Published in final edited form as:

Semin Nephrol. 2018 January ; 38(1): 88–97. doi:10.1016/j.semnephrol.2017.09.008.

Translating Knowledge Into Therapy for Acute Kidney Injury

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Summary

No therapies have been shown to improve outcomes in patients with acute kidney injury (AKI). Given the high morbidity and mortality associated with AKI this represents an important unmet medical need. A common feature of all of the therapeutic development efforts for AKI is that none were driven by target selection or preclinical modeling that was based primarily on human data. This is important when considering a heterogeneous and dynamic condition such as AKI, in which in the absence of more accurate molecular classifications, clinical cohorts are likely to include patients with different types of injury at different stages in the injury and repair continuum. The National Institutes of Health precision medicine initiative offers an opportunity to address this. By creating a molecular tissue atlas of AKI, defining patient subgroups, and identifying critical cells and pathways involved in human AKI, this initiative has the potential to transform our current approach to therapeutic discovery. In this review, we discuss the opportunities and challenges that this initiative presents, with a specific focus on AKI, what additional efforts will be needed to apply these discoveries to therapeutic development, and how we believe this effort might lead to the development of new therapeutics for subsets of patients with AKI.

Keywords

Precision medicine; therapeutic development; acute kidney injury

No therapies have been shown definitively to improve clinical outcomes in patients with acute kidney injury (AKI).^{1–4} Although there are many reasons for this, ranging from the paucity of preclinical models that accurately model the types of patients studied in AKI trials⁵ to weaknesses in preclinical and clinical study design,^{6,7} a common feature is that none of these discovery efforts primarily were human data-driven. An illustrative example of this comes from one of the more recent clinical studies of a melanocyte-stimulating hormone (MSH) agonist to prevent AKI in patients after cardiac surgery.⁸

A RECENT THERAPEUTIC FAILURE IN AKI

A synthetic α -MSH agonist, ABT-719 (formerly known as AP214), was evaluated for the prevention of AKI in patients undergoing cardiac surgery.⁸ This was a phase 2B study that

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Conflict of interest statement: none.

was powered properly to test the hypothesis that there is a dose-dependent protective effect of ABT-719 on the development of AKI in high-risk patients 72 hours after surgery. This study built on promising data from a small, unpublished, single-dose study of ABT-719 in cardiac surgery patients. It also was supported by a body of preclinical evidence indicating that α -MSH and AP214 have potent anti-inflammatory effects in a variety of experimental models, including dermal inflammation, inflammatory bowel disease, and arthritis, as well as models of ischemia reperfusion (IR) injury to the brain and kidney.^{9,10} AP214 and α -MSH subsequently were shown to enhance survival and ameliorate AKI in a mouse model of sepsis-associated AKI¹¹; in toxin-induced models of AKI in rats and mice^{12,13}; and, more recently, in IR-induced AKI in pigs.¹⁴ These effects are thought to be mediated by anti-inflammatory effects of melanocortin receptors (MRs), which are expressed in rodent and human monocytes and neutrophils, and possibly via direct protective effects resulting from activation of MR signaling in renal tubular epithelial cells.^{10,15} The required patient enrollment was reached in this large, well-conducted, multicenter clinical study, but unfortunately the study showed no effect of ABT-719 on the primary (early AKI) or secondary end points (90 days >25% reduction in estimated glomerular filtration rate, or urinary or plasma AKI biomarkers).⁸ In retrospect, it is notable that despite the strong supportive preclinical data, there were no published data indicating that MRs are expressed in human kidneys, or that there is aberrant activation of MSH/MR signaling in human kidneys after AKI. Another concern is that although published preclinical data in a variety of species from different laboratories have indicated that AP214 and α -MSH are protective after IR-induced AKI,^{10,16–18} it is not clear that IR injury is the mechanism of AKI after cardiac surgery with modern cardiopulmonary bypass machines.⁵ In addition, although approximately 40% of patients in the ABT-719 study had pre-existing chronic kidney disease (CKD), none of the preclinical studies were performed in AKI models with pre-existing CKD at the time the clinical trial was initiated. In this respect, it is notable that a study published after the ABT-719 study was initiated showed that α -MSH had no protective effects in rats with IR AKI subjected to subtotal nephrectomy to induce CKD.¹⁹ Thus, we would suggest that the reason the ABT-719 study failed to show benefit in humans may have been because: although the therapeutic target, the MSH/MR signaling axis, was validated in multiple preclinical models, we are unaware that it has been studied in human AKI; (2) in the absence of data on the effects of MR pathway activity in human AKI, there was no possibility of evaluating whether ABT-719 was in fact activating this pathway in these studies; (3) none of the preclinical models that were used to support the use of ABT-719 modeled the dominant pathobiology of injury that was likely to be occurring in the clinical study population; and (4) before starting the study, no preclinical studies had been performed using animal models with risk factors for AKI that were included in the clinical trial, including CKD.

AKI IS A HETEROGENEOUS CLINICAL SYNDROME

One of the challenges for therapeutic development in AKI is that it is classified based on simple measures of renal function that do not distinguish between patients with highly variable responses and pathobiology.²⁰ There are many different causes of AKI that often occur in combination with other comorbidities, such as CKD, diabetes, old age, and heart

failure, all of which may affect the underlying pathobiology and therapeutic responses after AKI.^{21,22} In addition, there are marked differences in individual responses to the same injury, with some otherwise indistinguishable patients showing complete functional recovery after AKI, while others have delayed or incomplete recovery.²³ This has implications for therapeutic development because it makes our ability to predict common molecular and cellular mechanisms of AKI nearly impossible. This in turn poses fundamental challenges to the use of modern, largely target-based drug discovery campaigns because these are predicated on a detailed knowledge of the causal cellular and molecular pathways of disease, and creates real challenges for the design of cost-effective clinical trials, because it is difficult to predict clinical outcomes in heterogeneous populations of patients presenting with AKI.²⁴ Finally, even if we had a better understanding of the underlying mechanisms of disease in more clearly defined subsets of patients, the ill-defined phenotypic heterogeneity of patient with AKI in clinical practice makes it nearly impossible to determine whether individual patients will engage the same primary molecular pathways and will respond to therapy in the same way.

