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Clinical Trials in Acute Kidney Injury: Current Opportunities and Barriers

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Summary Report

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OPENING REMARKS AND OBJECTIVES

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Acute kidney injury (AKI) has been a focus of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) for more than a decade. A meeting was held approximately 10 years ago at NIDDK to set an AKI research agenda. Changes have occurred in the past decade that make it an ideal time to review what has been accomplished and develop a research agenda for the future. Several clinical trials have provided critical information for setting the research agenda. The NIDDK must prioritize research in areas that likely will provide public health benefits in the near future.

Meeting organizers have brought together a diverse group of individuals interested in AKI. They include National Institutes of Health (NIH) researchers, academic researchers, nonprofit research organizations, pharmaceutical and device companies, and representatives from the American Society of Nephrology as well as from other Federal agencies such as the U.S. Food and Drug Administration (FDA). The NIDDK is interested in collaborations to identify emerging products that may be ready for clinical trials in patients with AKI. Over the past several years, the NIDDK's Division of Kidney, Urologic, and Hematologic (KUH) Diseases has undergone a paradigm shift that facilitates meaningful clinical trials, with support from various partners including industry. This shift, which began last year, includes establishing a dialog with industry and academics to discuss all sides of issues that can impact research directions.

The expectations for this meeting include comprehensive discussions on the latest AKI research, with a focus on new compounds that are ready for human AKI clinical trials. The breakout sessions are intended to produce practical information that can be translated into a publication and used widely to facilitate effective study designs.

At the 2000 AKI meeting, many discussions focused on the need for NIH-facilitated and supported clinical and translational AKI studies. NIH guidance was needed especially to overcome the significant barriers to planning and conducting AKI trials. These included patient-related, diagnostic, therapeutic, and financial challenges. Although many of these same barriers exist today, there has been progress. Concerning patient-related barriers, for example, progress has been made on a consensus AKI definition, understanding patient heterogeneity, defining useful measures of early diagnosis, stratification of risk, and the identification of endpoints that will satisfy requirements of patient care, the needs of industry, and regulatory groups, including the FDA.

The future AKI research agenda will depend on the ideas generated by participants and presented to the NIDDK during this meeting. Participants are encouraged to provide their perspective on barriers and problems in AKI research.

LESSONS LEARNED FROM AKI MEETINGS/TRIALS

Moderator: *John Stokes, M.D., Professor, Division of Nephrology, Department of Internal Medicine, University of Iowa, Iowa City, IA*

State of the Art Lecture: Challenges and Successes in AKI Trials—Past Trials and AKI Network (AKIN) Viewpoints

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Although a number of pharmacologic agents such as dopamine, fenoldopam, loop diuretics, atrial natriuretic peptide (ANP), insulin-like growth factor-1 (IGF-1), thyroxine, and erythropoietin have been successful in animal studies of established AKI, translation of success to human disease has been poor. For example, ANP, when used in combination with dopamine two days after ischemia-reperfusion injury (IRI) in an AKI animal, showed an improvement in kidney function and a reduction in serum creatinine concentration (SCr) (Conger et al., *Kidney Int* 1991). However, clinical benefit was not observed in clinical trials in humans. In an initial study, anaritide was associated with no improvement in dialysis-free survival in patients with non-oliguric AKI, although there was a trend toward benefit in oliguric patients (Allgren et al., *N Engl J Med* 1997). A second study, restricted to oliguric patients, was considered a negative trial, with anaritide administration improving dialysis-free survival from 15% to 21% ($p=0.22$) as compared to placebo (Lewis et al., *Am J Kidney Dis* 2000). Several factors may have contributed to the lack of benefit in these studies. In both studies, patients were given anaritide late in the course of AKI, when SCr levels ranged from 4-5 mg/dL and anaritide infusion was associated with an increased incidence of hypotension. In a more recent pilot study in which lower doses that were not associated with hypotension were administered earlier in the course of AKI in patients after cardiac surgery (mean SCr 2.3±0.1 mg/dL), ANP was associated with a decreased need for dialysis and improved dialysis-free survival (Sward et al., *Crit Care Med* 2004).

In animal studies, IGF-1 ameliorated the increase in SCr and mitigated the weight loss associated with AKI following IRI compared to controls (Miller et al., *Proc Nat Acad Sci* 1992). However, no benefit was observed when this agent was tested in a clinical trial (Hirschberg et al., *Kidney Int* 1999). Once again, however, patients were not enrolled into the trial until SCr levels were markedly elevated and IGF-1 infusion was associated with significant hypotension.

Recognition that increases in SCr are a lagging marker of kidney injury in patients with AKI has resulted in efforts to find early biomarkers of AKI. In this paradigm, the biomarker would identify patients at a stage of incipient damage, before there is a decline in glomerular filtration rate (GFR). Although there are numerous candidate biomarkers, none have been adequately validated in clinical trials. One study has, however, attempted to use such biomarkers to guide therapeutic intervention. In animal studies, administration of erythropoietin (EPO) prior to induction of IRI or after renal artery clamping but prior to reperfusion was associated with renal protection (Sharples et al., *J Am Soc Nephrol* 2004). Based on this and other animal studies demonstrating a benefit associated with early EPO administration in AKI, a clinical trial was conducted in which EPO or placebo was administered to patients based on elevations in urinary γ-glutamyl transpeptidase (GGT) and alkaline phosphatase (Endre et al., *Kidney Int* 2010). There was no difference in patients in the EPO arm versus placebo for the primary endpoint of SCr levels. For the secondary endpoint of urine output, the placebo group actually performed better than the EPO group. Several lessons can be drawn from this trial. First, for biomarkers to be used to guide clinical therapy they must be highly predictive, which was not the case for the biomarkers used. Second, the timing of the intervention needs to be appropriate. In the animal studies, EPO was only effective when given prior to reperfusion, which made it unlikely to be clinically beneficial in the setting of this trial. In addition, given the multiple pathways of organ injury in AKI, it may be naïve to think that targeting a single pathway will be sufficient.

To date, intervention trials have failed because of multiple factors, perhaps including delayed timing of intervention, use of interventions that are ineffective in established AKI, having benefits masked by side effects, using insufficiently robust biomarkers, enrolling the wrong study populations and targeting a single branch of a complex pathogenic pathway.

Prevention of AKI requires that the time of kidney injury can be anticipated and that patients at high-risk for developing AKI can be identified. Specific settings where this may be possible include AKI following cardiovascular surgery and following administration of radiocontrast. Although scoring systems to estimate the risk for developing AKI following cardiac surgery have been developed (Thakar et al., *J Am Soc Nephrol* 2005), these scores may not be sufficiently robust to identify the majority of patients who will develop AKI. For example, although the risk of AKI increases from less than 0.5% in the lowest risk patients to greater than 20% in the highest risk cohort of patients, the absolute number of patients developing AKI despite low risk-scores is similar to that in the high-risk group, even though there is a smaller number of high-risk patients. Better risk-prediction models are therefore required, especially if potential interventions are associated with significant cost or complications.

Over the past decade, multiple studies have tested whether N-acetylcysteine (NAC) prevents contrast-induced AKI (CI AKI). In a meta-analysis of 26 studies, NAC was found to have a marginally statistically significant benefit (Kelly et al., *Ann Intern Med* 2008). Eleven of the studies were positive, and 15 were negative. Sample size ranged from 24 to 487 patients, with a median of 95 patients. Even when the data were pooled, the combined studies had insufficient statistical power to detect a 20% difference in the rate of CI AKI. Studies that are too small do not provide researchers with meaningful answers.

In a recent large study presented at the November 2010 American Heart Association meeting, 2,308 patients undergoing angiography were randomized to receive either NAC or placebo. CI AKI occurred in 12.7% of patients in both treatment arms. Although this was a negative study, the implications of the study need to be considered in light of the enrolled patient population and co-interventions provided. The enrolled cohort was not at substantially increased risk of CI AKI, particularly as only a small minority of patients had significant baseline kidney disease. Furthermore, the baseline SCr was not ascertained in a consistent manner, which may have affected the ability to reliably detect small changes in SCr. Greater than 20% of patients received high-osmolality contrast agents, which are not generally used due to increased risk of CI AKI. This study illustrates how selection of the study population and management of co-interventions can impact study outcomes.

