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Drug discovery in Focal and Segmental Glomerulosclerosis

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Abstract

Despite the high medical burden experienced by patients with focal segmental glomerulosclerosis (FSGS), the etiology of the condition remains largely unknown. FSGS is highly heterogeneous in clinical and morphological manifestations. While this presents challenges for the development of new treatments, research investments over the last two decades have yielded a surfeit of potential avenues for therapeutic intervention. The development of many of those ideas and concepts into new therapies, however, has been very disappointing. Here, we describe some of the factors that have potentially contributed to the poor translational performance from this research investment, including the confidence we ascribe to a target, the conduct of experimental studies, and the availability of selective reagents to test hypotheses. We will discuss the significance of genetic and systems traits as well as other methods for reducing bias. We will analyze the limitations for a successful drug development. We will use specific examples hoping that these will guide a consensus for investment and drive greater translational quality. We hope that this substrate will serve to exemplify the tremendous opportunity for intervention as well as facilitate greater collaborative effort between industry, academia and private foundations in promoting appropriate validation of these targets. Only then will we have achieved our goal for curative therapies for this devastating disease.

Keywords

podocyte; proteinuria; focal segmental glomerulosclerosis

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CONFLICT OF INTEREST. A.F. is inventor on pending patents aimed to diagnose or treat proteinuric renal diseases. She stands to gain royalties from their future commercialization. A.F. is consultant for Hoffman-La Roche, Genentech, Janssen, Mesoblast, Abbvie, Boehringer Ingelheim, Alexion, Bristol Myers Squibb. N.P. is an employee of Pfizer.

Epidemiology and key clinical considerations

FSGS is a common cause of primary nephrotic syndrome, with a peak age of onset between 10–45 years; with ~2000 individuals reaching end-stage kidney disease (ESKD) each year¹. As prevalence and incidence can not be precisely estimated due to lack of population based studies, the implementation of longitudinal cohorts of patients affected by nephrotic syndrome as well as the development of national and international registries will be key to understand disease pathogenesis, estimate disease impact, stratify patients, develop alternative clinically meaningful outcome measures and design feasible clinical trials. This is even more important if we consider that the clinical disease course of patients with biopsy proven FSGS is highly heterogeneous, as well as the recurrence rates after transplant, the response to treatment and the morphological pattern as reviewed elsewhere². Successful drug development in the field of FSGS has also been hampered by the fact that the etiology of FSGS remains largely unknown (with 80% of cases being idiopathic) and little consensus as well as confidence on key interventional nodes have been reached, as we will discuss.

While FSGS, and more broadly CKD, represents a silent killer, and affected vulnerable populations such as underrepresented minorities and children are often characterized by a worse clinical outcome, the investment in experimental studies has been somewhat limited in contrast to other disciplines. First, this clearly reflects the influence of the public opinion in the community, which overall is more sensitive to specific areas of research characterized by high morbidity and mortality, such as cancer and heart disease. Interestingly, however, the risk for dying from CKD is equal or superior to certain forms of cancer³, yet, patients are surely more afraid to be informed they have cancer rather than CKD. While a cultural shift and education are needed to generate awareness about this, there is perhaps also a need to make the discipline more glamorous and attractive to researchers. Secondly, dialysis and transplantation remain a lucrative therapeutic alternative to preventive or curative strategies in kidney diseases, thus decreasing the attractiveness of drug development. Finally, the lack of alternative, regulatory acceptable outcome measures^{4–6} as well as methods for better stratifying patients on their risk profile has discouraged industry to invest in drug development and requires further studies. Alternative biomarkers are being explored, for instance the measurement of podocyturia and urinary podocyte mRNA, which maybe especially relevant in FSGS where damage to the podocyte appears to be a key determinant in the disease pathogenesis. These alternatives may help stratify patients, predict disease progression or sensitivity to drug intervention⁷ and certainly more investment as well as qualification is warranted.

The path moving forward

The limitations discussed in the prior paragraph are among the factors that have led to the limited success in the development of new effective drugs for kidney diseases. In fact Nephrology, among other subspecialties in Internal Medicine, has performed very poorly when it comes to conducting randomized clinical trials⁸. Table 1 summarizes ongoing clinical trials in FSGS or treatment resistant nephrotic syndrome. There is a relative paucity of innovative and tailored approaches in development. This may be due to insufficient understanding of the disease pathophysiology, or the confidence in translation of