MOLECULAR RECLASSIFICATION OF AKI

Most disease classifications are dependent on clinically observable and easily measurable parameters that do not necessarily reflect common pathogenic mechanisms of disease.²⁵ One of the goals of the National Institutes of Health (NIH) Precision Medicine Initiative is to reclassify complex diseases into more clearly defined categories that more closely reflect the cellular and molecular mechanisms of disease.²⁶ AKI is an example of a complex and often multifactorial syndrome that shows a high degree of interindividual variability in responses and outcomes.²¹ Despite this, AKI currently is classified by simple measures of renal function that do not take into account its underlying pathophysiological heterogeneity (Fig. 1).²⁰ In addition, because of concerns about the risks of renal biopsies in patients with AKI,²⁷ we rarely have the benefit of renal histology to determine whether there are common pathologic responses in these patients. The net result of this is that our current classification system for patients with AKI does not provide any insight into the molecular, cellular, or even basic histologic heterogeneity of this syndrome. As part of the NIH Precision Medicine Initiative, one of the stated goals of the Kidney Precision Medicine Project (KPMP) that recently was launched by the National Institute of Diabetes and Digestive and Kidney Diseases is to “ethically obtain and evaluate human kidney biopsies from participants with AKI to create a kidney tissue atlas, define disease subgroups, and identify critical cells, pathways, and targets for novel therapies.”²⁸ If achieved, this will provide an unprecedented opportunity for the renal community to develop a new molecular classification of AKI that accounts for the heterogeneity of this clinical syndrome. The focus of this review is to discuss how this may impact therapeutic discovery for AKI. However, before starting it will be helpful first to consider what these data might look like, how they might be organized, and how these data might be used to drive a molecular reclassification of human AKI.

Cellular and Molecular Interrogation of Human Kidney Biopsies

The KPMP aims to provide detailed molecular phenotyping of kidney biopsy specimens from patients with AKI that is integrated with simultaneous urine and plasma samples, deep

clinical phenotyping including long-term follow-up evaluation, and potentially the inclusion of genetic data from the affected patients.²⁸ The molecular phenotyping of kidney biopsies may include highly dimensional analyses not only of gene and protein expression profiles, but also of metabolites and lipids, all of which reflect changes in cell state and function. In addition, there will be a focus on analyzing changes in cell states and molecular profiles across different cell populations, such as epithelial cells, fibroblasts, and inflammatory cells, rather than the more traditional bulk tissue analyses in which individual cellular changes may be diluted. If successful, this will create a treasure trove of data that will be open to the scientific community and could be interrogated using a variety of advanced computational and analytical tools for systems-based network analyses of the data.²⁹

A Systems Biology Approach

Molecular and cellular analyses may show clear evidence that discrete molecular targets are activated or modified in specific cell types in subsets of patients with AKI. However, although this reductionist approach may show candidate targets for therapeutic discovery, it does not take into account the cellular heterogeneity and molecular interactions that will give rise to highly dimensional and interactive data sets in each biopsy. For example, this may include analyses of cell types and states, neighborhood effects, protein and gene expression and activation, and metabolites and lipids. More comprehensive, systems-based network analyses will be necessary to untangle and properly organize these data to identify common molecular networks and cellular phenotypes that influence cell function and disease phenotypes.^{29,30} Systems-based analyses provide much stronger support for the choice of a molecular target (or targets) driving complex cellular responses because they account for higher-order interactions, downstream responses, and compensatory pathways that may not be identified using reductionist approaches for single target discovery.³¹ In addition, systems-based analyses allow for the identification of common pathogenic or reparative cellular phenotypes that might be exploited in phenotype-driven discovery efforts. If successful, or if even a limited number of dominant pathogenic or reparative molecular networks or cellular phenotypes are identified in subsets of patients with AKI, this will pave the way for evaluating new, human data-driven drug discovery. In addition, by linking renal biopsy studies to simultaneously collected urine and plasma samples, it may be possible to use the KPMP data to develop new biomarker profiles (not necessarily restricted to proteins), that ultimately will enable us to determine the underlying molecular classification of AKI in individual patients without the need for renal biopsy. Finally, this systems biology approach has the potential to link AKI-associated molecular network analyses with human clinical and genetic data, enhancing our ability to predict molecular and therapeutic responses to AKI in individual patients (Fig. 2).

Opportunities and Challenges of the Precision Medicine Initiative

The strength of a multidisciplinary and open access approach to discovery is that it enables us to benefit from the use of diverse, state-of-the-art, analytic approaches from across different institutions. This will enhance the probability of success, but also poses significant informatics challenges: we will need to establish the best ways to format these data sets to support diverse analytical platforms and approaches. In addition, it is unknown whether dominant signals associated with common molecular profiles in patients with AKI can be

identified if we are able to obtain only a limited number of research biopsy specimens. This is complicated further by the fact that AKI is not a static condition, but involves rapidly changing events at distinct phases from initial injury to repair and fibrosis,²² and also commonly is associated with comorbidities such as diabetes, CKD, and old age.^{21,22} All of these effects fundamentally may influence the molecular heterogeneity of kidney biopsy biorepositories used for these discoveries. We have no way of knowing in advance whether dominant signals associated with common molecular networks in patients with AKI will be strong enough to be detectable in these samples above the inevitable biological noise. This is not to say that this effort should not be undertaken. Our current strategies for therapeutic discovery in AKI clearly are not working,¹⁻⁴ and this human data-driven approach for the molecular classification of even subsets of patients with AKI has the potential to transform our approach to therapeutic development (Fig. 2).

THERAPEUTIC DISCOVERY IN THE ERA OF KIDNEY PRECISION MEDICINE

Human data-driven molecular reclassification of AKI has the potential to transform our approach to therapeutic discovery and development. This may occur at a number of different levels that will be discussed separately later, as follows: (1) in early therapeutic discovery with the choice of targets, pathways, and/or phenotypic models; (2) the choice of preclinical models; and (3) in clinical study design.

