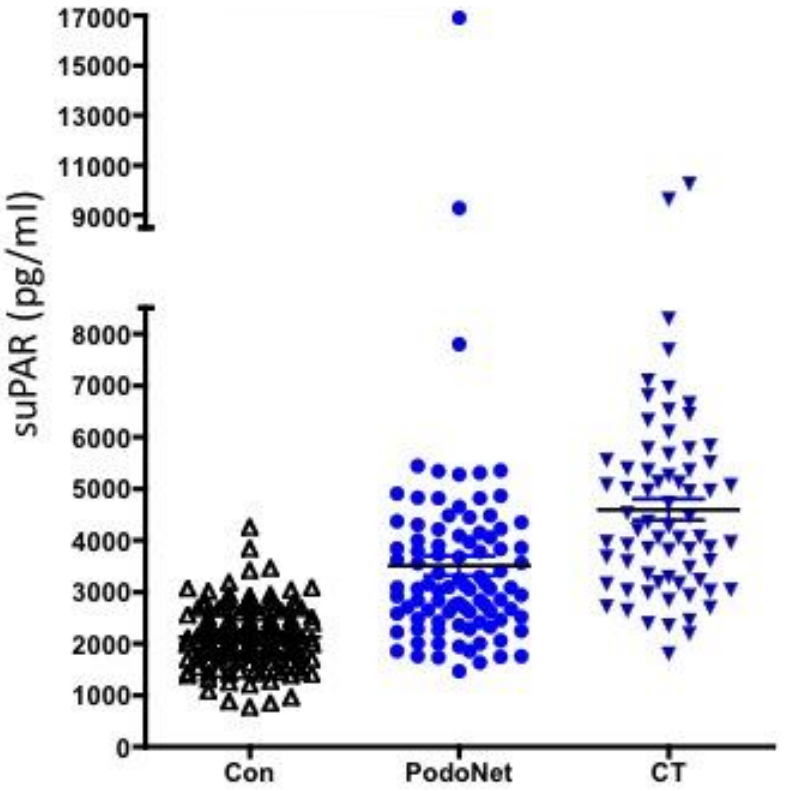




Wei C et al. Nature Med 2011

Elevated suPAR Associates with SRNS: Is sUPAR the Elusive Glomerular Permeability Factor?





Wei et al. Nature Med. 2011

Wei et al. JASN 2012

Amiloride

Well established **potassium-sparing diuretic**;
blocks epithelial sodium channel (ENaC) in collecting duct

Off-target effect:

- Inhibits uPAR expression in tumor-infiltrating lymphocytes and colon cancer cells
- Blocks cancer cell motility, metastasis formation

Nephrol Dial Transplant (2012) 27: 1746–1755

Amiloride off-target effect inhibits podocyte urokinase receptor expression and reduces proteinuria

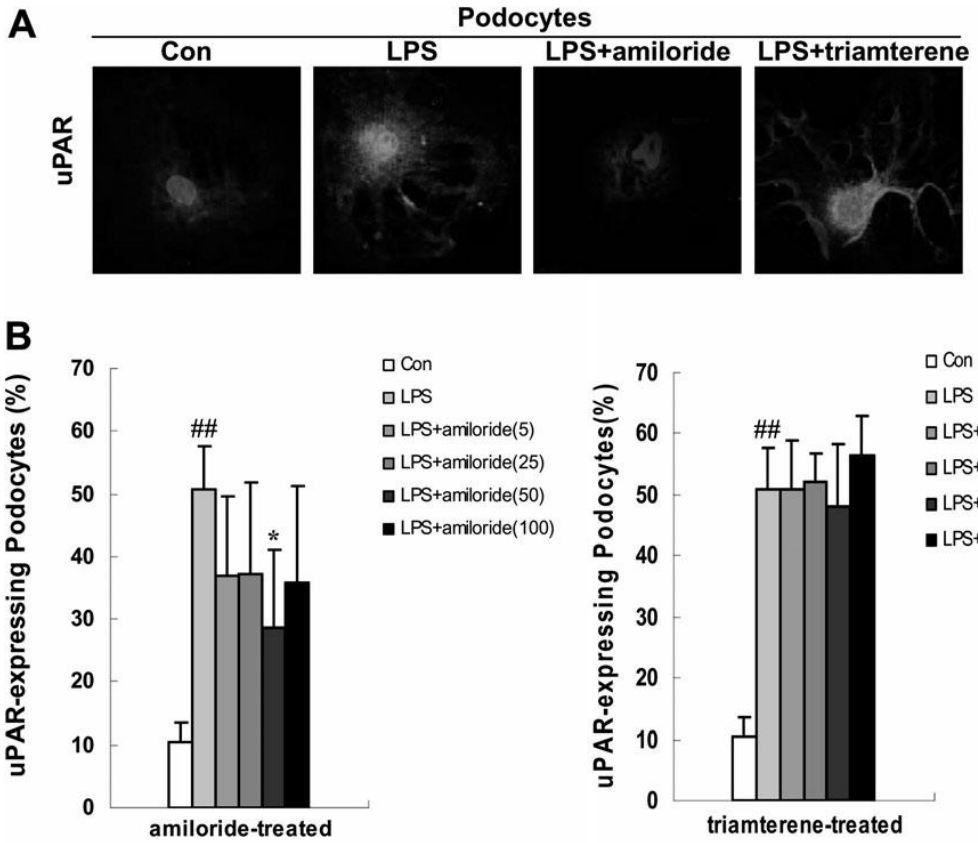
Bin Zhang[1,2,*], Shaoting Xie[1,2,*], Wei Shi[1] and Yun Yang[1]