Volume administration with isotonic bicarbonate also has been studied for the prevention of CI AKI. A small study reported impressive results showing bicarbonate administration was superior to saline administration (Merten et al., *JAMA* 2004). In this study, however, if the next patient to enter the bicarbonate arm of the study had developed CI AKI, the positive results would have been negated, which is a risk when too much credence is given to small studies. A review of bicarbonate studies found six positive studies and four negative studies, with approximately 2,000 patients. Studies ranged from 59 to 502 patients. As with the studies of NAC, these studies, individually and in aggregate, are underpowered to determine statistically significant findings. In addition, there is evidence of publication bias, with many unpublished small negative trials.

The development of CI AKI is associated with the progression of kidney disease, development of ESRD, and both short-term and longer-term mortality risk (James et al., *Kidney Int* 2010). Risk factors for the development of CI AKI are also risk factors for the development of these other adverse outcomes, including death, need for dialysis and accelerated progression of chronic kidney disease (CKD). Studies have not demonstrated that preventing the small changes in SCr that define CI AKI alter the risk for these more important outcomes. Future studies will need to be adequately powered to evaluate whether prevention of short-term changes in kidney function reduces the risk of these longer-term outcomes.

For future research, clinical trials should be based on plausible biological effects. Interventions must be appropriately timed. Risk models for identifying at-risk patients need to be refined and complications of interventions must be minimized. While small changes in SCr or other biomarkers may be appropriate endpoints for proof-of-concept studies, definitive trials need to demonstrate impact on clinically important outcomes.

In 2010, the Acute Kidney Injury Network (AKIN) met and discussed two important issues that are relevant to study design. The first related to the adjudication of AKI as an endpoint. There was consensus that a confirmed episode of AKI should be defined based on the RIFLE/AKIN criteria combined with at least one additional factor, which could include appropriate clinical context, a characteristic pattern of increase or decrease in SCr, a sustained increase in SCr, a response (or lack of response) to volume administration, or appearance of or a change in a validated associated clinical or laboratory biomarker. A diagnosis of severe AKI could be made based on RIFLE/AKIN stage, the need for renal replacement therapy (RRT) or other complications. The workgroup also discussed appropriate endpoints beyond the short-term changes in SCr and urine output that define AKI and proposed the use of a composite endpoint consisting of major adverse kidney events (MAKE). Components of this endpoint might include some or all of the following: death, need for RRT, renal hospitalization within 90 days, persistent decline in kidney function and progression of underlying CKD. In addition, cardiovascular endpoints could be considered given the linkage between cardiovascular and renal disease.

Discussion

An important issue is how best to determine high-risk patients so they can be recruited in clinical trials. AKI comprises a broad spectrum of disease processes, and current biomarkers do not consistently reveal patients' disease stage. Biomarkers for early diagnosis are needed, especially if they help identify risk level.

For clinical trial design, critical issues include locating enough patients to give trials the statistical power needed to develop accurate findings. The Institutional Review Board (IRB) process also takes time. For example, in the VA/NIH Acute Renal Failure Trial Network (ATN) study, it took some sites as long as 10 months to complete the IRB approval process, which is a considerable barrier for any clinical trial.

Moving a successful study from animals to humans presents many challenges, including considerations regarding drug dosing, size, clinical outcomes, and whether the study animals used are good models for human disease. In addition, long-term follow up with patients in clinical trials presents challenges for industry. It was suggested that a network should be established outside the NIH consisting of groups that are interested in AKI. Entities that have developed drugs for use in clinical studies should be able use the network to develop trials that can be conducted in centers. This would speed up the conduct of clinical trials as is done in cardiology research. NIH could offer support in establishing such a network.

Other issues related to clinical trials are whether composite endpoints should be used. The use of composite endpoints has contributed to the success of many cardiology trials to date. Using composite endpoints might allow shorter follow-up periods (60 or 90 days, rather than years). Ischemic AKI is a good single outcome, however, but most other endpoints need further development. In addition, there is a shifting patient demographic base that currently is not served because many centers have too few patients to conduct single-center trials.

Risk Assessment Analysis and Study Populations

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Risk stratification arranges patients according to the severity of their illness and provides researchers with the ability to predict outcomes from a given intervention. The usefulness of risk stratification is based on linking severity to specific outcomes. For AKI patients, changes in SCr are known to have clinical impacts in large populations of at-risk patients. The most severe clinical impact is the risk of increased mortality. AKI patients may be found in the hospital or community setting, with different prevalence and outcomes in different settings. In the developing world, infections are the primary cause of AKI. In the developed world, infection, drugs, and medical procedures are the primary causes.

There are many factors that increase the susceptibility to AKI, including modifiable and unmodifiable factors (Abuelo, *N Engl J Med* 2007). There also are multiple causes of low-perfusion states that are associated with increased AKI probability, including hypovolemia, cardiovascular causes, reduced vascular resistance, and local renal hypoperfusion. Challenges that AKI researchers continue to face include developing standard AKI definitions, classifications, and criteria for initiating therapy. The current use of variable definitions, data from selected populations, the need for agreement regarding a gold standard test for functional renal status, and undetermined clinical endpoints remain challenges for researchers.

There are various outcomes in patients with AKI that range from complete resolution of AKI without CKD to death, with dialysis an intermediate outcome that can occur in patients at either end of the outcome spectrum. The likelihood of a specific outcome depends on the severity of the condition and is related to whether the patient is identified in the community (better outcomes) or in hospital settings (worse outcomes). Kidney function measured by GFR is directly associated with outcomes, with worse outcomes in those patients with lower GFR (Cerda et al., *CJASN* 2007).

There are multiple risk scoring systems that predict outcomes in patients with AKI. In coronary artery bypass graft (CABG) patients, one risk factor is urine output, which independently predicts AKI and poor outcomes (Lin et al., *J Critical Care* 2004). Another risk score has been developed based on gender, congestive heart failure (CHF), ejection fraction (EF), chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM), level of kidney

function, and type of surgery (Thakar et al., *J Am Soc Nephrol* 2005). Many other risk scores are based on other factors, such as sepsis (Bagshaw et al., *Critical Care* 2008), the use of angiotensin-converting enzyme (ACE) inhibitors prior to cardiovascular surgery (Arora et al., *Clin J Am Soc Nephrol* 2008), complexity of patient groups (Kohle et al., *Critical Care* 2008), and location (Candela-Toha et al., *Clin J Am Soc Nephrol* 2008).

The question asked in a clinical trial will determine the approach to risk stratification. Risk stratification goals include identification of appropriate patients, delineation of responsive patients, cost containment, improving physician practice, improving patient education, and evaluating effectiveness of care. An important risk stratification tool is a database made up of representative samples of populations with accurate data elements.

A recent cohort study of more than one million patients in Canada with outpatient estimated glomerular filtration rate (eGFR) and/or urine protein tests found that information on eGFR and proteinuria should be used together when identifying patients at risk for AKI. The study also demonstrated that a previous episode of AKI provides long-term information on AKI (James et al., *Lancet* 2010). Based on this and other studies, it appears that kidney function (eGFR, SCr/cystatin, and proteinuria) may be the most important predictive factor of AKI incidence, AKI outcomes, and patient outcomes. These parameters, used in combination, may provide practical methods for determining risk stratification in patients. Many of the other risk stratification tools are associated with kidney function and proteinuria, such as age, cardiovascular disease (CVD) and diabetes mellitus.

In summary, there is a need to improve AKI definitions for use in clinical trials, and to define meaningful clinical endpoints. Patient selection and risk stratification are dependent on the specific clinical questions being asked, with simple questions being more useful. Development of a consistent framework will enhance the ability to conduct relevant observational and interventional studies. Validation and testing of the utility and feasibility of definitions and risk scores is critical.