Early Therapeutic Discovery

Target-based discovery—In the postgenomics era, many companies have used target-based approaches in which distinct molecular targets, usually proteins, are selected and used as the foundation for therapeutic discovery.³²⁻³⁴ This is performed using a known biologic agent that either activates or inhibits this pathway (eg, an antibody or synthetic hormone such as ABT-719), or through the discovery of small molecules from high-throughput screening campaigns using interaction-based screening, structure-based modeling, and/or selective functional assays of target engagement.^{35,36} Systematic, systems-based analyses of highly dimensional cellular and molecular data from human kidney biopsies will help identify with increased confidence which molecular targets are likely to be the primary drivers of key disease-associated cellular responses in subsets of patients with AKI. However, these analyses also may identify molecular pathways that are interconnected through the same hierarchical networks, some of which may cooperate with other drivers of disease-associated molecular responses in AKI, whereas others actually may counteract the effects of modulating other molecular targets in vivo.³¹ This systems biology approach is well recognized in the areas of cancer and antimicrobial therapeutics, in which there are numerous examples in which highly selective drug targeting has been found to be less effective clinically than nonselective or multitargeted approaches to therapy.^{37,38} This may be particularly important when dealing with the complex, multicellular, and dynamically changing tissue interactions that occur in the kidney after AKI, and would alter our approach toward early therapeutic discovery for AKI. For example, this might lead to the screening of mixture-based chemical libraries to identify mixtures of compounds that target multiple competing targets to mediate the desired response in vivo, or might lead to the use of modular approaches in drug design that incorporate more than one targeting structure.^{37,39}

Human data–driven drug repurposing—Another approach that has been used successfully to identify new applications of old drugs has been to exploit established drug-gene expression databases, such as the Connectivity Map and the Library of Integrated Network-based Cellular Signatures,⁴⁰ to identify drugs that induce the inverse molecular signatures to those in human disease.⁴¹ This has been used to identify new cancer therapies and antimalarial drugs,⁴² but in principle also could be applied after using the KPMP data sets to define distinct molecular signatures in defined subsets of patients with AKI. Although these drugs still will need to be validated in preclinical models, this has the advantage that repurposed drugs have well-established pharmacokinetics and toxicity profiles, so that the drug development timeline can be truncated substantially, an important concern for currently untreatable clinical conditions.⁴³

Phenotypic discovery—Despite the focus on target-based approaches for modern therapeutic discovery, in the area of small-molecule discovery, the majority of first-in-class new molecular entities approved by the Food and Drug Administration were discovered through phenotypic screens.^{32,33,44} The advantage of a phenotype-based screen is that it is target-agnostic. This means that phenotypic screens can be performed when there is little insight into the molecular basis of a disease, and may identify compounds that engage multiple targets that would be missed in traditional target-based screens.^{33,44} In addition, unlike target-based screens, phenotypic screens, particularly those performed in live animals such as *Drosophila*, *Caenorhabditis elegans*, and zebrafish, are dependent on the survival of cells and/or the model organisms being tested, so that these assays intrinsically are able to negatively select for compound with poor tissue permeability and toxicity,^{45–47} both of which may be discovered only late in the process of target-based discovery efforts. These assays also can be used as secondary screens for target-based drug discovery efforts to weed out compounds that do not promote the desired phenotypic response at an early stage in drug development because of poor permeability, toxicity, and harmful off-target effects. With the development of new instrumentation and imaging technologies, a variety of increasingly complex models are being developed for AKI drug discovery and toxicity screens, including: (1) induced pluripotent stem (IPS) cell-derived kidney organoids, which form interconnected renal epithelial structures that respond with toxin-induced markers of kidney injury⁴⁸; (2) three-dimensional and microphysiological systems (kidney on a chip) that incorporate cell and organ interactions as well as the flow effects on cell function^{49,50}; and (3) drug screens using whole animals,⁴⁷ notably zebrafish, which has ideal attributes for drug screening in kidney disease including primitive kidney structures and function, blood flow, and glomerular filtration, all of which model those seen in mammalian kidneys.^{51,52}

The key to success of these phenotypic screening assays are in the assay design and in the selection of physiological responses that model the desired therapeutic response in human beings.^{33,44} Human data–driven molecular reclassification of AKI would have a significant impact on assay selection and design. By using human data to determine which molecular networks to target in patients with AKI, parallel molecular analyses will need to be performed to identify the most relevant phenotypic assays (or combination of assays) that are dependent on activation of the same molecular networks that ultimately will be targeted in selective subsets of patients with AKI. With modern gene editing technologies, these

studies can be performed rapidly and without bias.⁵³ In this way we can avoid falling into the trap of believing that just because an assay superficially resembles the clinical phenotype we are targeting, that it is therefore necessarily the best assay for the therapeutic effort in question. A good example of this is with current excitement about the use of IPS cell-derived organoids for drug discovery and toxicity studies.⁵⁴ IPS cell-derived kidney organoids are excellent models of human embryonic kidney epithelium and interstitium, and can be injured with nephrotoxins to induce epithelial injury markers and interstitial cell reactions.⁴⁸ However, these organoids lack a blood supply, glomerular filtration, or tubular flow, and do not have immune responses, and therefore they may recapitulate only some of the molecular and cellular responses seen in adult kidneys after AKI.

Preclinical Models of Human Disease

The transition from therapeutic screen to clinical trial requires considerably more work to optimize absorption, distribution, metabolism, excretion, and toxicity, as well as therapeutic efficacy studies using a variety of different preclinical models.⁵⁵ Ideally, preclinical efficacy studies should be used to define the specific clinical scenarios that are planned for future therapeutic interventions. However, in practice, these kinds of studies rarely are performed in preclinical therapeutic efficacy studies for AKI.⁵ Moreover, concerns about data reproducibility and relevance to human disease have led some to question the validity of any preclinical experimental model that does not use human material for therapeutic discovery.⁵⁶ Data irreproducibility is a serious concern that is being addressed at many different levels by interested stakeholders but is beyond the scope of this review (the reader is referred to a recent review that discusses this problem in detail in the context of AKI research⁶). However, to argue that the lack of translation from experimental models to human disease occurs because preclinical models do not recapitulate the pathophysiology of a human disease, does not take into account the fact that we know so little about the cellular and molecular pathophysiology of human AKI that we cannot know which of the preclinical models is relevant or not relevant to the human conditions being treated. To put this another way, do we really intend to throw the baby out with the bathwater, or should we first try to get a better handle on what exactly it is we are trying to treat?