experimental efficacy studies in animals to studies in humans or the cost of clinical development. With this in mind, the National Institute of Health established in 2012 a National Center for Advancing Translational Science (NCATS). Through two initiatives, the “Discovering New Therapeutic Uses for Existing Molecules (New Therapeutic Uses)” (<http://www.ncats.nih.gov/ntu>), and “Pfizer’s Centers for Therapeutic Innovation (CTI) for NIH Researchers” (<http://www.ncats.nih.gov/cti>) NCATS has facilitated the engagement of multi-disciplinary physician scientists capable of conducting clinical studies and Pharma to support the translation of new ideas. While the first initiative enables access to a diverse portfolio of clinically safe but developmentally curtailed agents and aims to re-purpose an asset for an alternative indication, to date no nephrology programs have been sponsored. This is somewhat surprising given the ingenuity and talent of the nephrology community for exploring therapeutic re-positioning of existing agents, as we will describe below. This contrasts the appetite of most Pharma and biotech companies, for de novo discovery and development of highly differentiated as well as novel approaches. As academicians often lack the expertise in drug discovery and Pharma has limited access to patient samples and clinical databases supporting their development plan, this makes approaches like CTI very attractive for both parties. Indeed, both initiatives place partnership between industry, academia, potentially private foundations and government funds at the center helping to fast track programs with high probability of success. It will also allow for the early implementation of precision medicine initiatives and for their translatability into the arena of clinical care. The role of private foundations such as NephCure Kidney International, engaging patients and families will be key for the success of any development, primarily when rare diseases such as FSGS are being targeted. Furthermore, foundations that are willing to serve as a partnering mediator between the academic investigators and the investors will undoubtedly facilitate the development of new therapeutic strategies. Access to patient registries, developed either by private foundations or supported by the NIH via consortia, that track a longitudinal cohort of affected individuals, as successfully implemented via national and international study networks such as NEPTUNE and CureGN, will be tremendously powerful.

Strategies to identify clinically relevant targets

The glomerulus is a highly specialized structure that ensures the selective ultrafiltration of plasma so that essential proteins are retained in the blood⁹. Podocytes, the primary cellular targets in FSGS, are specialized glomerular epithelial cells whose numerous foot processes support the formation of the slit diaphragm^{10, 11}. Podocytes contribute to the glomerular filtration barrier through a tight regulation of actin cytoskeleton remodeling^{12, 13}. A large variety of new experimental tools have allowed for the identification of specific drug targets that are predominantly expressed by podocytes in health and disease^{14, 15}. In fact, while nephrin was the only component of the glomerular filtration barrier when first discovered in 1998, a large body of new podocyte specific proteins and potential targets have now been identified^{16, 17}.

However, target validation has remained a problem and new strategies to increase the probability of success in drug development remains a matter of intense debate. Therefore, while we do not intend to discuss each of the new targets under experimental and clinical

investigation for FSGS, we will provide some examples on how effective validation of new targets may lead to successful drug development. Some guidance criteria for strategic decisions, target selection and experimental assessment are summarized in Figure 1 and discussed below.

Validation of clinical targets based on genetics

The discovery of Mendelian inherited genes, such as mutations in nephrin, podocin, etc, that pre-dispose individuals to FSGS signposted the significance of the podocyte in proteinuric disease. Their study has provided insight into potential mechanistic causes of proteinuric disease and opened avenues for patient stratification. Many of these genes have been studied in the context of transgenic and knockout systems, providing potentially important disease-relevant models for efficacy studies.

It has been increasingly recognized that the study of disease causing as well as protective alleles can yield to a rich source of important therapeutic targets. Recent industry analyses of clinical success and failure have indicated that portfolios that are rich in assets derived from genetic validation have a greater chance of success than those derived without^{18–20}. Many of the genes that have been shown to contribute to proteinuric disease are not easily or obviously targetable. Remarkably little investment has been made in developing approaches against known casual genes. If we take TRPC6 as an example, multiple gain-of function mutations have been mapped to FSGS^{21, 22}. TRPC6 is a non-selective cation channel that sits at the slit diaphragm. It is a member of a class of channels that are believed to be chemically tractable drug targets. As hyperactivation of the channel is believed to promote podocyte motility, foot process effacement and apoptosis, antagonism of the channel might be desirable in a disease setting. Indeed, there is supporting evidence of increased expression of the channel in other glomerular diseases suggesting that this is a pathway that might have a key disease role²³. However, for several potential reasons this target has not received much attention. Firstly, targeted knockdown or knockout mice alone do not appear to have a significant renal phenotype^{24, 25}, suggestive of potential compensation by other TRP channels or pathways²⁶. To this latter point, TRPC5 has also emerged as a potential podocyte target based on some pre-clinical observations²⁶ and we await evidence that TRPC5 activity is altered in human FSGS in order to underwrite the significance of these findings compared with what is known about TRPC6. As TRPC6 appears to be activated in an AngII-dependent fashion, if channel blockade is not superior to ACE inhibition or ARB treatment alone, then a TRPC6 antagonist might not have the additional clinical horsepower. TRPC6 is also expressed in many other cell types, and this raises potential safety concerns, especially cardiovascular safety. Finally, the quality and availability of selective TRPC6 tools has limited the validation of this approach; even ML204, which was used to assess the potential contribution of TRPC5 in podocytopathy, has significant polypharmacology at the concentrations tested that might limit interpretation of the pre-clinical findings^{26,27}. The barriers for initiating any de novo drug discovery program are considerable.