Discussion

Biomarkers could potentially add to risk stratification by identifying those who are at risk of developing AKI and those who have AKI and are in a specific stage of disease progression, although no biomarker yet exists for either situation other than SCr.

Resources for AKI Studies—Experience From an O'Brien Center

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The NIDDK-funded University of Alabama/University of California at San Diego UAB/UCSD O'Brien Center for AKI research was established to advance the understanding of AKI pathophysiology, expand diagnostic specificity, and increase AKI therapeutic and preventive approaches. The O'Brien Center focuses on AKI pre-clinical, clinical, and translational research that will be required for the development and operation of multidisciplinary collaborative networks. The Center's clinical focus is to provide support for the design and conduct of clinical research, genomics, and the collection of samples from patients with and identification of biomarkers for AKI. A database system is used to collect and coordinate clinical data as part of the clinical research effort. Biomarker investigations include functional biomarkers such as creatinine and cystatin C; structural biomarkers such as NGAL, IL-8, microalbumin, and KIM-1; and cytokines such as IL-6 and IL-10, and TNF-alpha. Once biomarkers are identified, they can be evaluated for validation and potential use in clinical trials. Genomic studies for genetic susceptibility and gene modifiers are the focus of ongoing research. The O'Brien Center also has a biorepository for use in AKI studies. Activities supported through the O'Brien Center include observational studies, interventional studies, and health services research. The available infrastructure is conducive to each study design.

Over the last decade, a variety of observational and interventional studies have been conducted in AKI. The Program to Improve Care in Acute Renal Disease (PICARD), a multicenter study of 618 AKI patients, is an example of an observational study that has provided considerable information on the design and conduct of clinical studies in AKI (Mehta et al *Kidney Int* 66: 1613-21; 2004). An analysis of the PICARD study and other recent studies in AKI (ATN, RENAL, ASSESS AKI) has highlighted several issues pertinent to clinical trials in these fields. These include: a substantial amount of time is needed to design, conduct, and analyze AKI trials; observational trial results must be complete before informing a decision to conduct an interventional trial; data used to assess trial benefits and adverse events may not represent prevalent standard of care and variations in care that affect outcomes; trial results may not be generalizable to broader populations due to changes in natural history over the time the trial is conducted; and trial sites disband after the trial is over.

To address some of these issues, the clinical core of the UAB/UCSD O'Brien Center has initiated an international multicenter registry of AKI that captures clinical data on hospitalized patients with AKI using a web based system. Data include information on the incidence, prevalence, and risk factors of AKI, including details of RRT (indications, modality, operations, dose, and outcomes) and the post-AKI natural history, management, progression, and outcomes. There is a centralized data repository with individual and pooled cross-sectional and longitudinal data, and international multicenter projects. Additionally, participating centers have the option to collect biological samples that can be stored and analyzed for various biomarkers of AKI.

Preliminary data from the AKI registry provide evidence that the information can be used to inform the pre-trial design, conduct and post-trial assessment of clinical studies, making the research more efficient and cost-effective. The registry project also emphasizes the opportunity afforded by collaborations across centers to pool information to advance knowledge and improve management of patients with AKI.

In summary, several resources currently exist at the UAB/UCSD O'Brien Center for enhancing AKI clinical and translational research. Future clinical and translational research in AKI will require the development of collaborative networks of investigators drawn from various disciplines that are willing to share their expertise and resources. The AKI registry offers an opportunity to standardize approaches for AKI studies and enhance collaboration across centers. Support is needed to maintain and enhance the AKI registry infrastructure, which includes support for data entry from participating centers. In addition, the AKI registry could be used to design and conduct future AKI clinical trials. To develop the O'Brien Centers concept into a national network for AKI research and clinical trials a shared effort is needed. Continued investment and enhancement of the infrastructure will be required to maintain and advance these resources for wider application. Benefits of such an effort likely will be greater than individual efforts and will serve the community well to improve care and outcomes related to this devastating disease.

CHALLENGES IN CLINICAL TRIAL DESIGN (I)—PLANNING STUDIES FOR AKI

Moderator: *Glenn M. Chertow, M.D., M.P.H., Professor of Medicine, Division of Nephrology, Stanford University School of Medicine, Palo Alto, CA*

Lessons for Nephrologists From Ophthalmology—Choosing Endpoints That Work and Are Accepted

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The FDA readily recognizes outcome variables that are associated with a loss of function, such as loss in visual acuity. The Diabetic Retinopathy Study (DRS), the first major collaborative clinical trial in ophthalmology, used severe visual acuity loss (VA < 5/200 at two consecutive visits 4 months apart) as their main outcome variable. This outcome was appropriate for assessing new treatments for proliferative diabetic retinopathy (PDR) before effective treatments were available. Without treatment there was a high rate of severe functional loss in patients with PDR (>50% in 5 years). However, the DRS demonstrated the benefit of laser treatment for PDR. Because of the success of laser treatment, severe visual acuity loss became an inappropriate outcome variable, since less than 3% of patients would lose this much vision in five years. However, moderate visual acuity loss, especially from diabetic macular edema (DME) was still a frequent occurrence. An eye chart and method of measuring visual acuity was developed such that one could assess the proportion of eyes that had a 50% reduction in their visual acuity or a "doubling of the visual angle" (e.g. going from 20/20 to 20/40 or 20/50

to 20/100). Approximately 36% of untreated eyes with DME involving the center of the macula were likely to lose this much visual acuity or more over three years. The Early Treatment Diabetic Retinopathy Study (ETDRS) demonstrated that those who received immediate focal laser treatment for diabetic macular edema were approximately 50% less likely to develop moderate visual acuity loss than those with no focal laser treatment.

Newer treatments of diabetic retinopathy that include drugs could add additional benefit to standard laser treatments. Because of the success of laser treatment, outcomes other than change in visual acuity are necessary to demonstrate a treatment benefit on retinopathy progression. One such outcome that has been approved by the FDA is a standardized approach to document worsening of diabetic retinopathy. Using this diabetic retinopathy scale that was developed based on natural history information from the ETDRS and from epidemiologic studies, new treatments can be tested for their ability to slow the progression of diabetic retinopathy. The first major study to use the outcome variable of "retinopathy worsening", based on these retinopathy severity levels, was the Diabetes Control and Complications Trial (DCCT). In the DCCT, tight control of blood glucose statistically significantly slowed diabetic retinopathy progression based on the grading scale. In addition, tight control of blood glucose also statistically significantly slowed the progression of diabetic nephropathy and neuropathy. These positive results may have been missed, however, if long-term follow up had not been conducted, as the benefits on nephropathy and neuropathy were not recognized until 5 years, several years after the results for retinopathy became statistically significant.

The FDA prefers functional outcome variables such as "severe visual loss" or definite worsening of visual acuity, such as moderate visual loss (3-line VA change). However, they recognize the need for other outcome variables because of the success of photocoagulation in reducing the risk of functional vision loss. Based on reliable natural history information, from both clinical trials and epidemiologic studies of patients with diabetic retinopathy, they have accepted surrogate outcomes such as the development of a three-step worsening of diabetic retinopathy, the development of PDR in eyes starting with moderate diabetic retinopathy and the development of "center-involved" macular edema in eyes that did not have "center-involved" macular edema at baseline.

Discussion

The diabetic retinopathy experience demonstrates that developing outcome variables is a dynamic process that must change as new treatments are developed that modify the severity of the outcomes of the disease process. If researchers were forced to use blindness as the functional outcome for assessing new treatments of diabetic retinopathy, the studies would take decades and would be impossibly large because of the low rates of that outcome, given new standards of care. New outcome variables that link surrogate outcomes to functional loss can be based on careful natural history collection in untreated arms of clinical trials and epidemiologic studies. Using these surrogates, new treatments can be evaluated in smaller studies and shorter study durations, making it possible to continue to develop new safe and effective treatments.