Better modeling of human AKI—The majority of preclinical studies used to support therapeutic interventions in patients with AKI have failed to incorporate the types of injury that are likely to occur in these patients.⁵ For example, the clinical trial of ABT-719 to prevent AKI in patients undergoing cardiac surgery⁸ was supported by preclinical research in models of sepsis-associated AKI (cecal ligation and puncture),¹¹ and IR-induced AKI in rodents and pigs.^{9,10,14} However, at the time the clinical studies were initiated, there were no data on ABT-719 efficacy in preclinical models of cardiac surgery, and no data on the efficacy of ABT-719 in preclinical models of AKI with pre-existing CKD. It might be argued that there were more data to support a study on the effects of ABT-719 in the prevention of AKI in patients with polymicrobial (gut-associated) sepsis, or that additional data on the efficacy of ABT719 in the prevention of AKI in the setting of CKD might have directed patient recruitment toward subsets of patients who would have been more likely to respond favorably to this therapeutic intervention. In the absence of a better understanding of the human condition, this pragmatic approach of advancing therapeutic trials using

therapeutic agents that are effective in multiple models of AKI in the presence of defined risk factors currently seems to be the most reasonable approach. However, in reality, we have so little understanding of the cellular and molecular mechanisms of AKI in human beings that it is impossible to know whether attempts to mimic the types of patients being evaluated in these clinical trials more closely will increase their likelihood of success.⁵⁷

Molecular modeling of human AKI—It is anticipated that the KPMP will provide us with new, human data–driven insight into cellular and molecular networks that influence both beneficial and detrimental cellular responses in patients with more clearly defined AKI phenotypes. This could and should have a major impact on our approach toward selecting the best preclinical models to assess the likely therapeutic efficacy in defined subsets of patients with AKI. Armed with this knowledge, focused cellular and molecular phenotyping of panels of preclinical AKI models, both with and without commonly associated AKI comorbidities, would identify which models, or, more likely, which combination of models, best reflect the molecular networks that might be targeted in patients with AKI. To effectively integrate these preclinical drug discovery efforts with the KPMP initiative, academia, funding agencies, and industry would do well to form collaborative partnerships that will catalogue the molecular and cellular underpinnings of a broad range of preclinical models of AKI. In this way, therapeutic discovery would be synchronized with the optimal choice of assays and models that reflect the molecular underpinnings of subtypes of AKI that would be targeted for therapeutic intervention studies.

Preclinical biomarkers and target engagement studies—There is a drive to discover not only predictive and prognostic biomarkers for early AKI, but also to identify pharmacodynamic biomarkers that will enable us to monitor whether therapeutic interventions efficiently are engaging their desired targets or responses in patients with AKI.⁵⁸ This is particularly important given the marked differences in drug metabolism and dosing between species⁵⁹: a drug that is effective in one species may be ineffective in another because it is metabolized more rapidly, or does not engage its molecular or cellular target as efficiently. By linking molecular discovery with serum and urinary biomarkers in AKI, the KPMP may enable us to identify protein, lipid, and/or metabolic biomarkers that predict whether specific cellular and molecular targets are being activated in the kidneys of patients with AKI. However, preclinical studies still are required to prove that modification of these biomarker profiles by the therapeutic interventions reflect both therapeutic efficacy and target engagement in the kidney. Currently, very few preclinical therapeutic intervention studies for AKI have included the use of biomarkers to evaluate whether the therapeutic target or pathway has been engaged effectively by the intervention.⁵ Moving forward, it will be important that preclinical studies that are used to model the molecular pathways being targeted in patients with AKI also are used to validate the use of biomarkers as indicators of target engagement.

An integrated approach for preclinical therapeutic development—No single preclinical model of AKI is likely to incorporate the spectrum of cellular and molecular networks that are activated or modified in patients with AKI at different time points during and after the initiating injuries. Therapeutic focus on subsets of patients at defined stages of

AKI progression, for example, during the repair phase after injury, will narrow the spectrum of responses that need to be modeled. However, the complexity of these responses is unlikely to be matched perfectly in the pathophysiological responses associated with any single preclinical model of AKI. For this reason, we believe that an integrated approach should be taken that incorporates human data to direct the development and application of a range of different models throughout the therapeutic development pipeline. To illustrate how this might work, we have provided an example of the workflow that we currently are using for the development of phenylthiobutanoic acid (PTBA) analogs (Fig. 3). PTBA analogs are small molecules that we discovered in collaboration with Dr. Neil Hukriede from the University of Pittsburgh, using a phenotypebased screen in zebrafish larvae.^{60–63} PTBA analogs are nontoxic histone deacetylase inhibitors that halt the progression of AKI to CKD when given late after the initial injury in multiple experimental models of AKI.⁵¹ The goal of our current studies is to identify and develop new PTBA analogs with more favorable pharmacologic and toxicity profiles that are most effective in accelerating recovery and reducing CKD progression in preclinical models of AKI that reflect the types of injury and repair processes that occur in patients with AKI during the recovery phase after injury. Our specific focus is in the prevention of CKD progression during recovery in patients with severe AKI. For these studies, our initial proliferation-based phenotypic assay in zebrafish larvae is complemented by target-based modeling based on the histone deacetylase selectivity of PTBA, as well as in vitro pharmaceutical profiling to optimize the pharmacologic properties, including solubility, permeability, and stability of new compounds in an iterative process of compound design, synthesis, and testing. Compounds with favorable properties are moved into rodent models using pharmacokinetic-based dosing in a robust model of IR-AKI that allows us to evaluate the effects of a short-term interventions on long-term post-AKI renal fibrosis and CKD.⁶⁴ Compounds with favorable pharmacokinetics and efficacy in the IR-AKI model then are passed through a secondary, cell-based screen, involving the use of human IPS cell-derived kidney organoids to assess effects on nephrotoxin-induced kidney injury, before progressing to in vitro safety profiling. These assays will extend our initial mouse IR-AKI screen by determining whether compounds that are effective and nontoxic in mouse IR-AKI, also are likely to be effective and nontoxic in human beings. Pharmacokinetic, efficacy, and toxicity data from these studies will feed back into the iterative compound design, synthesis, and testing process to optimize PTBA analogs further. Analogs that are selected to move forward will next be tested using a panel of toxic and obstructive models of AKI in both male and female mice and rats, both with and without associated AKI comorbidities including old age and diabetes, to determine whether these influence the efficacy of new PTBA analogs in reducing renal fibrosis and CKD progression after AKI.