Increasingly researchers are trying to apply the knowledge of the underlying systems biology, transcriptional and omic networks with the genetic causes to re-purpose known drugs for therapeutic use^{28, 29}. This has been most elegantly demonstrated in patients with

steroid resistant nephrotic syndrome and mutations in *ADCK4*³⁰. *ADCK4* appears to localize to mitochondria, to interact with COQ6. Comparisons with related proteins and COQ mutant phenotypes suggested that *ADCK4* promotes CoQ₁₀ biosynthesis and Q10 deficiency might be driving the phenotype of individuals harboring *ADCK4* mutations. Supporting *in vitro* studies and preliminary human data strongly suggest for this small group of individuals with *ADCK4* mutations a restoration of renal function can be achieved with Q₁₀ replacement. This early success suggests that more examples will emerge from the application of integrated functional genomics and the investigational pharmacopeia.

Validating clinically relevant targets through experimental studies

It is postulated that a hallmark of proteinuric disease is the sustained loss of podocytes from the glomerular tuft, either by injury or impaired function. As these cells have a poor capacity for self-renewal^{31, 32}, the loss of functional podocyte mass on glomerular function is potentially irreversible, leads to progressive scarring and ultimately ESKD. Elegant experimental studies performed by the Wiggins group³³ have shown that selective depletion of 30–40% of glomerular podocyte is sufficient to induce an overt proteinuria and progressive glomerulosclerosis that is phenotypically reminiscent of the clinical situation. These observations, coupled with the high phenotypic penetrance of mutations in key proteins necessary for podocyte function in individuals that present with primary FSGS, have brought the podocyte to the center stage in the visualization and development of therapeutic approaches that seek to either preserve podocyte mass or restore function.

The goal of either an unbiased phenotypic screening approach or hypothesis-driven assessment of any potential target is to conduct studies that generate data that meaningfully signpost a pharmacological effect, offer an insight to differentiation compared with standard of care and a directionally compelling rationale for efficacy in the patient. There are several critical elements that are needed for the development and execution of a podocyte screening funnel; these include:

- a) disease relevant, human based *in vitro* assays and functional endpoints;
- b) orthologous reagents and *in vivo* systems that can be used to evaluate a compound(s) efficacy and concentration/dose response.

Of the >100 podocyte targets and pathways that have been identified from the phenotypic evaluation of transgenic animals, human genetics and systems biology approaches as potential causal contributors to nephrotic syndrome, the task to replicate the observation for each one and evaluate their therapeutic potential would be both time-consuming and cost prohibitive in the absence of sensitive, reproducible as well as predictive semi-high throughput assays. Podocytes are terminally differentiated cells and screening requires potentially large volumes of them. As primary cultures derived from sieved glomeruli are limited by tissue availability (human) or de-differentiate generating heterogenous populations in culture during expansion, many groups have therefore taken advantage of conditionally immortalized human and rodent lines^{34, 35}. However, these cells lack the distinctive morphology of the interdigitated foot process. Whether this is due to the clonal selection, the performance of these cells in the absence of an endothelium and glomerular basement matrix, the conventional 2D structure and lack of microfluidics or just simply that

these cells do not express a full repertoire of podocyte proteins, especially those that form the structure of the slit diaphragm, is not clear. New cell lines and systems for deriving podocytes are being developed^{36, 37} and may offer promise with respect to some of these deficiencies, but as we will discuss we are still far from a system that offers all of the desired features of bio-mimicry. Should we achieve the perfect conditions to culture podocytes, standardized protocols will need to be developed to assess the correlation between *in vitro* read outs of podocyte function and *in vivo* disease observation. Notwithstanding these limitations, some groups have been successful in deriving assays that might mimic some of the mechanistic processes associated with proteinuric disease^{38, 39} and that can be used for drug screening purposes⁴⁰. It will be interesting to determine if high throughput imaging system of podocyte function *in vivo* can be developed, and the scientific progress of this technique has been reviewed elsewhere⁴¹.