Lessons for Nephrologists from Rheumatology—Trial Design Choices and Consequences

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To illustrate the consequences of clinical trial design choices, three drug development case studies were reviewed to show how science-based and feasibility-related decisions played out in the development process. The cases reviewed were zoledronic acid (Reclast™) for post-menopausal osteoporosis and prevention of clinical fractures after hip fracture; Prosaptide™ for treatment of AIDs-related neuropathic pain; and Pegloticase (Krystexxa™) for refractory gout.

Bisphosphonates were used in daily dosing regimens in osteoporosis and oncology treatments in the late 1990s with good success. Researchers noticed the benefit of bisphosphonates for strengthening bones through remodeling and reducing fractures, but assumed dosing of these agents had to be timed within the period of the three-month bone remodeling cycle. A Phase 2 trial was designed specifically to investigate the use of bisphosphonates when dosed in a paradigm outside the bone remodeling cycle, that is, a single cumulative annual dose of 4 mg administered every three, six, or every 12 months. The frequency of dosing cycle was not relevant, as all dose regimens provided the same high degree of effectiveness in terms of bone mineral density and bone turnover marker outcomes. Animal studies of long-term observation following a single dose of zoledronic acid confirmed its prolonged effectiveness, but it was unknown if the unprecedented once per year dose regimen of a bisphosphonate would result in a fracture reduction benefit. A placebo controlled Phase 3 osteoporosis fracture trial was designed to determine the effectiveness of a once per year regimen, designating vertebral and hip fracture endpoints as ordered co-primary endpoints. The trial was designed with two strata. Stratum I were participants with no current osteoporosis therapy. Stratum II participants were on therapy with selective estrogen receptor modulators (SERMs) or calcitonin hormone replacement therapy (HRT) at baseline. Results of the fracture study, using an annual infusion of zoledronic acid for 3 years, showed that treatment significantly reduced the risk of vertebral, hip and other fractures (Black et al., *N Engl J Med* 2007). As a corollary, it was shown that use of a placebo control group in this population can be managed ethically, and pre-study assumptions about the standard of care in the community may be flawed.

A subsequent trial investigated the use of zoledronic acid in women with previous hip fracture (Lyles et al., *N Engl J Med* 2007). This trial, which required careful normalization of vitamin D levels after hip fracture in the elderly study population, found that an annual infusion of zoledronic acid within 90 days after repair of a low-trauma hip fracture was associated with a reduction in the rate of new clinical fractures and improved survival.

Lessons learned from these trials included: selection of practice setting and specialty of Principal Investigator (PI) is critical for success, practical considerations must have practical solutions and international participation is needed to recruit for very large studies. Study population key characteristics must be addressed (e.g., vitamin D deficiency and required time to full normalization). In addition, be ready for surprises (i.e., all-cause mortality benefit).

Prosaptide ™, a 24-amino acid neuropeptide, has been investigated in animal models for diabetic neuropathy and AIDS-related neuropathy with some success. A pilot Phase 2 clinical trial was implemented in patients with diabetic neuropathy, but the trial was under-powered and the inverted U dose response that resulted could not be explained. A second pilot Phase 2 trial six weeks in duration was implemented in patients with AIDS-related neuropathy, which demonstrated that Prosaptide ™, although anecdotally effective in improving analgesia, failed in a futility analysis when the trial was 50% completed (Evans et al., *PLoS One* 2007). Further development of Prosaptide ™ was not continued, and the company was able to save millions of dollars in otherwise futile effort.

Pegloticase (Krystexxa™) is a pegylated modified recombinant porcine uricase administered in an intravenous (IV) infusion every 2 weeks in patients with refractory gout, an orphan indication. Phase 2 trial results, with Pegloticase given open label, indicated that there was a clinical benefit not previously reported, elimination of gout tophi, accompanying normalization of uric acid throughout a 12 week period. Subsequently, two placebo controlled Phase 3 trials were designed, with liberal entry criteria pre-approved by the FDA in a Special Protocol Assessment. The design did not include a Data Safety Monitoring Board (DSMB), study stopping rules, or stratification for serious co-morbidities. Results were impressive, with normalization of uric acid within hours and elimination of tophi, reduction in swollen and tender joints, reduction in frequency of gout flares and significant improvements in pain and function (Sundy et al., *Arthritis and Rheumatism* 2008). A 2:2:1 randomization scheme was used with two dose regimens (every 2 weeks or every 4 weeks) versus placebo. Cardiovascular Serious Adverse Events occurred only in the treatment arms, but because the majority of patients in the trials had pre-existing co-morbidities such as diabetes, myocardial infarction, cardiac arrythmias, hypertension or dyslipidemia, and the number of adjudicated events was small, and because heavily skewed randomization was employed, it was not possible to discern whether the observed cardiovascular adverse events had any relationship to study drug. Pegloticase was approved by the FDA based on these trials, with a requirement for a post-marketing 500 patient observation trial to gather additional safety information among this difficult to treat population.

Challenges and Successes in the ICU—Choosing Interventions and Endpoints
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The Acute Respiratory Distress Syndrome Network (ARDSnet) is a multicenter network charged with efficient testing of promising agents, devices, or strategies, especially if they are unlikely to be studied by industry. The trials are designed to be of sufficient size and importance to change practice. ARDSnet was established in 1994 with funding from the National Heart, Lung and Blood Institute (NHLBI). The first patients were accrued in 1996 in a placebo controlled trial of ketoconazole combined with a trial of lower versus traditional tidal volumes for patients with Acute Lung Injury (ALI) using a 2 x 2 factorial design. The DSMB ended the ketoconazole trial early (January 1997) after determining that ketoconazole was ineffective and stopped the lower tidal volume trial early at 861 patients for efficacy. This landmark trial has fundamentally changed practice (ARDS Network, *N Engl J Med* 2000). The ARDSnet went on to complete five additional studies with a total enrollment of over 2,600 patients during its first

funding cycle. These trials have changed fluid management practices and have clarified the role of corticosteroids, pulmonary arterial catheters, and positive end expiratory pressure for the management of patients with ARDS.

Success of ARDSnet may be attributed to sound preclinical work that facilitated trial design as well as efficient trial methodologies (factorial design) and concomitant testing of early and late intervention strategies. When Phase 2 data were judged to be insufficient, the ARDSnet used a Phase 2/3 roll over design and retained the Phase 2 patients in the Phase 3 analyses subject to external independent review. In addition, explicit methodologies and replicable methods facilitated research by other groups to build upon the Network's findings. Problems identified in ARDSnet include controversies related to control group selection for the tidal volume trial and missed opportunities for partnering with pharmaceutical companies because of timing/conflicts with ongoing trials.

Endpoints for clinical trials in the intensive care unit (ICU) are largely determined by the research question. For Phase 3 studies conducted under an IND, endpoints are determined by/with the FDA. Mortality (28 days or longer) and composite endpoints (ventilator-free days up to day 28) are endpoints that are conventionally accepted. The 28-day endpoint is less relevant as modern ICU care is both reducing overall mortality and delaying many deaths beyond this endpoint. Examples include a recent ICU trial on intensive versus conventional glucose control in critically ill patients (NICE-SUGAR Investigators, *N Engl J Med* 2009). A statistically significant difference favoring less intensive glucose control did not emerge until 90 days. Evidence-based changes in ICU care are lowering mortality globally, making it difficult to estimate placebo mortality and thus sample size necessary to detect the anticipated treatment effect. The PROWESS-Shock trial for patients with sepsis used an event-driven adaptive design that allowed for a larger sample size if the observed aggregate mortality was lower than anticipated, which proved to be the case half way through the trial (35% expected versus ~27% observed) (Thompson et al. *Int Care Med* 2010). Finally, contemporary ICU trials are increasingly measuring both mortality and long term functional status and quality of life to assess the durability and full impact of the interventions. For example, the PROWESS-Shock endpoints include a primary endpoint of 28-day all-cause mortality. Secondary endpoints included 90-day and 180-day mortality, changes in organ function over time, and quality of life as measured by Euro-QoL-5 and SF-12 scales after 6 months (Finfer et al., *Int Care Med* 2008).