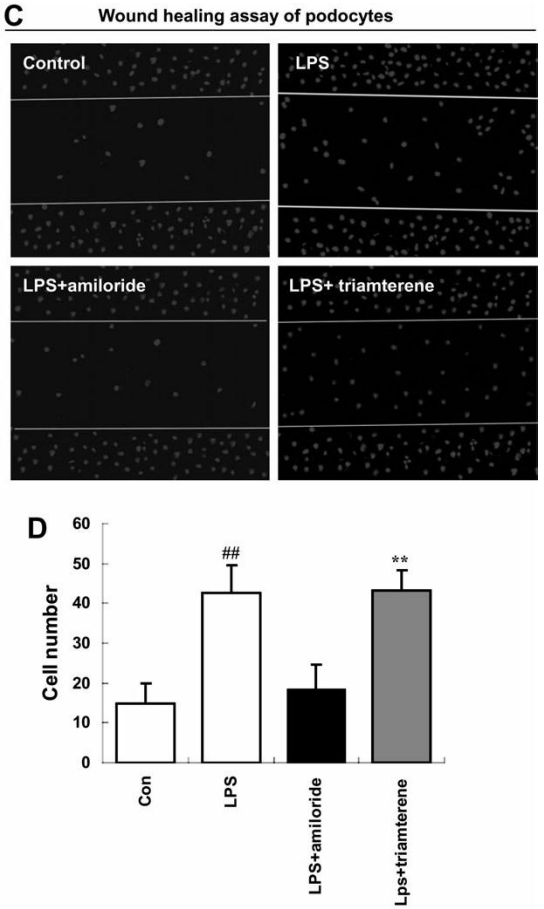
[1]Department of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China and
[2]Southern Medical University, Guangzhou, China

Abstract
The urokinase receptor (uPAR) and its soluble form play a key role in the pathogenesis of focal segmental glomerulo-

urokinase receptor (uPAR) expression in tumor-infiltrating lymphocytes [3] and colon cancer cells [4, 5]. uPAR is a glycosylphosphatidylinositol-anchored protein that has

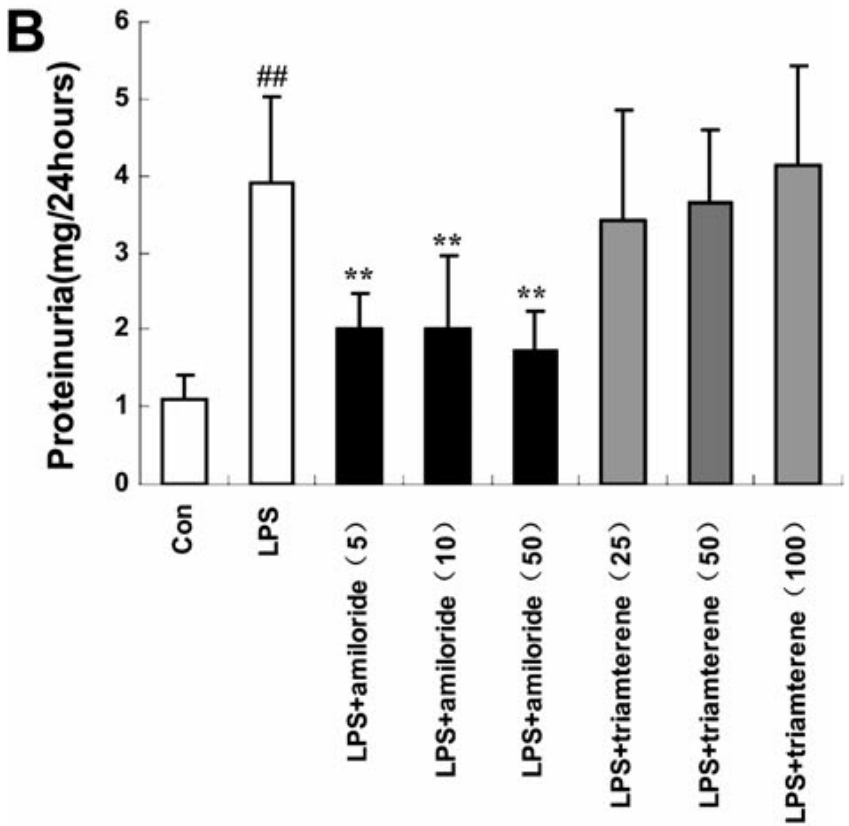
Specific inhibition of Podocyte uPAR Expression and Motility by Amiloride

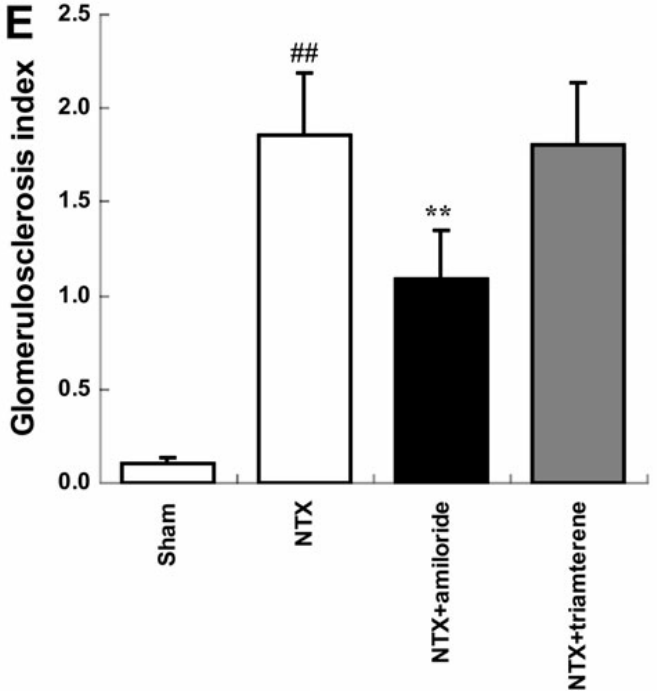




Zhang et al. NDT 2012

Amiloride Attenuates Proteinuria and Glomerulosclerosis in Mouse Disease Models





Zhang et al. NDT 2012

Is there a Potential for Drug Repurposing in Rare Kidney Diseases ?