The desire to have both rodent as well as human systems stems from two important observations. Firstly, one can not be confident of the translation of data generated in a rodent system to a patient in the absence of an experimental characterization in an equivalent human assay, for instance if there is a difference in receptor expression or pathway redundancy between systems. Much of the emphasis of the research community has focused on rodent derived podocytes as a primary screening assay, reflecting a desire to characterize a mechanism prior to assessment in a rodent *in vivo* efficacy model or that the human cells are more tricky to work with³⁵. Secondly, not all compounds or reagents that one might use to interrogate a human target will have specificity and selectivity for the orthologous rodent target. There are many instances where the failure to understand the orthologous pharmacology of a reagent has generated misleading data.

Studies with cultured podocytes have principally focused on changes in actin cytoskeleton, for instance loss of F-actin stress fibers, which accompany a response to injury, usually induced with puromycin^{40, 42, 43}. This process is believed to underlie the increase in motility and loss of structural stiffness, which accompanies podocyte effacement and loss from the glomerular capillary into the urine. The development of sensitive high content screening (HCS) imaging has enabled the automation and quantitation of F-actin fibers in hundreds of cells/well by decoration with Alexa-labeled phalloidin. With the right reagents it would be possible to develop assays with alternative imaging and functional endpoints, for instance the localization of components of the slit diaphragm at the plasma membrane, the phosphorylation of VASP-1⁴⁴, activation of TRP channels^{26, 45}, accumulation of lipid droplets³⁹ to complement conventional measures of cell morphology, survival, mitochondrial integrity, etc. The increasing availability of relatively affordable HCS technologies (e.g Perkin Elmer Opera) in academic settings are likely to transform the screening and triage of potential therapeutic targets for nephrotic syndrome, whether using podocytes or other cell types. However, these assays take considerable effort and care to develop to ensure robustness and specificity. Ideally the goal of such a system would be for the assay to report a Z' of 0.6, that is an effect size that is greater than >10 standard deviations of assay variability; but in practice a Z' >0.4 is usable. Despite the wide utilization of changes in actin cytoskeleton as a measure of effect, only one group have reportedly developed a murine podocyte HCS assay⁴⁰ that could be used to conduct a phenotypic screen. In these studies, puromycin was used to elicit a concentration-dependent

decrease in cellular stress fibers and synaptopodin expression; an effect that could be attenuated by mizoribine as a positive control. Many companies and organizations have invested in developing libraries and ‘tool boxes’ of compounds with known pharmacology and specificity, for instance well-characterized development candidates and FDA approved drugs. In this study, the screen of a library of >2000 of chemically active compounds obtained from several commercial sources yielded a number of interesting hits. Whilst one might have expected certain classes of compounds to be identified, for instance ROCK inhibitors or other modifiers of the actin cytoskeleton, what was more compelling about the list of hits was that the vast majority did not have a known podocyte target pharmacology, for instance were antimicrobial drugs, or had a potency in the assay that exceeded that of its known pharmacology. Should we be more drawn to the targets that validate our own expectations and biases or observations that were unexpected? The paths to validation in both instances are somewhat similar. For the (apparently) known pharmacology there is always a need to independently validate the hit with a structurally distinct chemotype that has similar pharmacology at the receptor. For those examples without an obvious mammalian target, there is a desire to explore the structure-activity relationship of close-in analogues, which might reveal more potent adducts that could be used as bait in a chemical approach or other to identify the target, as has been recently achieved for thalidomide⁴⁶. Notwithstanding the value to the patient that could be realized by either opportunity, in the academic community there is likely to be less interest and fanfare of a target identified in an unbiased screen for which there was already published precedence. Similarly, if approved drugs already exist, the incentives and costs associated for repurposing these assets presents significant challenges, even before the consideration of the safety of those agents in the patient population. The critical niche then may be the lower hanging fruit of an unprecedented example where perhaps there are some potential good quality chemical or biological leads available with scope for innovation or that might fall under the NCATS initiative. With the potential utility of a PAN injury assay demonstrated, the opportunity afforded for RNAi or mAb library screening or access to more comprehensive chemical libraries through industry partnership should now be seen as a key driver in the expansion of commercially attractive targets.