In summary, state-of-the-science clinical trials for ARDS, sepsis, and AKI should take a full measure of the short and long term impacts of interventions and consider adaptive designs given changing ICU care and improving outcomes. Results from the presented trials suggest that mortality remains the most compelling endpoint; however, 28-day mortality is an incomplete measure of the disease burden of sepsis and ARDS. Composite endpoints need to be developed and validated, and should include patient-centered outcomes (Spragg et al., *Am J Respir Crit Care Med* 2010).

Challenges and Successes in Sepsis

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Sepsis is a significant cause of mortality in ICU patients, especially those with AKI. Results from an international guideline-based performance improvement program, the Surviving Sepsis Campaign, which included data from 165 centers from January 2005 until March 2008 and 15,022 patients with severe sepsis, indicated that the Campaign guidelines resulted in sustained, continuous quality improvement in sepsis care, with a non-significant reduction in mortality (Levy et al., *Int Care Med* 2010). Another prospective, multicenter, observational trial of 2,796 patients with severe sepsis found that patients with renal dysfunction suffered 45.7% mortality in hospital compared to 31.2% of patients without renal dysfunction (Ferrer et al., *Am J Respir Crit Care Med* 2009). Similar results (40.5% to 23.8%) were found in the PROWESS-Shock trial (Bernard GR, et al., *N Engl J Med* 2001).

Sepsis regulatory guidance in 2000 included a requirement for a primary endpoint of 28-day all-cause mortality (improvement in organ dysfunction was not acceptable). No longer term follow up was required. A single, adequate and well-controlled Phase 3 trial was acceptable for registration. Subgroup analyses were largely considered hypothesis generating. FDA acceptance on a single study to show efficacy was limited to situations where a clinically meaningful effect is observed, such as mortality, permanent morbidity, or prevention of a disease with a potentially serious outcome. Confirmation of the results in a second trial would be practically or ethically impossible. In 2010, FDA guidance for sepsis still includes the 28-day all-cause mortality requirement, but longer term follow up now is required. FDA strongly encourages two adequate and well-controlled Phase 3 trials to support registration.

Lessons from the PROWESS drotrecogin alfa (activated human protein C) trial demonstrated reduced mortality compared to placebo, except in the first quartile of the Acute Physiology and Chronic Health Evaluation (APACHE) II classification, as well as reduced relative risk in subgroups. Disease severity rankings also were improved with drotrecogin alfa. In PROWESS, there were protocol violations that subsided as the trial progressed, and investigators improved their understanding and implementation the longer they conducted the trial. PROWESS also confirmed that there is a learning curve associated with conducting clinical trials.

The Administration of Drotrecogin Alfa (Activated) in Early Stage Severe Sepsis (ADDRESS) Trial investigated efficacy and safety of drotrecogin alfa in adult patients with early stage severe sepsis. ADDRESS was an international, multicenter, randomized double-blind, parallel, placebo-controlled study at 516 sites in 34 countries. It included 11,444 adult patients with early stage severe sepsis (e.g., an APACHE II score <25 or with one sepsis-induced organ failure). Treatment was with 24 μg/kg/h drotrecogin alfa or placebo for 96 hours with a primary objective of 28-day all-cause mortality. Randomization was stratified by site and by baseline use of low-dose heparin for deep venous thrombosis (DVT) prophylaxis. ADDRESS was stopped early after a futility analysis showed no differences between the treatment and control groups (Abraham et al., *N Engl J Med* 2005).

A continuing challenge in clinical trials is when drugs have positive benefits for treated conditions but unpleasant or dangerous side effects, for example, the bleeding seen in the ADDRESS trial, which make them unusable in the clinical setting. Another concern is improvements in some outcomes such as organ function but no change in mortality. This occurred in the ARDSnet methylprednisolone trial, which demonstrated early improvement in weaning and cardiovascular function, but no improvement in mortality, and an increased incidence of serious and non-serious adverse events (Steinberg et al., *N Engl J Med* 2006;354:1671-1684). The Corticosteroid Therapy of Septic Shock Study (CORTICUS) reported reversal of shock with hydrocortisone administration, but no improvement in mortality and increased incidence of secondary infections (Sprung et al., *N Engl J Med* 2008).

To provide perspective, there is continued high mortality of severe sepsis in the real world setting and a very high incidence of AKI associated with increased mortality. Improved survival remains the regulatory endpoint and most likely will require two Phase 3 trials with adequate subgroup sizes in power calculations and stopping rules. Site performance is extremely important. Enrolled patients must be reviewed carefully. Protocol violations must be monitored carefully. The inclusion/exclusion criteria must provide an identifiable patient population to which the study results can be applied in the real world setting. "Seeing a drug work" may not be feasible within the complexity of severe sepsis.

Discussion

It is critical during the regulatory process that the appropriate data are collected and analyzed so the correct measures show efficacy. The efficacy hurdle must be overcome. Cost-effectiveness also needs to be considered in the new health care milieu. Treatments should be medically, socially, and financially palatable to be accepted. Comparative effectiveness research (CER) will be part of this equation. A time-to-event analysis is another critical aspect of results that are expected from AKI clinical trials.

In addition, PIs and nurses must be trained so that they understand complex protocols, such as those present in AKI trials. One approach is to check daily with those implementing complex trials before a trial begins. The devastating first-patient effect that presented in these trials confirms the importance of ensuring that everyone involved in the trial knows how to conduct the trial. Protocol violations cannot be tolerated.

Why Good Trials Go Bad

John Lachin, Sc.D., Professor of Biostatistics, Epidemiology and Statistics, The Biostatistics Center, The George Washington University, Rockville, MD

A clinical trial is designed to test a specific hypothesis as to whether a difference in treatment assignment results in a difference in outcomes. When conducting a statistical test of a hypothesis, two types of errors may occur. A false positive (type I) error occurs when there really is no difference in the general population but a statistically significant test result is obtained. By chance this can occur with probability α that is pre-specified, such as $\alpha = 0.05$. A false negative error occurs when a difference truly exists but the test result is non-significant, and occurs with probability β. The complement is the power of the study, or the probability $1-\beta$ of a true positive statistical test result. In the setting of classification or prediction, the power is equivalent to the sensitivity of a diagnostic test and the probability $1-\alpha$ is the specificity.

The α-level of a trial is protected only when the statistical test is conducted of a pre-specified hypothesis, with a specified outcome, and using a pre-specified statistical procedure that is applied to the full cohort randomized into the study. Various factors can introduce biases that affect α, among them missing data. While statistical methods can be applied under specific assumptions, the only unquestionably unbiased study is one with no or minimal missing data. This is achieved using an intent-to-treat design wherein all patients randomized are evaluated as objectively as possible and are included in all analyses (Lachin, *Clinical Trials*, 2000). If a patient leaves a trial before its planned end, the patient must be also be included in follow-up analyses.

Various factors may affect power. One is the precision or reliability of an outcome measure. The reliability coefficient ρ equals the proportion of total variation between subjects due to variation in the true values. $1-\rho$ equals the proportion of variation caused by random errors of collection, processing, and measurement. The reliability of an outcome measure has significant statistical consequences (Lachin, *Clinical Trials*, 2005). As the value of ρ decreases, study power decreases and the required sample size increases, the maximum correlation with other measurements decreases, and the ability to correctly classify subjects into different populations decreases.

Every study population consists of a mixture of potential biological responders versus non-responders, and the two cannot be distinguished before starting the trial. As the fraction of responders decreases, the power decreases sharply. Often a population of high-risk subjects is selected to increase the number of outcome events in order to increase power. However, a high-risk cohort could have less power if many subjects are non-responders, such as those who will have the outcome regardless of the effectiveness of treatment on other mechanisms.

For the analysis of an outcome event, the required sample size depends on the hazard rate in the control group and the follow-up period. For example, for a trial with a 3 year recruitment period and 5-year follow up period, and a control group hazard rate of 0.02 / year, approximately 2,500 subjects are needed to provide 90% power to detect a hazard ratio or relative risk of 0.6.