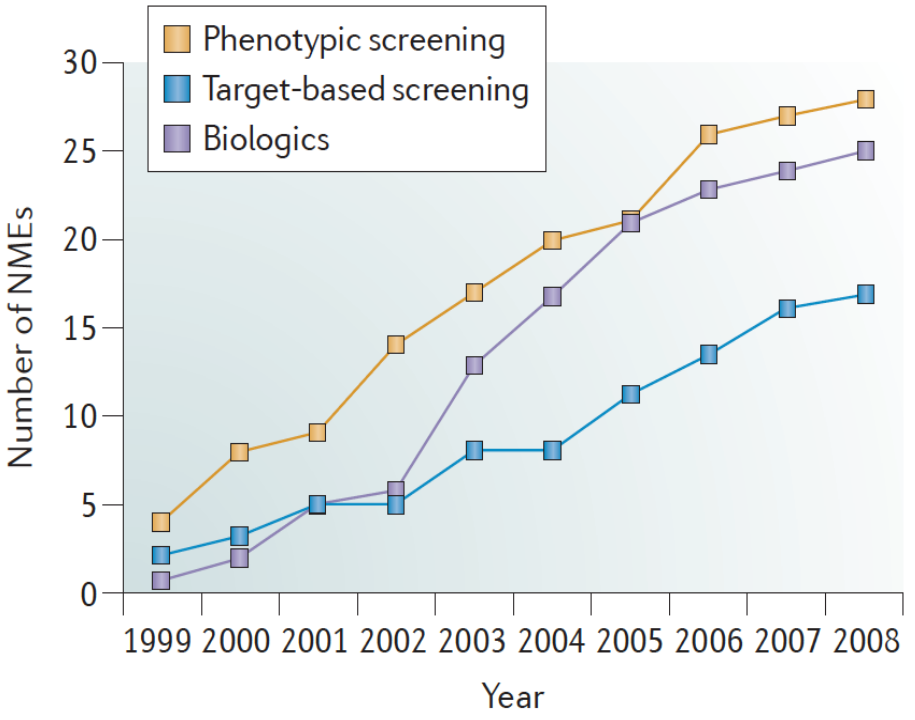
The hard way: High-throughput phenotype screening

Drug Discovery Strategies

Recent pharmaceutical research experience:

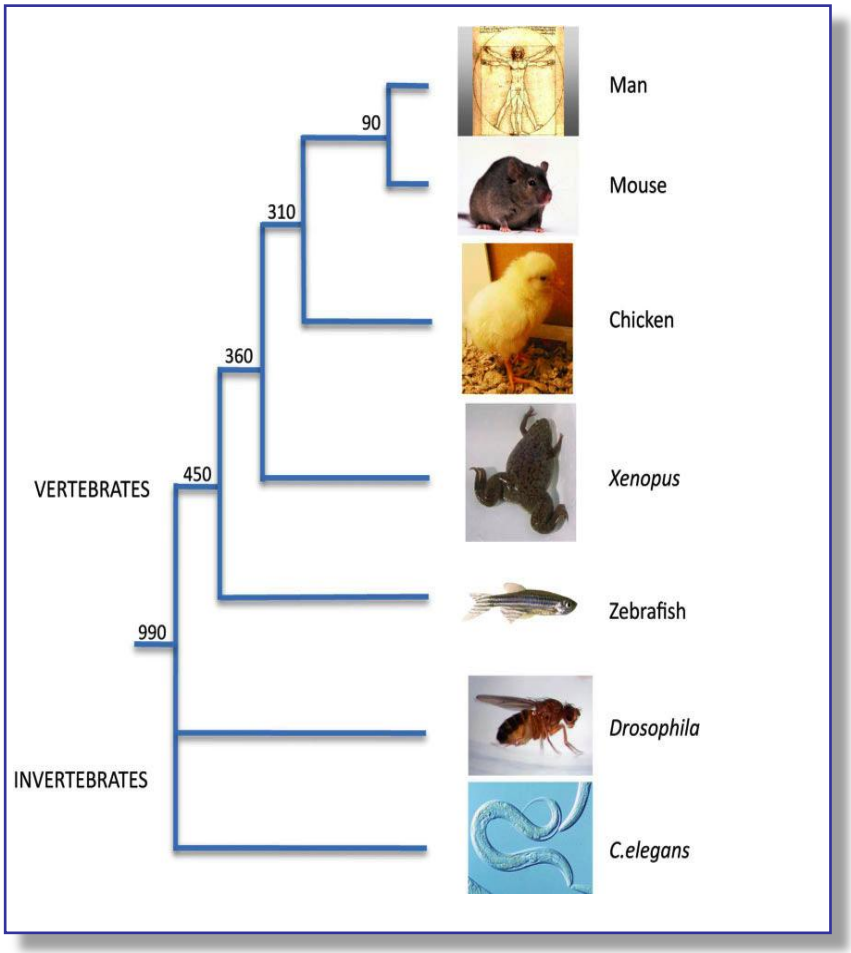
Screening for phenotype modification more efficient than target-based screening tailored to molecular mode of action