How might the kidney precision medicine initiative impact this workflow? Our current choice of screening assays and preclinical models has been empiric, based on our limited understanding of proliferation-based repair processes that occurs after AKI. However, it is unknown whether the targets, or any of the zebrafish, rodent, or human models of AKI, however realistic, engage the same molecular and cellular networks that are activated or otherwise modified in patients with AKI, specifically during recovery and repair, which may occur days after the initiating injury. The expectation is that molecular and cellular network

data from the KPMP, combined with molecular and cellular discovery across a range of different preclinical models of AKI, will influence our choice of targets, models, and pharmacodynamic biomarkers at every stage of this drug discovery process (Fig. 3, indicated with red arrows). For example, if it is determined that human IPS cell-derived kidney organoids do not model the molecular responses accurately that occur during the repair phase after AKI in human beings, this assay will be dropped in favor of more closely related rodent and/or cellular models. This will require not only that the KPMP will be able to catalog the spectrum of cellular and molecular responses that occur in the human kidney at different stages after the initiating injury, but also that parallel studies are performed that will provide insight into which molecular and cellular pathways are engaged at different stages after injury in a broad range of screening assays and preclinical models of AKI. To be effective it is essential that both human and experimental data are openly accessible; that they can be cross-referenced easily; and that efforts are made in academia, funding agencies, and industry to avoid the development of impenetrable data silos that would limit access to these large data sets by the entire research community.

Clinical Trial Design

Finally, although not the primary focus of this review, it would be remiss of us not to discuss how the KPMP might impact clinical study design, particularly given the emphasis that has been placed on the need to harmonize models of AKI with the scenarios that ultimately will be tested in clinical trials (Fig. 4).⁵⁷

Patient selection—By facilitating the molecular reclassification of human AKI, particularly if this can be linked with clearly defined clinical, genetic, and/or predictive biomarkers, the KPMP may enable us to identify patients for recruitment into clinical trials who are most likely to respond favorably to specific therapeutic interventions (ie, those targeting specific molecular networks associated with these subgroups of patients) (Fig. 2). However, given the often multi-factorial and dynamic nature of cellular events after AKI, this also presents a serious challenge to the KPMP, particularly given the challenges of obtaining single, let alone sequential, renal biopsy specimens in patients with AKI. This may be a particular challenge in patients during the early phases of injury when risks of biopsy may be greater.⁶⁵ That said, some patients with AKI are more likely to undergo renal biopsy for diagnostic purposes as part of their standard care, particularly patients with delayed recovery after severe AKI.²⁷ This subset of patients would be exposed to only the additional risk of an extra research biopsy core, rather than being exposed to the risks associated with a renal biopsy procedure performed for research purposes only. Time will tell which groups of patients will be recruited through the KPMP, but if it is limited to patients with delayed recovery after AKI, this may be a favorable subset of patients as an initial focus for therapeutic development. Not only may there be shared mechanisms of recovery and repair between patients with different causes for their AKI,⁶⁶ but also more data may become available through the KPMP for their molecular reclassification, providing subsequent focus for human data-driven therapeutic development.

Drug metabolism—Another aspect of the broader NIH Precision Medicine Initiative that could be exploited to streamline AKI clinical trial design is the use of personalized genetic

information in patients to predict differences in drug excretion and metabolism, and to determine effective therapeutic dosing, independently, in each patient.³⁰ Some of these data currently are being used in clinical practice for some drugs,^{67,68} but with more detailed understanding of genetic variants associated with differences in drug excretion and metabolism, genetic data also could be used prospectively to develop personalized dosing regimens for specific drugs based on each patient's genetic information.

Pharmacodynamic biomarkers—One of the limitations of the clinical trial using the α -MSH agonist, ABT-719, to prevent AKI in patients undergoing cardiac surgery, was that the study did not include the use of biomarkers to determine whether the doses of ABT-719 used were sufficient to activate the MSH/MR signaling axis in the kidneys of these patients.⁸ This weakness was mitigated by including the use of three doses of ABT-719 in the clinical trial design: even if the lowest dose had no effect, we might have expected to have seen some effects at higher doses. However, this expectation makes the assumption that any dose of ABT-719 can activate this pathway in human beings, and if it does not, how could ABT-719 have been expected to have any beneficial effects in patients with AKI? If a biomarker had been used to determine what dose of ABT-719 was sufficient to activate the MSH/MR signaling axis in the kidneys of patients with AKI, this information also could have been used as part of an adaptive design to reduce sample size during the course of the study.⁵⁸ This weakness in study design is not unique to the ABT-719 trial. A recent review of this topic failed to identify a single AKI intervention study that has used pharmacodynamic biomarkers as readouts for interventions.⁵⁸ By linking molecular phenotyping of patients with AKI with simultaneously collected urine and plasma samples, the KPMP may allow us to discover candidate biomarkers that reflect activation or modification of defined molecular events in the kidneys of subsets of patients with AKI. Although these urinary or plasma biomarkers still would need to be validated as pharmacodynamic biomarkers for specific therapeutic interventions in independent patient cohorts and in preclinical models, identification of these biomarkers has the potential to radically enhance clinical study design for AKI in this new era of precision medicine-based therapeutic discovery.