For a 0.04 / y hazard rate, approximately 1,300 subjects are needed. In the Diabetes Control and Complications Trial (DCCT), hazard rates over 6.5 years of follow-up for retinopathy progression (0.022 / y), microalbuminuria (0.036 / y), and albuminuria (0.006 / y) illustrate the real-life challenges in clinical trials.

One suggestion for increasing power, or reducing sample size or duration, is to use a combined outcome defined as the time to the first of one of a set of events (e.g., retinopathy progression and microalbuminuria in the DCCT). However, if a component is not affected by treatment to the same degree as the other components, a combined outcome can dilute the difference between groups. That is, the total number of events may increase but the hazard ratio could be closer to 1.0, thus reducing power. The number of each type of event that could be prevented in the course of the study, as well as the total number of events, must be considered. In the DCCT, for example, a combined retinal and renal composite outcome event would increase the number of events (relative to an analysis of retinopathy alone as the outcome), but the added events do not offset the dilution of the hazard ratio, and power is not increased.

Another suggestion has been to use a surrogate outcome, such as a biomarker like the eGFR. This can be risky because there may be limited information about the surrogate. For example, an outcome may have several causal pathways, only one of which may be reflected by the surrogate, or the surrogate may not be in any of the causal pathways (Fleming and DeMets, *Annals of Internal Medicine*, 1996). Criteria for the validation of a surrogate have been proposed, such as showing that the full effect of treatment on the true clinical outcome is captured by the treatment effect on the surrogate (Prentice, *Statistics in Medicine*, 1989). A high correlation between the surrogate and the true outcome is necessary, but alone is not sufficient. The surrogate must be linked directly to the causal pathway by which the treatment affects the outcome.

Surrogates may also be misleading. Even if there is a perfect correlation ($r = 1$) between the surrogate and the true outcome, the treatment effect on the surrogate and the outcome could be in opposite directions if the nature of the association of the surrogate and the true outcome (e.g., slope) differs between groups.

For a quantitative surrogate outcome, it is also important to show the association between the surrogate outcome and the risk of the primary clinical outcome, such as quantifying the change in the risk of end-stage renal disease per 5 mL/min/1.73m^2 in eGFR.

In conclusion, the following points were summarized:

- Intention-to-treat designs with complete follow up are needed to reduce bias.
- Outcome measures of known high precision (reliability) should be employed.
- Inclusion of biological non-responders decreases power and increases the required number of subjects that are needed for trials.
- High-risk populations could include a number of non-responders, and could reduce power.
- Combined outcomes may not increase power.

- Surrogate outcomes may require a smaller sample size but should first be validated. Rigorous statistical criteria to validate a surrogate require an initial study using the definitive clinical outcome as well as the surrogate.
- A study of treatment effects on a quantitative measure (such as eGFR) could require smaller sample size, but data should exist to demonstrate that the treatment affects this measure by a mechanism tied to clinical outcomes.

Discussion

The argument for conducting early and late therapy clinical trials can be illustrated by the DCCT, in which there was no evidence of benefit over the first 3 years of follow-up. If patients had not been followed up to 4 years and beyond, the highly beneficial treatment effect on retinopathy would have been missed.

Power calculations during clinical trial design usually are based on various unknowns including the control group hazard rate, the rate of recruitment, and losses to follow-up. This impacts all aspects of the design. For evaluation of sample size, researchers must provide specifications of the expected values of various trial parameters (e.g. hazard rate) based on the best science available, and then monitor these values during the trial to ensure that power is not degraded. An alternative process is to implement a maximum information design (Lachin, *Clin Trials*, 2005). The advantage of this approach is that if the initial specifications of these design parameters are wrong, the trial can continue until the necessary information is obtained without the need to revise the study protocol.

The most controversial efficacy issue in AKI research is whether the surrogate SCr is valid. Small changes in SCr can achieve statistical significance. This would be expected to reflect a difference in eGFR. However, the real investigation should focus on whether a treatment that produces changes in SCr, or in eGFR, will also have a positive impact on survival.

CHALLENGES IN CLINICAL TRIAL DESIGN (II) – PLANNING STUDIES FOR AKI

Moderator: *David Warnock, M.D., Professor, Department of Medicine, University of Alabama at Birmingham, AL*

Lessons for Nephrologists—AKI Clinical Trials—An FDA Viewpoint

Aliza Thompson, M.D., Medical Officer, U.S. Food and Drug Administration, Silver Spring, MD

Dr. Thompson discussed clinical trials in AKI from a regulatory standpoint, noting that her views do not necessarily represent the views of the FDA.

From a regulatory perspective, the FDA follows the requirements set forth in the 1962 Kefauver-Harris Drug Amendment, which states that for a drug to be approved there must be substantial evidence of effectiveness consisting of evidence from "adequate and well-controlled" trials demonstrating that a drug will have the effect it purports or is represented to have. The courts later added that the effect must be of therapeutic/clinical significance (1986 Warner-Lambert Co v M Heckler E). In practice, this has come to mean an effect on an endpoint that represents how a patient feels, functions, or survives, or a surrogate endpoint that is expected to reliably predict an effect on such a clinically meaningful endpoint.

Acute changes in serum SCr are a hallmark of AKI and transient changes in SCr have been proposed as endpoints for drug approval in clinical trials of AKI. SCr is a biomarker but also is a marker of organ function and for this reason is held in higher regard than perhaps some other biomarkers. Nonetheless, patients do not perceive changes in SCr per se, and while patients ultimately may become symptomatic as a result of a loss of kidney function, the elevation in SCr in itself is not what is making them sick. Hence, effects on transient changes in SCr represent a "clinically meaningful benefit" only to the extent that they reliably translate into a clinical benefit that a patient can perceive. Although observational studies suggest poor outcomes in patients with small and transient changes in SCr, whether or not the observed changes in renal function cause poor outcomes (or simply are markers of sicker patients) is not clear. Thus, small and transient changes in SCr currently are not accepted as a surrogate endpoint for establishing the efficacy of drugs being developed for AKI. For small and transient changes in SCr to be accepted, there likely would need to be data from intervention trials showing that a treatment's effect on such changes in SCr reliably predicts the treatment's effect on clinical outcomes.

Possible endpoints to consider in Phase 3 trials of AKI include dialysis, death, an irreversible loss of renal function (i.e., a marked and sustained reduction in renal function at a time point remote from the time of acute injury), and other important outcomes (e.g., hospitalization). Composite endpoints are acceptable, and endpoints should be selected based on the effect(s)/benefits the drug is expected to have. In terms of what constitutes a "marked" irreversible loss of renal function, the definition of "marked" may not be the same for all development programs, and consideration should be given to the safety profile/perceived risks of the drug when defining appropriate endpoints for a particular development program. When to call the loss in renal function "irreversible" is another important issue. While showing drug effects on outcomes remote from an intervention can provide compelling evidence of efficacy, looking at endpoints remote from an intervention can also introduce noise, making it harder to establish a drug's benefit. This concept is illustrated by The Platelet Glycoprotein IIb/IIIa in

Unstable Angina: Receptor Suppression Using INTEGRILIN® Therapy (PURSUIT) study, in which eptifibatide was administered for up to 72 hours in patients presenting with acute coronary syndromes. The PURSUIT study showed a highly statistically significant effect on the composite endpoint of death and myocardial infarction at 3 days. Although by days 7 and 30 the absolute number of events was greater in both treatment arms, the finding/difference between treatment arms was no longer as statistically significant (eptifibatide drug label). In AKI trials in which a drug is administered for a short period of time, a similar problem may arise if one looks at endpoints remote from the intervention.

Other considerations in establishing efficacy include ensuring that studies are powered adequately to show an effect (e.g., conducting event-driven trials); enriching studies for patients likely to experience the event of interest and also likely to respond to the therapeutic intervention; and studying more than one dose in Phase 3 studies, which offers more opportunities to get the dose right and can sometimes provide compelling evidence of efficacy.