First-in-class drugs



Swinney & Anthony, Nat Rev Drug Disc 2011

Screening Suitability of Common Animal Models



	C. elegans	*Drosophila*	Zebrafish	*Xenopus*	Chicken	Mouse
Brood size	250-350	80-100	100-200	1000-5000	1	5-8
Costs per embryo	low	low	low	low	medium	high
High-throughput multiwell-format screening	good	good	good	good	poor	poor
Access to embryos	good	good	good	good	good	poor
Micromanipu-lation of embryos	limited	limited	fair	good	good	limited
Genome	known	known	known	known*	known	known
Genetics	good	good	good	fair*	none	good
Knockdowns (RNAi, morpholinos)	good	good	good	good	limited	limited
Transgenesis	good	good	good	good	poor	good
Evolutionary distance to man	very distant	very distant	distant	intermediate	intermediate	close

Wheeler & Brändli Dev Dyn 2009

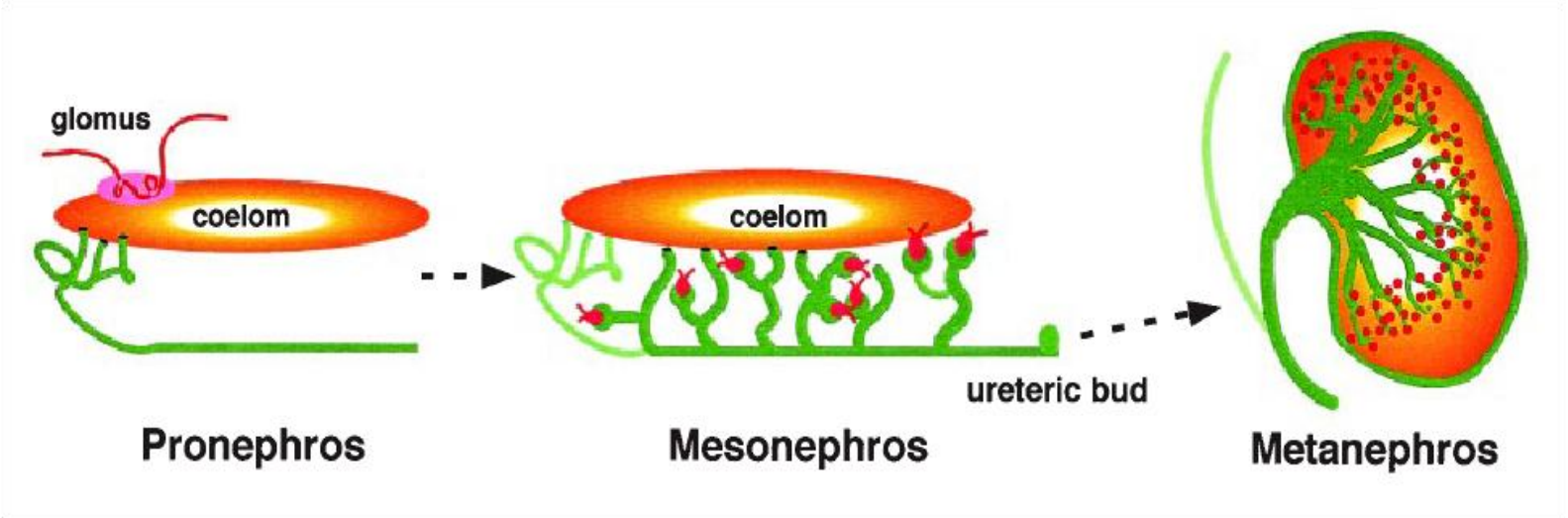
Model Organisms & Model Kidneys

Xenopus tadpole

Mouse

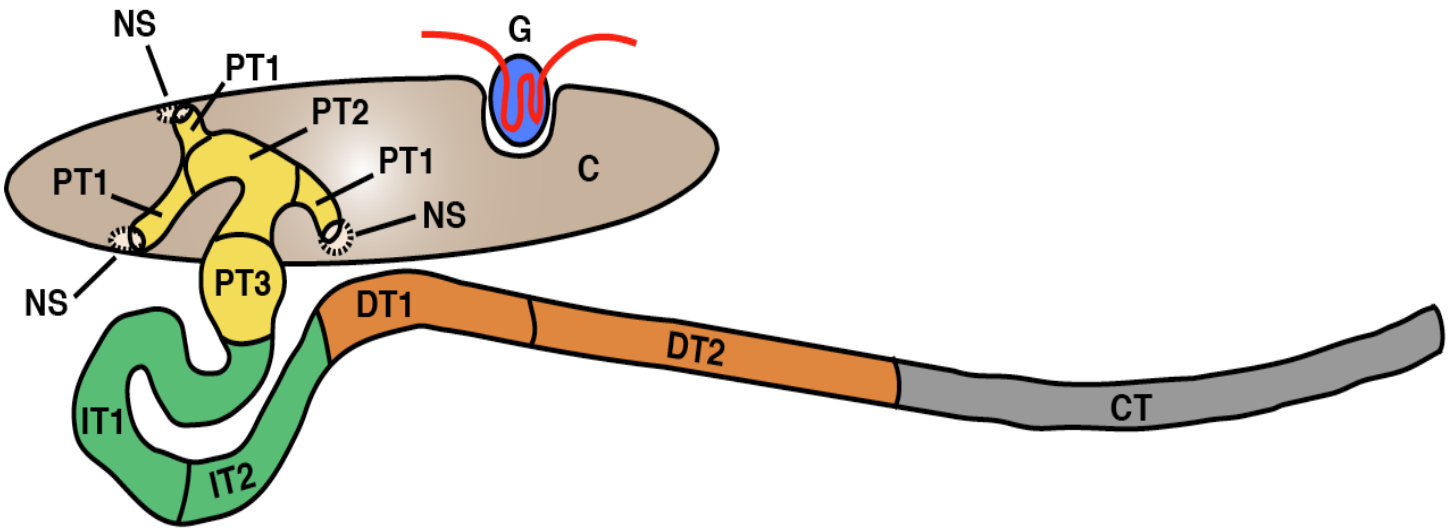
1 nephron

30’000 nephrons



Chan & Asashima, Clin Exp Nephrol 2000

Segmental Organization of the Pronephros



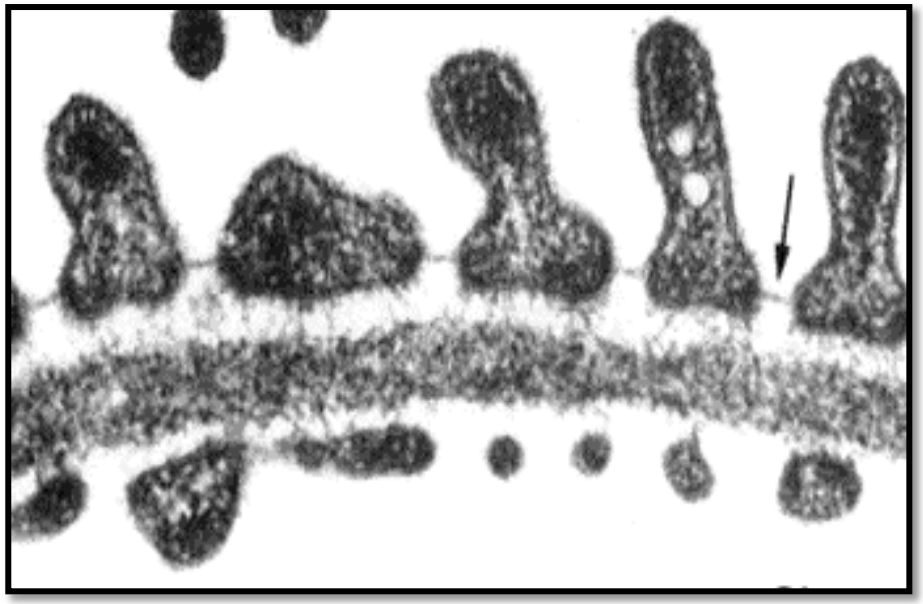
Four segmented tubular compartments defined:

- **Proximal tubule (PT1, PT2, PT3)**
- **Intermediate tubule (IT1, IT2)**
- **Distal tubule (DT1, DT2)**
- **Connecting tubule (CT)**

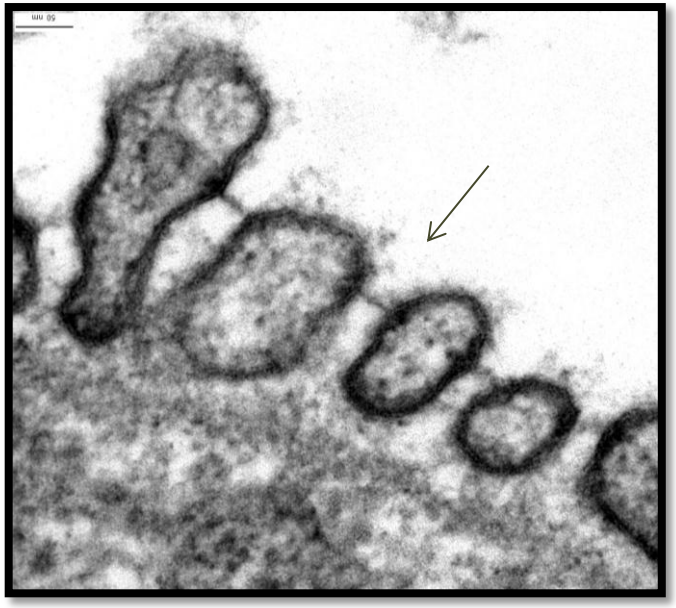
Raciti *et al.* (2008) Genome Biol. 9, R84