CONCLUSIONS

Ultimately, the success of the KPMP in influencing the direction of therapeutic discovery for AKI will require a coordinated effort between all of the interested stakeholders including industry, academia, funding bodies, federal regulators, and patient advocacy groups.⁶⁹ Integration of the complex multidimensional molecular and cellular data from the KPMP with clinical data and biomarkers will have to be performed in an open manner and formatted in such a way that it will support diverse analytic platforms at different institutions and companies. Parallel efforts will be required to develop new molecular classifications for patients with AKI, and to exploit the KPMP data sets to identify new predictive, prognostic, and pharmacodynamic biomarkers that can be used to define which subsets of patients are most likely to respond to specific therapeutic interventions. Finally, and importantly, the success of this initiative also will require a concerted and coordinated but currently unfunded effort to define and catalog the molecular and cellular pathways that are activated or otherwise modified in diverse preclinical models of AKI. This will allow therapeutic

development efforts to focus on the most relevant preclinical models that recapitulate the molecular and cellular networks that are being targeted for therapy.

Acknowledgments

Financial support: Supported by National Institutes of Health grant RO1DK112688 and American Heart Association grant 16GRNT31310011 (M.d.C.); and by National Institutes of Health grants RO1DK51265, RO1DK095785, RO1DK62794, and R24DK103067 (R.H.).

References

1. Billings FT, Shaw AD. Clinical trial endpoints in acute kidney injury. *Nephron Clin Pract.* 2014; 127:89–93. [PubMed: 25343828]
2. Okusa MD, Molitoris BA, Palevsky PM, et al. Design of clinical trials in acute kidney injury: a report from an NIDDK workshop—prevention trials. *Clin J Am Soc Nephrol.* 2012; 7:851–5. [PubMed: 22442188]
3. Weisbord SD, Gallagher M, Kaufman J, et al. Prevention of contrast-induced AKI: a review of published trials and the design of the prevention of serious adverse events following angiography (PRESERVE) trial. *Clin J Am Soc Nephrol.* 2013; 8:1618–31. [PubMed: 23660180]
4. Molitoris BA, Okusa MD, Palevsky PM, et al. Design of clinical trials in AKI: a report from an NIDDK workshop. Trials of patients with sepsis and in selected hospital settings. *Clin J Am Soc Nephrol.* 2012; 7:856–60. [PubMed: 22442184]
5. Skrypnik NI, Siskind LJ, Faubel S, de Caestecker MP. Bridging translation for acute kidney injury with better preclinical modeling of human disease. *Am J Physiol Renal Physiol.* 2016; 310:F972–84. [PubMed: 26962107]
6. de Caestecker M, Humphreys BD, Liu KD, et al. Bridging translation by improving preclinical study design in AKI. *J Am Soc Nephrol.* 2015; 26:2905–16. [PubMed: 26538634]
7. Weisbord SD, Palevsky PM. Design of clinical trials in acute kidney injury: lessons from the past and future directions. *Semin Nephrol.* 2016; 36:42–52. [PubMed: 27085734]
8. McCullough PA, Bennett-Guerrero E, Chawla LS, et al. ABT-719 for the prevention of acute kidney injury in patients undergoing high-risk cardiac surgery: a randomized phase 2b clinical trial. *J Am Heart Assoc.* 2016; 5:8.
9. Chiao H, Kohda Y, McLeroy P, Craig L, Housini I, Star RA. Alpha-melanocyte-stimulating hormone protects against renal injury after ischemia in mice and rats. *J Clin Invest.* 1997; 99:1165–72. [PubMed: 9077523]
10. Kohda Y, Chiao H, Star RA. alpha-Melanocyte-stimulating hormone and acute renal failure. *Curr Opin Nephrol Hypertens.* 1998; 7:413–7. [PubMed: 9690041]
11. Doi K, Hu X, Yuen PS, et al. AP214, an analogue of alpha-melanocyte-stimulating hormone, ameliorates sepsis-induced acute kidney injury and mortality. *Kidney Int.* 2008; 73:1266–74. [PubMed: 18354376]
12. Deng J, Kohda Y, Chiao H, et al. Interleukin-10 inhibits ischemic and cisplatin-induced acute renal injury. *Kidney Int.* 2001; 60:2118–28. [PubMed: 11737586]
13. Kolgazi M, Arbak S, Alican I. The effect of alpha-melanocyte stimulating hormone on gentamicin-induced acute nephrotoxicity in rats. *J Appl Toxicol.* 2007; 27:183–8. [PubMed: 17216604]
14. Simmons MN, Subramanian V, Crouzet S, et al. Alpha-melanocyte stimulating hormone analogue AP214 protects against ischemia induced acute kidney injury in a porcine surgical model. *J Urol.* 2010; 183:1625–9. [PubMed: 20172543]
15. Jo SK, Lee SY, Han SY, et al. alpha-Melanocyte stimulating hormone (MSH) decreases cyclosporine a induced apoptosis in cultured human proximal tubular cells. *J Korean Med Sci.* 2001; 16:603–9. [PubMed: 11641530]
16. Kwon TH, Frokiaer J, Fernandez-Llama P, Knepper MA, Nielsen S. Reduced abundance of aquaporins in rats with bilateral ischemia-induced acute renal failure: prevention by alpha-MSH. *Am J Physiol.* 1999; 277:F413–27. [PubMed: 10484525]