The Randomized Evaluation of Long-term Anticoagulant Therapy (RE-LY) study that compared warfarin with dabigatran for the prevention of stroke and noncentral nervous system systemic embolism in patients with non-valvular atrial fibrillation (Connolly et al., *N Engl J Med* 2009). In this trial, two doses of dabigatran were studied. Relative to warfarin and the lower dose of dabigatran, the higher dose of dabigatran significantly reduced the incidence of the composite endpoint. The finding of a dose-response relationship provided further support for the efficacy of the higher dose of dabigatran (and arguably made "two trials" out of one).

While establishing efficacy is critical, safety is also important. Some AKI settings are likely to have a high background rate of adverse events, making it difficult to discern drug-related toxicities. Safety data from studies in animals and healthy subjects (Phase 1 dose-escalation studies) and other populations can inform our understanding of potential risks/toxicities in patients with AKI. Experience with other members of the pharmacologic class also is of interest. The size of the safety database in AKI will depend in part upon efficacy endpoints being studied in Phase 3 programs. A larger database will be needed if the program seeks to establish effects on less important clinical endpoints. There is less tolerance for risk (real or perceived) in development programs predicated on surrogate endpoints. Independent DSMBs are also important for ensuring the safety of study subjects because they can review data at a high level and make comparisons across treatment arms in near real-time.

Discussion

Clinical trial design and conduct and clinical practice are changing, and the FDA seeks to keep apace with what is needed by the research community and clinical practice. However, the FDA does not accept endpoints simply because they are being used elsewhere; they must be clinically meaningful or expected to reliably predict clinically meaningful outcomes.

There is interest in the co-development of drugs designed for use in combination. The FDA is aware of this issue (and since this conference a draft guidance has been issued). There should be some assurance that the drugs being combined are each contributing to the efficacy expected, and the safety of the individual components should be characterized.

An issue at the heart of discussions for this meeting was the lack of standardized criteria for initiating dialysis. Current criteria are subjective, and the FDA and clinical community struggle with this issue. Similar subjectivity surrounds endpoints such as length of hospital stay and dialysis-free days, but these may be the best that can be done at this time. Adjudicated need for dialysis is one way to alleviate uncertainty surrounding the decision to initiate dialysis, but the concept needs to be developed further and accepted by the scientific community.

Lessons for Nephrologists—Primary Prevention Studies—A Design Viewpoint
Dr. Chertow

Failure in clinical trials includes the inability to reach a definitive conclusion. Trial success is definitive, informs clinical practice, and does not depend on whether the intervention yielded a benefit. Confusing an issue further rather than informing it may be the worst form of failure. With regard to design issues, it is important to balance internal and external validity. Internal validity is the ability of a study design to determine the effect of treatment on the (primary) outcome variable(s). External validity is the ability to generalize study results to clinical practice in the target population.

General design issues for both treatment and prevention trials include sensible inclusion and exclusion criteria, defined delivery of interventions (open-label versus randomized, and blinded versus non-blinded), well-defined primary and secondary outcomes, and appropriate sample sizes. In AKI prevention trials, it is not known when AKI can be predicted in the clinical setting. Possibilities include before radiocontrast administration, before cardiothoracic or vascular surgery, or before chemotherapy. The low event rate contributes to this problem because it makes it difficult to identify a high-risk population, and CKD is the dominant risk factor. Also unknown is whether it is possible to determine the feasibility of a trial or factors that impact external validity.

Primary outcomes in persons with established AKI include death, death or the need for dialysis, and death or dialysis or sustained reduction in kidney function. The feasibility of an AKI prevention trial is based on the determination of sample size, which itself depends on the incidence rate, effect size, α errors (typically 0.05), and 1-β errors (typically 0.80 to 0.90).

The challenges in AKI trials are exemplified by a study on the prophylactic role of NAC in patients undergoing coronary and/or peripheral angiography and/or angioplasty (Tepel et al., *N Engl J Med* 2000). This study was, however, a source of controversy among researchers because power calculations were not included, the study included only 83 patients, and the incidence of CI AKI was 21% in controls. In addition, the p-value of 0.01 was based on one patient in the treatment arm acquiring CI AKI, but nine did so in the control arm. A more appropriate sample size for this study, given power calculations and effect size, would have been 572 subjects for a

20% incidence rate in controls and 928 subjects to show a more realistic effect estimate of 40%. Although the trial was considered an analytical success because the intervention yielded a statistically significant result, an academic success because the manuscript was published in a prestigious journal, and an influential success because it changed clinical practice, the trial actually was a failure by definition on the basis of not providing definitive results and the fact that it confused rather than informed clinical practice.

A similar situation occurred with another study published in 2003 regarding hemofiltration for the prevention of CI AKI (Marenzi et al., *N Engl J Med* 2003), which had many of the same problems as the Tepel study. The Marenzi study, though an analytic and academic success, was an influential failure because it did not change clinical practice and the results were not plausible or valid.

For an AKI trial to have an incidence rate of 5%, an effect size of 20%, and α-error of 0.05, and a power of 0.90, a sample size of 18,462 with no drop-outs, drop-ins, or protocol violations would be required. For a 50% effect, the required sample size would be 2,482. For a 90% effect, a sample size of 638 would be needed. This raises the question of the feasibility of AKI prevention trials. Such trials are dependent on identifying very high-risk target populations (20% or more) and a reasonably common event ("liberal" definition of AKI). Major challenges exist for design, recruitment, internal validity, and external validity. For example, how compelling is a reduction in the fraction of patients with eGFR < 30 mL/min who experience a change in SCr of 0.3 mg/dL? Previous intervention trials have failed because the expectations may have been too high, there were non-homogeneous disease processes, there was an inadequate understanding of the underlying disease mechanisms, the focus on the kidney was insufficient, and because of "Einstein's insanity." (Einstein's definition of insanity was to continue to do the same thing repeatedly while expecting different results.)

All AKI clinical trials are challenging. Prevention trials are especially challenging because of the relatively low event rates and difficulty in predicting the timing of injury. Designers of AKI prevention trials should be mindful of study power, and of whether the intervention is successful. It also is important to clearly define what is meant by success and to be mindful of the danger of the p-value.

Success will come with adequately powered clinical trials with definitive results. Failure will come with inadequately powered clinical trials, regardless of the results. Preliminary positive results must be confirmed, and it is imperative to focus on the kidney. Death is an outcome that cannot be ignored, but there is a need to focus subsequent trials on the evolution of AKI and sustained loss of kidney function.

Discussion

It is important to conduct good Phase 2 trials to inform Phase 3 trials. It is expected that Phase 2 studies will identify problems in study design that can be corrected in the design of Phase 3 studies.

The lack of definitions for staging disease is a barrier in designing AKI trials. Some data exist that inform the definitions, but the definitions need to be further clarified by well-designed studies.

Lessons for Nephrologists—Secondary Prevention Studies—A Design Viewpoint
James Tumlin, M.D., Professor of Medicine, Department of Internal Medicine, University of Tennessee College of Medicine, Chattanooga, TN

The critical time for AKI treatment is during the 2 to 12-hour initiation phase of the condition. Past clinical trials have focused on this phase for secondary prevention. Previously reviewed milestones in AKI trials include the trial that showed no improvement in patients for dialysis-free survival or mortality with the ANP anaritide (Allgren et al., *N Engl J Med* 1997). Issues that confused the results included the 48-hour duration of drug treatment and the 24-hour infusion time, the fact that the population was heterogeneous, and a failure to define an endpoint (when to dialyze). In addition, SCr levels of 4.6 mg/dL at entry were considered high. Another milestone trial also investigated 61 bypass patients at a lower dose of anaritide and showed better results for dialysis-free survival and time to dialysis (Sward et al., *Crit Care Med* 2004).