We should also be cognizant that puromycin may not be the most appropriate disease adjuvant in this setting. It will be interesting to know from ongoing efforts if the phenotype of human podocytes exposed to the sera of affected patients can be utilized as a tool for drug library screening, as suggested by prior studies³⁹. Still, the simplicity and reproducibility of a puromycin-induced cytoskeletal change, while attractive and has some clinical precedent, may not adequately mimic the genetic mechanisms of disease or the ‘circulating factor’ contribution. The target of puromycin in this setting is not known – whether ER stress, induction of autophagy or other – and this limits the confidence of this phenocopy to predict outcome in all nephrotic syndrome patients regardless of cause. To address this, there may be advantages in developing model systems incorporating known genetic mutations, for instance in podocin or TRPC6, which intrinsically drive a disease phenotype or subjecting cells to sera from FSGS patients to assess antagonism of the ‘circulating factor’ component^{38, 44}. Furthermore, as puromycin aminonucleoside nephrosis (PAN) is commonly used as a model of human minimal change nephropathy in rats induced by

injection of puromycin, one should be wary of the value of approaches identified by screening for prevention of an *in vitro* puromycin phenotype and assessed in a PAN model. An *in vivo* assessment in an alternative disease-relevant model, such as LPS challenge, or one of the many genetic models of disease⁴² may provide more meaningful translational confidence from *in vitro* data. Alternatively, as circulating factors are involved in the pathogenesis of podocyte injury in FSGS, the use of appropriately controlled patient sera may set the stage for a clinically relevant phenotypic readouts that can be utilized for drug discovery and not solely as non invasive prognostic marker^{38, 39}.

Better still, if podocytes are challenging to maintain in culture, show diminished expression of key podocyte proteins, lack the distinctive morphology and functional characteristics, then perhaps these 2D systems should be evolved to more sophisticated microfluidics systems. Podocytes *in vivo* are subjected to considerable fluctuations in pressure, indeed both podocytes and isolated glomeruli show increased TRPC6 evoked currents and Ca²⁺ signaling in response to stretch⁴⁵, suggesting that flow is an important in podocyte function in normal physiology and perhaps in disease also. Podocytes are nourished by paracrine factors released by endothelial cells and sit on a unique glomerular basement membrane that is not mimicked in static 2D cultures. Recent studies^{47, 48} have indicated that cells fabricated into a microfluidic environment more closely mimic the *in vivo* physiological situation. So far the attempts to develop a similar system mimicking the glomerulus that could be used to study the effects of AngII or other triggers of podocyte injury on filtration have not been successful. Earlier studies using sieved isolated whole glomeruli⁴⁹ had suggested that it might be possible to functionalize glomerular permeability screens, but development of these systems, even for calcium imaging^{26, 45} has proven equally challenging or are limited by throughput. Despite the caveats and limitations of isolated immortalized podocyte cultures, there is still much innovation that can be made in supporting the identification and triage of novel therapeutic approaches using the guidance described above.

If *in vitro* studies are designed to identify potential targets and test robustly the pharmacology, then the next objective is often to qualify the target in an *in vivo* setting. Genetic models are particularly attractive as genocopy mimics of the known human mutations (e.g. nephrin, PKCε and CD2AP knockout) lead to persistent proteinuria and glomerulosclerosis⁴². Similarly systems, such as those in the zebrafish, that can be used for screening suppressors or enhancers of a particular phenotype or in pharmacological screens⁵⁰ are also being increasingly used. However, because of availability, speed and cost, the PAN and LPS models appear to be the work horses of the community, although the models have a reversible phenotype and no efficacy/endpoint criteria that constitute a clinically meaningful effect have ever been formally recommended. In both instances, there is also a need for PAN/LPS titration, which has the potential to impact model performance and institution-to-institution replication. A number of disciplines have observed relatively poor translational performance of pre-clinical experimental studies^{51–53}, and the renal community are no less immune to the impact of data that are not reproducible⁵⁴. The confidence of the community to seek to invest and develop new therapies is entirely dependent on the quality of the experimental research that underpins it. The absolute requirement of any study, therefore, is that the data can be replicated by others at different

sites and independently by groups with different levels of expertise. Several features of inadequate design and execution have emerged that we shall briefly discuss here to raise awareness, namely:

- 1) **experimental bias:** the lack of animal randomization (for instance a tendency to 'dose cohorts' rather than randomly assign animals to groups) and blinding in both in-life and data analysis phases can influence, both positively as well as negatively, the outcome of the study. This is particularly impactful for studies that are dependent on qualitative or semi-quantitative endpoints, for instance the selection of EM or histology images to support mechanism and efficacy endpoints.
- 2) **statistical rigor:** it's imperative to design the study with adequate power around a specific magnitude change in a primary endpoint, so that appropriate numbers of animals can be assigned to a given cohort. This assumes that a group has sufficient prior experience with a model, in particular the animal and endpoint variability. For models where there is high intrinsic variability or where the phenotype is acutely driven and subject to variable around the measurement day, this can result in study designs with large numbers of animals that might mean the study has to be randomized in phases. By convention studies are often conducted in a prophylactic dosing setting, rather than after the establishment of a disease phenotype, which might be preferred, and the sample sizes for a therapeutic dosing regimen may be very different from a prophylactic dosing regimen. A positive control, to set a reference benchmark on efficacy, is also desirable.
- 3) **evidence of pharmacology:** the utilization of well characterized tools is essential as no compound is exquisitely selective at all concentrations⁵⁵. There is a strong desire for studies to test a dose-response and provide evidence of functional target engagement (proximal pharmacology) independently of the efficacy readout and confirm that the target is expressed in the desired compartment. This is important when one considers the potential difficulty of targeting of proteins expressed on the cell surface of the podocyte and how, for instance, a therapeutic antibody might access this cellular compartment from the vasculature or retain durable exposure at the site of action. Where many transgenic model systems which knockout or overexpress a gene/protein in the podocyte appear to have a renal phenotype, the absence of a correlative pharmacological intervention dilutes the significance of any phenotypic observation.