17. Deng J, Hu X, Yuen PS, Star RA. Alpha-melanocyte-stimulating hormone inhibits lung injury after renal ischemia/reperfusion. *Am J Respir Crit Care Med*. 2004; 169:749–56. [PubMed: 14711793]
18. Jo SK, Yun SY, Chang KH, et al. alpha-MSH decreases apoptosis in ischaemic acute renal failure in rats: possible mechanism of this beneficial effect. *Nephrol Dial Transplant*. 2001; 16:1583–91. [PubMed: 11477159]
19. Skott M, Norregaard R, Birke-Sorensen H, et al. Development of intestinal ischemia/reperfusion-induced acute kidney injury in rats with or without chronic kidney disease: cytokine/chemokine response and effect of alpha-melanocyte-stimulating hormone. *Kidney Res Clin Pract*. 2014; 33:79–88. [PubMed: 26877955]
20. Chawla LS. Disentanglement of the acute kidney injury syndrome. *Curr Opin Crit Care*. 2012; 18:579–84. [PubMed: 23079617]
21. Bellomo R, Kellum JA, Ronco C. Acute kidney injury. *Lancet*. 2012; 380:756–66. [PubMed: 22617274]
22. Molitoris BA. Therapeutic translation in acute kidney injury: the epithelial/endothelial axis. *J Clin Invest*. 2014; 124:2355–63. [PubMed: 24892710]
23. Chawla LS, Bellomo R, Bihorac A, et al. Acute kidney disease and renal recovery: consensus report of the Acute Disease Quality Initiative (ADQI) 16 Workgroup. *Nat Rev Nephrol*. 2017; 13:241–57. [PubMed: 28239173]
24. Liu KD, Glidden DV. Clinical trials for acute kidney injury: design challenges and possible solutions. *Curr Drug Targets*. 2009; 10:1190–5. [PubMed: 19715539]
25. Loscalzo J, Barabasi AL. Systems biology and the future of medicine. *Wiley Interdiscip Rev Syst Biol Med*. 2011; 3:619–27. [PubMed: 21928407]
26. Collins FS, Varmus H. A new initiative on precision medicine. *N Engl J Med*. 2015; 372:793–5. [PubMed: 25635347]
27. Fiorentino M, Bolignano D, Tesar V, et al. Renal biopsy in 2015—from epidemiology to evidence-based indications. *Am J Nephrol*. 2016; 43:1–19. [PubMed: 26844777]
28. NIDDK. [Accessed on: Oct 3rd 2017] Kidney Precision Medicine Project (RFA). 2016. Available from: <https://www.niddk.nih.gov/research-funding/research-programs/kidney-precision-medicine-project-kpmp>
29. Wang RS, Maron BA, Loscalzo J. Systems medicine: evolution of systems biology from bench to bedside. *Wiley Interdiscip Rev Syst Biol Med*. 2015; 7:141–61. [PubMed: 25891169]
30. Singer DR, Zair ZM. Clinical perspectives on targeting therapies for personalized medicine. *Adv Protein Chem Struct Biol*. 2016; 102:79–114. [PubMed: 26827603]
31. Maggiora GM. The reductionist paradox: are the laws of chemistry and physics sufficient for the discovery of new drugs? *J Comput Aided Mol Des*. 2011; 25:699–708. [PubMed: 21698487]
32. Swinney DC, Anthony J. How were new medicines discovered? *Nat Rev Drug Discov*. 2011; 10:507–19. [PubMed: 21701501]
33. Lee JA, Berg EL. Neoclassic drug discovery: the case for lead generation using phenotypic and functional approaches. *J Biomol Screen*. 2013; 18:1143–55. [PubMed: 24080259]
34. Eder J, Sedrani R, Wiesmann C. The discovery of first-in-class drugs: origins and evolution. *Nat Rev Drug Discov*. 2014; 13:577–87. [PubMed: 25033734]
35. Mullard A. 2015 FDA drug approvals. *Nat Rev Drug Discov*. 2016; 15:73–6. [PubMed: 26837582]
36. Mullard A. 2016 FDA drug approvals. *Nat Rev Drug Discov*. 2017; 16:73–6. [PubMed: 28148938]
37. Medina-Franco JL, Giulianotti MA, Welmaker GS, Houghten RA. Shifting from the single to the multitarget paradigm in drug discovery. *Drug Discov Today*. 2013; 18:495–501. [PubMed: 23340113]
38. Hopkins AL. Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol*. 2008; 4:682–90. [PubMed: 18936753]
39. Zhang W, Pei J, Lai L. Computational multitarget drug design. *J Chem Inf Model*. 2017; 57:403–12. [PubMed: 28166637]
40. Musa, A., Ghorraie, LS., Zhang, SD. A review of connectivity map and computational approaches in pharmacogenomics. *Brief Bioinform*. 2017 Jan 9. pii: bbw112. <http://dx.doi.org/10.1093/bib/bbw112>. [Epub ahead of Print]

