Designing Strategies for Therapeutic Progress in Rare Kidney Diseases

Franz Schaefer
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
Targeted Rare Kidney Disorders

WP1:
Nephrotic glomerulopathies

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

**Lectin Pathway**
- Immune Complex Clearance
- Microbial Opsonization

**Classical Pathway**

**Alternative Pathway**

**C3**
- Natural Inhibitors
- Amplification
- \( C_3 + H_2O \) - ALWAYS ACTIVE (Chronic)

**C5**

**C5a**
- Potent Anaphylatoxin
- Chemotaxis
- Proinflammatory
- Leukocyte Activation
- Endothelial Activation
- Prothrombotic

**C5b-9**
- Membrane Attack Complex
- Cell Lysis
- Proinflammatory
- Platelet Activation
- Leukocyte Activation
- Endothelial Activation
- Prothrombotic

**Consequences**
- Anaphylaxis
- Inflammation
- Thrombosis

**Consequences**
- Cell Destruction
- Inflammation
- Thrombosis
Eculizumab binds to C5 and inhibits membrane attack complex formation.
Eculizumab 'Repurposing'

DISEASES ASSOCIATED WITH COMPLEMENT ACTIVATION

- Paroxysmal nocturnal hemoglobinuria
- aHUS
- Membranoproliferative glomerulonephritis
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...

**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


**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 modifying 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%
- CNI sensitivity
- Post-tx recurrence

Steroid-resistant NS
- 15%
- T cell disorder
- Circulating ‘permeability’ factor
- Structural anomalies of glomerular filtration barrier

[Diagrams and illustrations related to renal structures are included, showing podocytes, endothelial cells, and glomerular basement membranes.]
Urokinase plasminogen activator receptor (uPAR): Modifying Matrix Composition and Cell Motility

Podocyte uPAR is required for FP effacement and proteinuria

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

Elevated soluble uPAR causes albuminuria in mice

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

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

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

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Abstract

The urokinase receptor (uPAR) and its soluble form play a key role in the pathogenesis of focal segmental glomerulosclerosis (FSGS). The modified uPAR and biological 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

Swinney & Anthony, Nat Rev Drug Disc 2011
## Screening Suitability of Common Animal Models

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Wheeler & Brändli Dev Dyn 2009
Model Organisms & Model Kidneys

**Xenopus tadpole**

- 1 nephron

**Mouse**

- 30,000 nephrons

The diagram illustrates the development of the kidney from pronephros to metanephros, showing the transition from a single nephron in the pronephros to multiple nephrons in the metanephros.
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

Zebrfish
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 platform)

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

**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

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**Albrecht and Drummond, 2005**

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

**ELISA measurement of GFP in water after podocyte injury**
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
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/+  
  - Expression of R138Q mutant podocin → podocytopathy

- **Inducible knock-out model**
  - *Nphs2* flox/flox, CRE +
  - Tamoxifen injection
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

[Graphs showing the effects of treatment on glomerular sclerosis index and podocyte number per glomerulus for control and R140Q knock-in models at different weeks of observation.]
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