- 4) **replication:** Whether knockout or transgenic animals, DNA, recombinant proteins, antibodies or small molecule compounds, a pre-requisite of publication should be that these reagents are made available to the community to facilitate replication and develop further evidence of significance of a given axis. Experimental protocols and methods should be sufficiently detailed and unambiguous that someone with sufficient expertise could repeat the studies. If we are to build a consensus on which approaches the community should invest

in, a mechanism for replication should be actively encouraged. Where these criteria are not met, it is clear from the industrial experience of the last few decades^{18, 52, 56} that the development of new medicines is stifled or leads to clinical failure.

Our dependence on, sometimes multiple, rodent efficacy models is driving up research costs without significant translational impact. The many studies that have generated data which have never been replicated or were poorly conducted serves only to propagate uncertainty at a time when the community would benefit greatly from some notion of consensus and quality. Figure 2 highlights some of the potential targets for drug development in FSGS.

CONCLUSIONS

FSGS and other glomerular diseases are an underestimated killer and there is a strong need for new therapeutic developments. Several new potential therapeutic targets have now been identified through human genetics or experimental studies. Yet, it will be only through a strong interaction across different disciplines on perhaps agreed mechanisms that will allow for data replication and success. A prioritization strategy to test clinically relevant hypothesis should be agreed by the scientific community. Strategies that are designed on compelling human biology to develop approaches focused on the modification of major disease nodes that are likely to benefit a large proportion of patients are also desirable. Finally, guidelines on experimental models, screening methods, biomarker research and clinical proof of mechanisms studies will be needed to fast track the development of successful drugs for FSGS and other glomerular diseases.^{57, 58}

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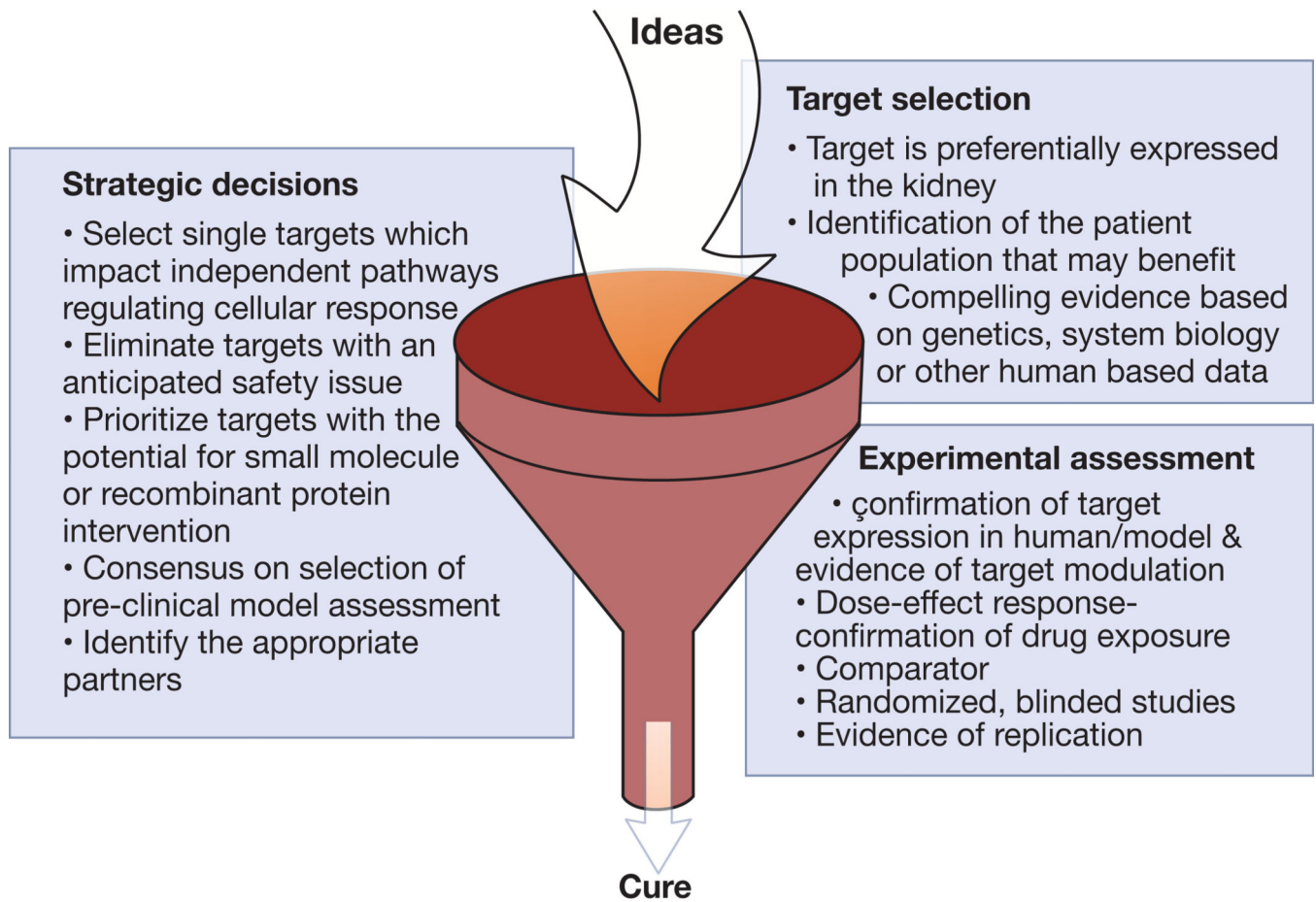