Similarity of Glomerular Ultrastructure

Human

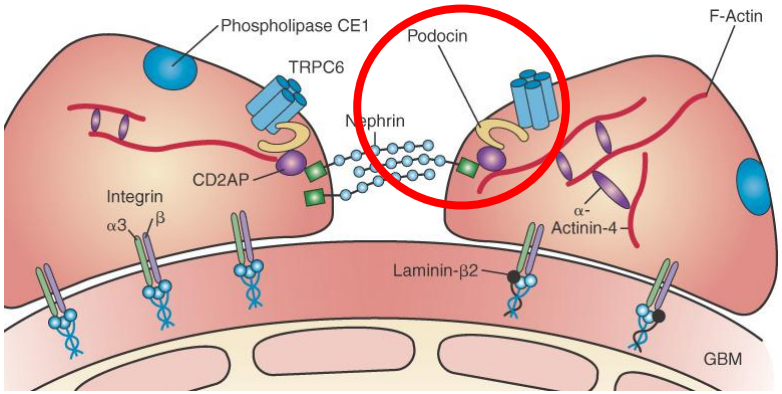


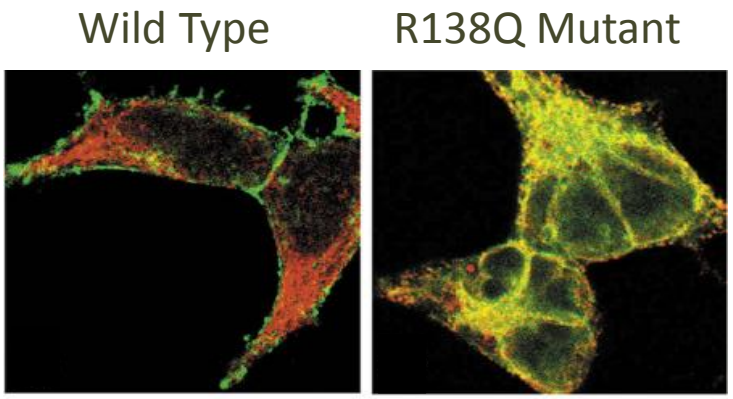
Zebrafish

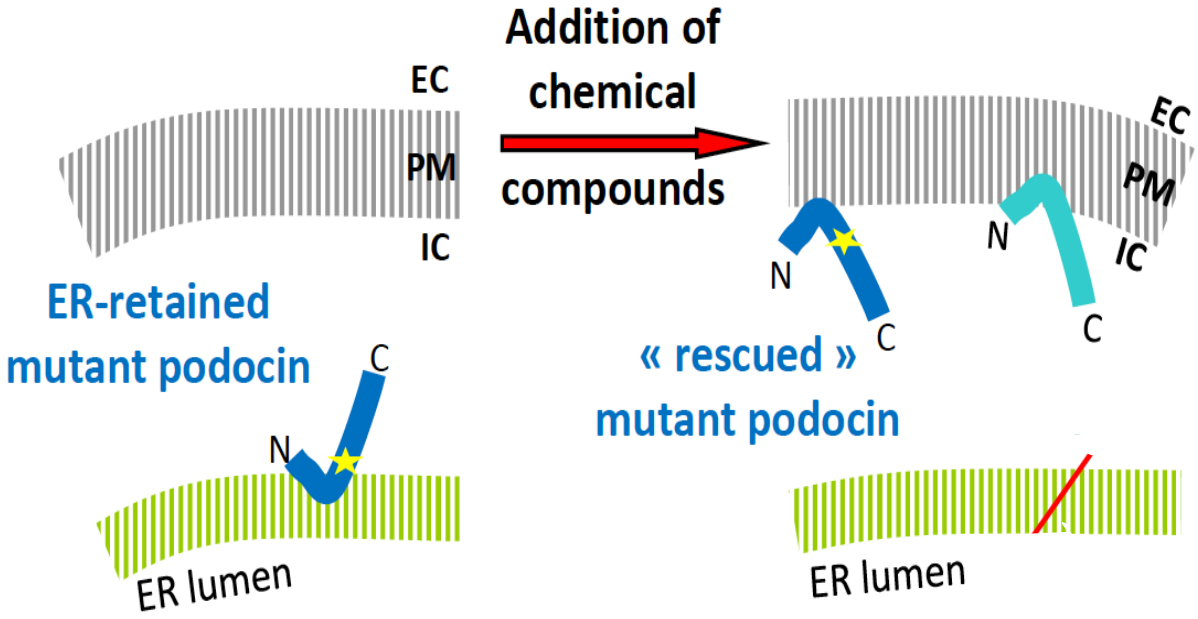


Strategy for Hereditary Nephrotic Syndrome:

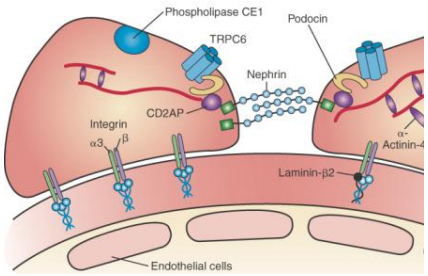
Restoring Plasma Membrane Targeting of Mutant Podocyte Proteins







Strategy for Hereditary Nephrotic Syndrome: Restoring Plasma Membrane Targeting of Mutant Podocyte Proteins



Step 1: High-Throughput Compound Library Screening in Cell Assay

Establishment of cellular models in hTERT-RPE1 cells:
- untagged WT and R138Q podocin (the most common mutation)
- V5-tagged WT and S366R nephrin

Miniaturization/automation of screening steps (BFX plateform)

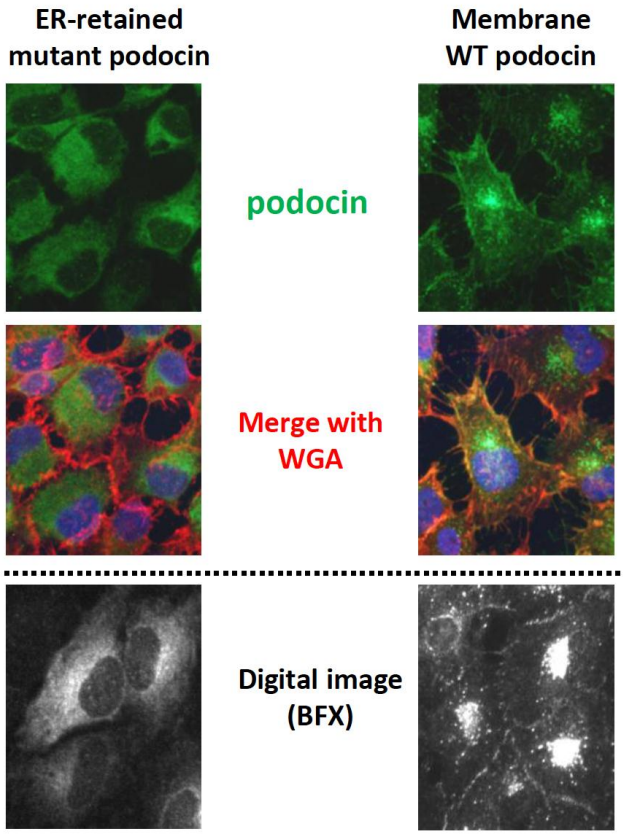
Screening of chemical libraries:

- **Prestwick** (1,200 molecules, 90% being marketed drugs and 10% bioactive alkaloids or related substances)

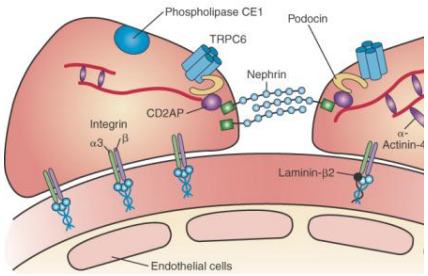
- **Chembridge DIVERSet E** (16,320 drug-like, small molecules, with maximum pharmacophore diversity

- **Enzo FDA-approved drug library** (ref:BML-2841, 800 compounds)

Functionality of "rescued" mutant proteins and characterization of "hits"



Strategy for Hereditary Nephrotic Syndrome: Restoring Plasma Membrane Targeting of Mutant Podocyte Proteins



Step 2a: Candidate Compound Screening in Zebrafish model

Nphs2 knock-down by morpholino:

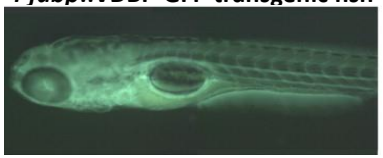
- severe phenotype with cardiac edema, proteinuria and tubulocystic dilation

Albrecht and Drummond, 2005

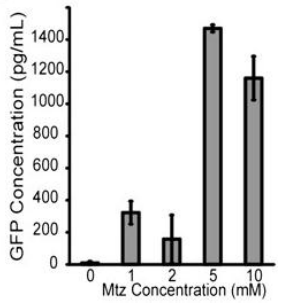
VDBP-GFP fish line

- expression of vitamin D–binding protein fused to GFP
- under control of liver-specific *l-fabp* promoter allowing secretion into blood
- detection of GFP as a tracer of proteinuria: ELISA and fluorescence imaging

***l-fabp*::VDBP-GFP transgenic fish**

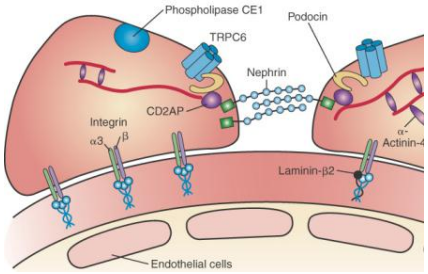


ELISA measurement of GFP in water after podocyte injury



SEVENTH FRAMEWORK PROGRAMME

Strategy for Hereditary Nephrotic Syndrome: Restoring Plasma Membrane Targeting of Mutant Podocyte Proteins



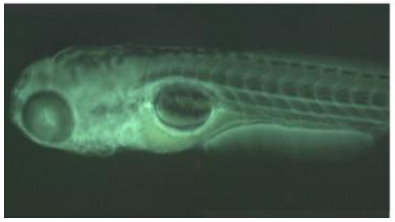
Step 2a: Candidate Compound Screening in Zebrafish model

Establishment of Zebrafish models:

- **null allele model** : *nphs2* knock-out line
- **transgenic models**:
  - WT and R138Q podocin-tagged GFP transgenic lines
  - *Nphs2* null line expressing the VDBP-GFP protein

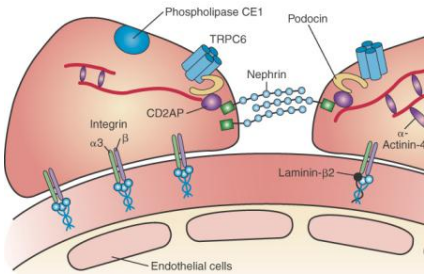
Podocin KO

X



VDBP-GFP fish

Strategy for Hereditary Nephrotic Syndrome: Restoring Plasma Membrane Targeting of Mutant Podocyte Proteins



Step 2a: Candidate Compound Screening in Zebrafish model

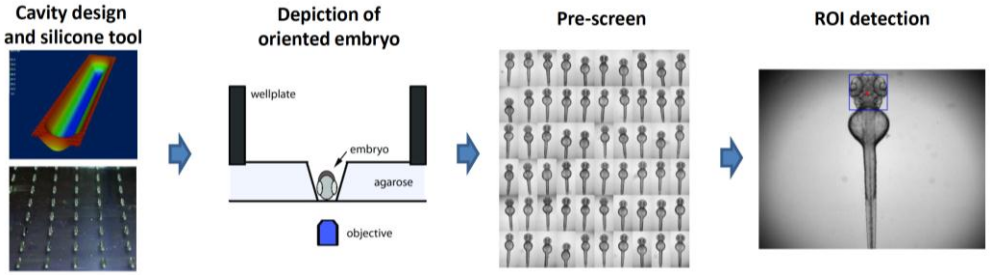
High-throughput drug screening

to identify molecules rescuing phenotype (null allele model)
or retargeting mutant podocin to plasma membrane (transgenic model)

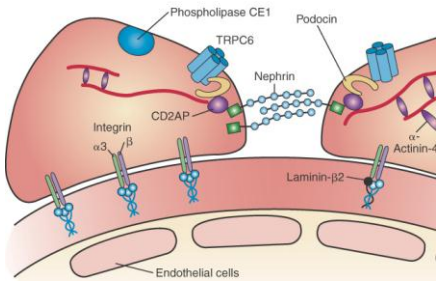
Fully automated screening system using excretion of fluorescent protein and/or pattern recognition (edema, tubulocystic phenotype)

European Zebrafish Resource Center at KIT:

- automated egg sorting into multiwell plates
- automated feature detection and imaging for high resolution screening
- measurement of excretion of fluorescent protein (VDBP-GFP)