41. Chen B, Butte AJ. Leveraging big data to transform target selection and drug discovery. *Clin Pharmacol Ther.* 2016; 99:285–97. [PubMed: 26659699]
42. Li J, Zheng S, Chen B, Butte AJ, Swamidass SJ, Lu Z. A survey of current trends in computational drug repositioning. *Brief Bioinform.* 2016; 17:2–12. [PubMed: 25832646]
43. Cha, Y., Erez, T., Reynolds, IJ., et al. Pharma perspective on drug repurposing. *Br J Pharmacol.* 2017 Mar 29. <http://dx.doi.org/10.1111/bph.13798>. [Epub ahead of print]
44. Vincent F, Loria P, Pregel M, et al. Developing predictive assays: the phenotypic screening "rule of 3". *Sci Transl Med.* 2015; 7:293ps215.
45. Strange K. Drug discovery in fish, flies, and worms. *ILAR J.* 2016; 57:133–43. [PubMed: 28053067]
46. Williams CH, Hong CC. Zebrafish small molecule screens: taking the phenotypic plunge. *Comput Struct Biotechnol J.* 2016; 14:350–6. [PubMed: 27721960]
47. Giacomotto J, Segalat L. High-throughput screening and small animal models, where are we? *Br J Pharmacol.* 2010; 160:204–16. [PubMed: 20423335]
48. Morizane R, Bonventre JV. Kidney organoids: a translational journey. *Trends Mol Med.* 2017; 23:246–63. [PubMed: 28188103]
49. Wilmer MJ, Ng CP, Lanz HL, Vulto P, Suter-Dick L, Masereeuw R. Kidney-on-a-chip technology for drug-induced nephrotoxicity screening. *Trends Biotechnol.* 2016; 34:156–70. [PubMed: 26708346]
50. Maschmeyer I, Lorenz AK, Schimek K, et al. A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. *Lab Chip.* 2015; 15:2688–99. [PubMed: 25996126]
51. Hukriede, N., Vogt, A., de Caestecker, M. Drug discovery to halt the progression of AKI to CKD: a case for phenotypic drug discovery in AKI. *Nephron.* 2017 Jun 15. <http://dx.doi.org/10.1159/000476079>. [Epub ahead of print]
52. Swanhart LM, Cosentino CC, Diep CQ, Davidson AJ, de Caestecker M, Hukriede NA. Zebrafish kidney development: basic science to translational research. *Birth Defects Res C Embryo Today.* 2011; 93:141–56. [PubMed: 21671354]
53. Lander ES. The heroes of CRISPR. *Cell.* 2016; 164:18–28. [PubMed: 26771483]
54. Shi Y, Inoue H, Wu JC, Yamanaka S. Induced pluripotent stem cell technology: a decade of progress. *Nat Rev Drug Discov.* 2017; 16:115–30. [PubMed: 27980341]
55. Barton P, Riley RJ. A new paradigm for navigating compound property related drug attrition. *Drug Discov Today.* 2016; 21:72–81. [PubMed: 26404453]
56. Pound P, Bracken MB. Is animal research sufficiently evidence based to be a cornerstone of biomedical research? *BMJ.* 2014; 348:g3387. [PubMed: 24879816]
57. Okusa MD, Rosner MH, Kellum JA, Ronco C. Acute Dialysis Quality Initiative XIII Workgroup. Therapeutic targets of human AKI: harmonizing human and animal AKI. *J Am Soc Nephrol.* 2016; 27:44–8. [PubMed: 26519086]
58. Parikh CR, Moledina DG, Coca SG, Thiessen-Philbrook HR, Garg AX. Application of new acute kidney injury biomarkers in human randomized controlled trials. *Kidney Int.* 2016; 89:1372–9. [PubMed: 27165835]
59. Martignoni M, Groothuis GM, de Kanter R. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert Opin Drug Metab Toxicol.* 2006; 2:875–94. [PubMed: 17125407]
60. Skrypnik NI, Sanker S, Skvarca LB, et al. Delayed treatment with PTBA analogs reduces postinjury renal fibrosis after kidney injury. *Am J Physiol Renal Physiol.* 2016; 310:F705–16. [PubMed: 26661656]
61. Cianciolo Cosentino C, Skrypnik NI, Brilli LL, et al. Histone deacetylase inhibitor enhances recovery after AKI. *J Am Soc Nephrol.* 2013; 24:943–53. [PubMed: 23620402]
62. de Groh ED, Swanhart LM, Cosentino CC, et al. Inhibition of histone deacetylase expands the renal progenitor cell population. *J Am Soc Nephrol.* 2010; 21:794–802. [PubMed: 20378823]

63. Novitskaya T, McDermott L, Zhang KX, et al. A PTBA small molecule enhances recovery and reduces postinjury fibrosis after aristolochic acid-induced kidney injury. *Am J Physiol Renal Physiol*. 2014; 306:F496–504. [PubMed: 24370591]
64. Skrypnyk NI, Harris RC, de Caestecker MP. Ischemia-reperfusion model of acute kidney injury and post injury fibrosis in mice. *J Vis Exp*. 2013; 78 2013.
65. Corapi KM, Chen JL, Balk EM, Gordon CE. Bleeding complications of native kidney biopsy: a systematic review and meta-analysis. *Am J Kidney Dis*. 2012; 60:62–73. [PubMed: 22537423]
66. Palant CE, Amdur RL, Chawla LS. The acute kidney injury to chronic kidney disease transition: a potential opportunity to improve care in acute kidney injury. *Contrib Nephrol*. 2016; 187:55–72. [PubMed: 26882009]
67. Peterson JF, Field JR, Unertl KM, et al. Physician response to implementation of genotype-tailored antiplatelet therapy. *Clin Pharmacol Ther*. 2016; 100:67–74. [PubMed: 26693963]
68. Weeke P, Roden DM. Applied pharmacogenomics in cardiovascular medicine. *Annu Rev Med*. 2014; 65:81–94. [PubMed: 24111889]
69. Linde PG, Archdeacon P, Breyer MD, et al. Overcoming barriers in kidney health-forging a platform for innovation. *J Am Soc Nephrol*. 2016; 27:1902–10. [PubMed: 27127187]

Figure 1.

Current classification of AKI. Current clinical classification of AKI relies largely on shared functional responses and outcomes that do not take into account how the varied and often poorly defined causes of injury interact with the patients' own genetic background and relevant comorbidities to define the underlying pathophysiology of injury and repair in an individual patient after AKI.

Figure 2.

Molecular reclassification of AKI through the precision medicine initiative. Systems-based approaches will be used to integrate highly dimensional tissue and patient-derived data sets to define subsets of patients with shared molecular responses to injury after AKI. This in turn could lead to the molecular reclassification of subsets of patients with AKI, the identification of patient-specific therapeutic targets, and the identification of genetic and biological markers of these molecular subsets of patients. It is anticipated that, if successful, this integrated, systems-based approach to interrogate patients with AKI will significantly enhance both the selection of suitable therapeutic targets and phenotypes for preclinical development, and our ability to select responsive patients for clinical trials of patients with AKI.

Figure 3.

An integrated approach for therapeutic development of small molecules. Workflow is indicated by blue arrows, and red arrows indicate critical points for human molecular data-driven modeling. Abbreviation: IPSC, induced pluripotent stem cells; PK, pharmacokinetics.

Figure 4.

Use of molecular classification to optimize patient selection and clinical trial design. If successful, it is anticipated that the precision medicine initiative will identify clinical parameters and genetic and biological markers that are predictive of distinct molecular responses to injury in patients with AKI. By integrating these findings with preclinical study design, we will be able to select patients who are most likely to respond to the specific therapeutic intervention being tested. Additional benefits of this approach may include the development of biomarkers that are predictive of target engagement by the therapeutic intervention (pharmacodynamic [PD] biomarkers), and the use of patient genotypes to predict drug metabolism and therefore optimal pharmaceutical dosing to achieve target engagement in the kidney.