Figure 1. Considerations in intellectual and experimental triage of ideas to target validation

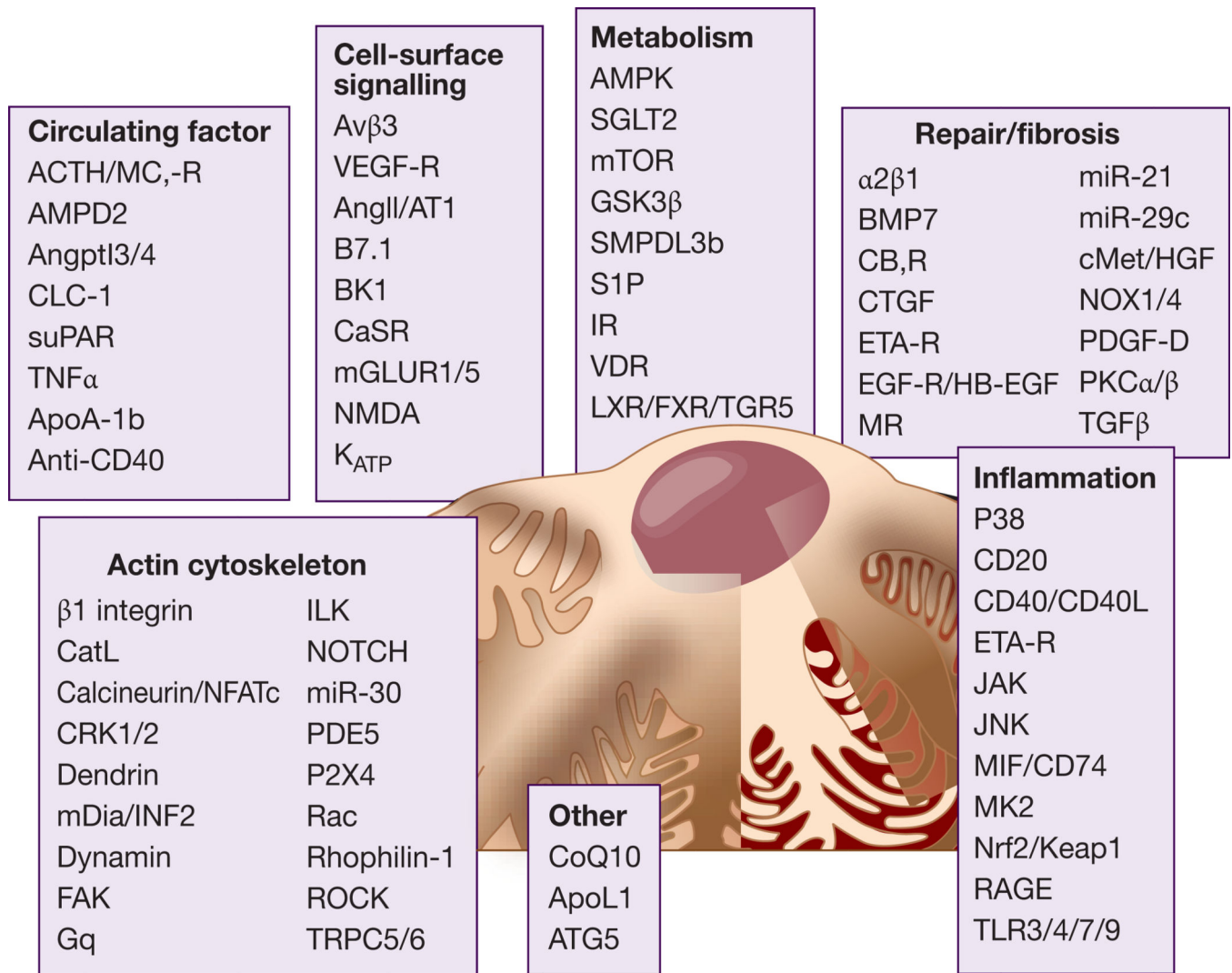


Figure 2. FSGS: clinical and potential experimental targets

Some of the targets that are either in clinical development or that are supported by experimental studies are shown and subdivided into seven categories: 1) Cell-surface signalling, 2) Metabolism, 3) Actin cytoskeleton, 4) Circulating factors, 5) Inflammation, 6) Repair/fibrosis, 7) Others. A limited number of supporting citations about these targets is provided in supplemental materials.

Table 1
A snapshot of ongoing clinical trials in FSGS captured from ClinicalTrials.gov

Identifier (NCT)	Indication	Study design	Asset	Mechanism	Sponsor	Stage	Comments
02382874	FSGS	Single arm open label pilot safety study (n=5)	Allogenic AD- MSC Transplant	Not known	Royan Institute	I	No substantial supporting pre-clinical data
02255857	FSGS	Open label POM study (n=35) Primary = change in UACR at 10 wks	Liposorber LA-15	Not known	Kaneka Pharma America LLC	Ib	No substantial supporting pre-clinical data
02000440	FSGS	Single arm open label POM study (n=24). Primary = 50% reduction in UACR at 24 wks	Losmapimod	p38 kinase inhibition	GSK	Ib	Pathway appears to be altered in FSGS. Some supporting pre-clinical data ^{7,38}
02585804	FSGS	Single arm open label POM study (n=12) Primary = change in inulin GFR at 8 wks	Dapagliflozin	SGLT2 inhibition	AZ	Ib	Rationale is based on observed effects of SGLT2 inhibitors on renal hemodynamics in other diabetic populations (e.g. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. ⁵⁹
01164098	FSGS (post-transplant)	Randomized open label study (n=60) Primary = Prevention of proteinuria recurrence at 4 wks	Rituximab	CD20 B cell depletion SMPDL3b	Genentech/ University Miami	II	Supported by clinical and pre-clinical observations ³⁸
01573533	FSGS (treatment resistant)	Open label study in treatment Primary = remission & change in UACR at 12m	Rituximab	CD20 B cell depletion SMPDL3b	Genentech Mayo	II	Supported by clinical observations
02394106	Nephrotic syndrome (treatment resistant)	Double-blind, two-parallel-arm, placebo-controlled randomized study (n=50) Primary = remission and change in UACR at 6m	Ofatumumab	CD20 B cell depletion	Istituto Giannina Gaslini	II	Supported by clinical observations
01613118	FSGS	Multi-dose randomized placebo control	RE-021/ sparsentan	ETRA/ARB	Retrophin	II	ETRA's appear to improved UACR and

Identifier (NCT)	Indication	Study design	Asset	Mechanism	Sponsor	Stage	Comments
02257697	Nephrotic syndrome	efficacy study (n=100) Primary = change in UACR at 8 wks Randomized controlled open label study (n=238) Primary = remission at 52 wks	Mizoribine	IMPDH Podocyte target not known	Asahi Kasei Pharma	III	fibrosis in pre-clinical models. As a class, though ETRAs have been associated with liver and CV AEs Pre-clinical supporting data ⁴⁰

Legend. NCT: National Clinical Trial; POM: proof of mechanism; UACR: Urinary albumin to creatinine ratio; wks: weeks; AD-MSC: adipose tissue derived mesenchymal stem cells; IMPDH: Inosine-5'-monophosphate dehydrogenase; ETRA: Endothelin Receptor antagonists; CV AEs: cardiovascular adverse events