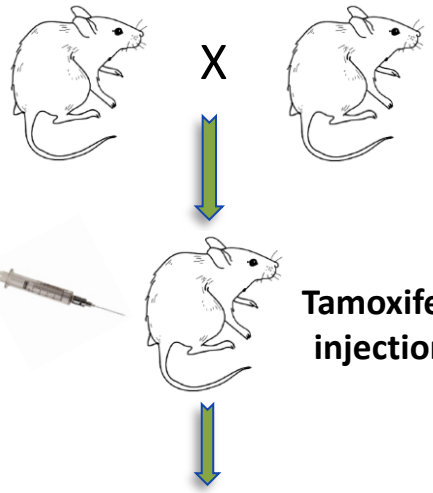


Strategy for Hereditary Nephrotic Syndrome:
Restoring Plasma Membrane Targeting of Mutant Podocyte Proteins



Step 2b: Testing of candidate compounds in a knock-in mouse model

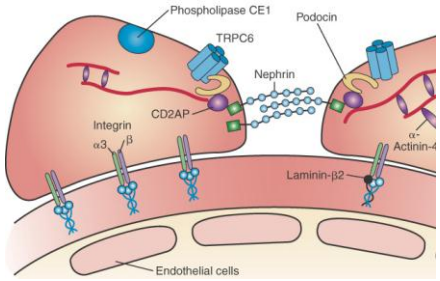
Constitutive knock-in model
Nphs2 R138Q/+



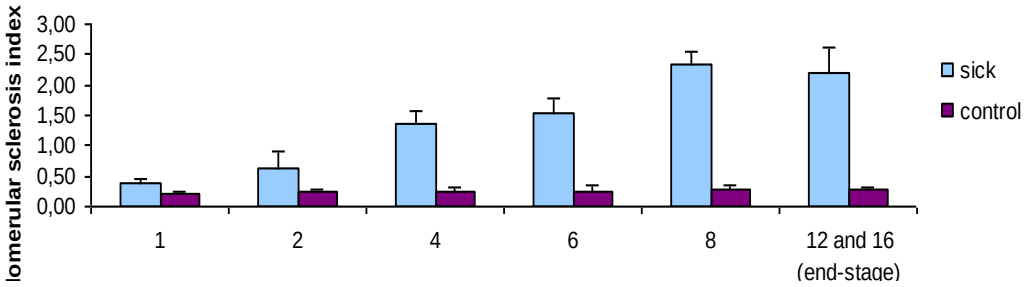
Inducible knock-out model
Nphs2 flox/flox, CRE +

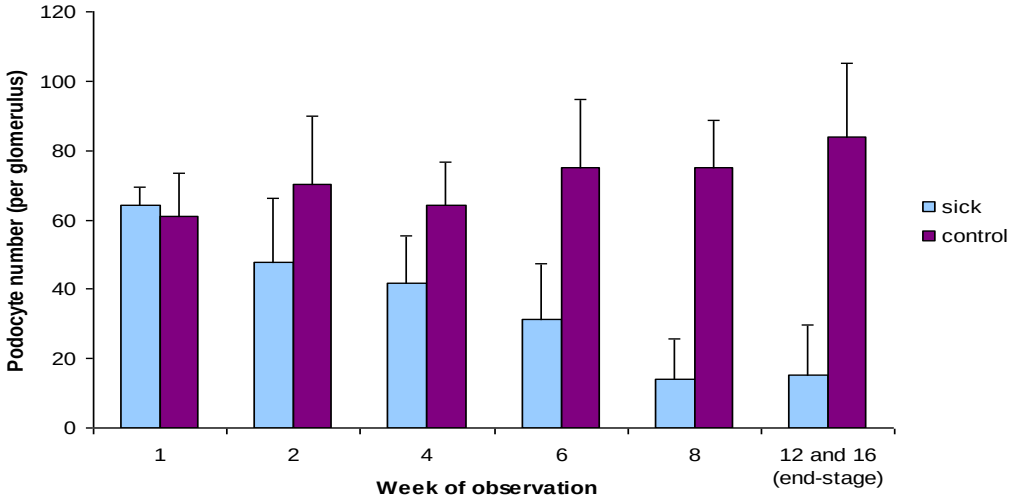
Expression of R138Q mutant podocin ➔ podocytopathy

Strategy for Hereditary Nephrotic Syndrome:
Restoring Plasma Membrane Targeting of Mutant Podocyte Proteins

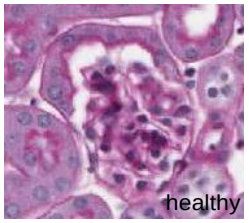


Step 2b: Testing of candidate compounds in a knock-in mouse model

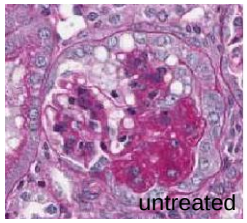




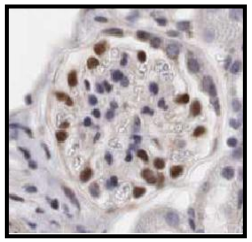
Control



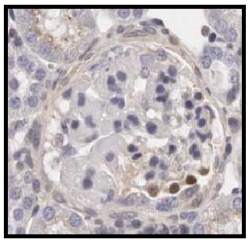
R140Q



Control



R140Q



Drug repurposing is expected to play a major role in the search for new treatments for rare kidney diseases

Three major strategies are emerging:

- Extending existing applications within main mode of drug action
  -> Low regulatory thresholds required

- Utilizing, or building upon, off-target effects of established drugs
  -> Broad, public knowledge base required

- Large-scale phenotype screens
  -> Development of suitable, refined models
  -> accessibility of small compound libraries