A primary prevention trial, the Nesiritide Administered Peri-Anesthesia in Patients Undergoing Cardiac Surgery (NAPA) study, of 303 patients with CHF after bypass surgery in 54 centers, investigated the use of nasiritide with endpoints of SCr at discharge or mortality at 30 and 180 days (Mentzer et al., *J. Am Coll. Cardiol* 2007). The study found that with nasiritide, there was a significant reduction in post-operative SCr in patients with CKD and improved patient survival at day 180.

A strategy to improve drug delivery in CKD and AKI patients is the *Benephit*® catheter, which has been shown to safely administer infusions up to 72 hours and allows concentrated exposure of therapeutic agents directly into the renal circulation. It also can allow combination therapy directed towards multiple AKI pathways. Drugs that have been delivered using the *Benephit*® catheter include ANP, dopamine, BNP, fenoldopam, adenosine, and prostacyclin. A trial using this system comparing extra-renal (IV) and intra-renal fenoldopam administration found a significant increase in renal arterial flow velocity using the catheter (Grube et al., *EuroInterv* 2005). Another study found that dopamine and fenoldopam could be administered more efficiently using the renal catheter rather than IV administration **(**Manrohan et al., *J. Am Col. Cardiol* 2006). The Manrohan study also found that there were beneficial dose-dependent changes in renal vascular resistance with the catheter compared to IV administration.

New approaches for AKI include investigations of apoptosis disruption in post-ischemic AKI, which involves the use of novel agents being developed from the Thrasos Compound

Library. Animal studies using derivatives of BMP-7 have been shown to reduce ischemia in AKI.

During the inflammatory phase of AKI, between 12 and 24 hours, macrophage and neutrophil infiltration into the interstitium has become a target for intervention. Past studies have shown that α-melanocyte stimulating hormone (α-MSH) protects against ischemic ATN and reduces SCr levels soon after administration (Chiao et al., *J Clin Invest* 1997). Using this information, novel drugs are being developed that address the inflammatory phase of AKI.

An AKIN-NIH-industry consortium that resembles the ARDS network and the Lupus Clinical Trials consortium has been suggested to spur AKI research. Also, there is a need to revisit some of the earlier trials to develop protocols that are based on subsequent research. Many of those trials appeared to fail but taught important lessons that could be applied to future clinical trials.

Discussion

One challenge in AKI trials is the timing of access to patients. If patients do not receive treatment quickly, the chance of success may be reduced dramatically. Some researchers develop relationships with surgeons to be alerted when patients are coming out of surgery. This is an important step toward developing successful treatments and reducing mortality.

Another challenge is treating patients with hypotension from the surgery or from the treatment. This generally is a transient problem that requires attention in developing entry criteria for trials. As shown in past studies, it can be overcome, but it also can be a serious problem if left unaddressed.

Patients accrued from primary centers (i.e., the community) as opposed to secondary centers (i.e., treatment facilities) tend to fare better in clinical trials. Perhaps different protocols should be developed for type of treatment setting. One issue is that the length of time it takes patients who come to primary centers to get to secondary or tertiary treatment facilities typically falls outside the window for conventional AKI treatment protocols. This issue needs further discussion, which should include whether patients are being treated at the correct phase of AKI.

Another issue that should assist in identifying patients who may benefit from treatments is the development of biomarkers. This is especially true for patients with pneumonia who develop AKI.

DECEMBER 3, 2010

DRUG TARGETS

Moderator: *Sudhir Shah, M.D., Division Director, Professor of Medicine, Department of Internal Medicine and Nephrology, University of Arkansas for Medical Sciences, Little Rock, AR*

Drugs Targeting Endothelial and Epithelial Cells

Dr. Molitoris

The functional pathophysiological phases of AKI include: (1) an at risk phase with kidney hypoperfusion; (2) an initiation phase, characterized by a precipitous drop in GFR over approximately 24 hours; (3) an extension phase manifested by microvascular injury with obstruction, coagulopathy, and inflammation; (4) maintenance, characterized by cellular de-differentiation, migration, and proliferation and the lowest level of GFR over 1-2 days; and (5) recovery, characterized by a rise in GFR accompanied by re-differentiation and re-polarization of epithelial cells. The entire event occurs over several days depending on the severity of injury, but is rarely a smooth progression followed by a smooth regression.

By examining the pathophysiology of the kidney proximal tubules, it is possible to see what is happening in the cell and where drugs are distributed within the tubules. Some researchers feel that proximal tubular cells are also sensing cells because they are present in the kidney where filtration of plasma delivers Danger-Associated Molecular Patterns (DAMPs) and proximal tubules reabsorb and process them (Wu et al., *JCI* 2007).

In an animal ischemic model of AKI, small interfering (or silencing) RNA (siRNA) was investigated for its role in inhibiting p53 in proximal tubular (PT) cells and preventing ischemic AKI. A study in rats with repetitive kidney ischemia, initiated at 30-day intervals, found that sip53 protected GFR and minimized proteinuria. In a second study, ischemic-induced CKD rats were studied. The goal of the study was to see if siRNA prevention of p53 upregulation in PT cells would prevent or minimize ischemic AKI. Results indicated that sip53 did attenuate AKI in this model with one treatment, with SCr returning to baseline in siRNA treated but not placebo treated rats.

A study of p53 inhibition also showed that fibrosis was minimized, with arrest of the G2/M cell cycle (Yang et al., *Nat Med* 2010). In addition, prolonged G2/M arrest attributable to Taxol administration led to increased fibrotic response in an IRI model of AKI. A recent study of myofibroblasts in kidney fibrosis found that pericytes, not epithelial cells, are the origin of myofibroblasts (Humphreys et al., *Am J Pathol* 2010). Data from the study indicate that therapeutic strategies directly targeting pericyte differentiation in vivo may productively impact fibrotic kidney disease.

The endothelial cell involvement in AKI is well known. Studies of the microvasculature have shown that all regions are affected by vascular drop out, with the medulla and inner stripe most affected. This leads to hypoxia, hypertension, and proteinuria during CKD progression

(Basile et al., *Am J Physiol* 2001, 2003, 2007). Ischemic injury causes reduced flow in the renal vasculature, which occurs following the first 4 hours after injury, after which time the injury may be too established for effective treatment. There is also recent evidence of endothelial cell mesenchymal transition in this ischemic model (Basile, et al., *Am J Physiol* 300(3) F721-33, 2011).

Cross talk also is an important part of the microvascular environment. Studies of vascular flow show that multiple cell types transmit information affecting specific outcomes. Endothelial cells send messages to epithelial cells and vice versa. During the inflammatory process, cross talk is critical for directing the immune response. This also suggests a wide range of possible new therapeutic compounds that can take advantage of this cell:cell cross talk.

Discussion

The injury to the tubules is mediated by white cells as the blood flow decreases. Heparin can prevent the tubules from walling off and keeps them open.

A question was raised about renal fibrosis in rat models, and whether the injury has acute effects on other organs. The model used healthy rats, which may be equivalent to a normal human who has transient AKI and recovers. In an unhealthy rat, such as in a diabetic rat model, it may not be the same, just as injury may be associated with different outcomes in people with multiple comorbidities.

The largest window of opportunity for effective treatment is drug dependent. Since the vasculature appears to take 4 hours to begin disintegration in the severe injury model, this obviously is a window for treatment. Studies show that treatment within the first 2-4 hours may be better than later. This makes it imperative that there be rapid diagnostic methods and therapeutic delivery of drugs quickly during the treatment window.

Drugs Targeting Inflammatory Cells

Mark Okusa, M.D., Professor, Department of Medicine, University of Virginia, Charlottesville, VA

Activation of the innate immune system leads to early IRI with subsequent inflammation. Although this appears to be a normal response to injury, it is the overzealous, uncontrolled inflammation that contributes to AKI. This "collateral" damage is similar to the process that occurs following the inflammatory response to infections. Thus blocking the early immune response may attenuate tissue injury. Tissue resident immune cells, such as macrophages and dendritic cells, along with infiltrating cells, such as proinflammatory macrophages, lymphocytes, and neutrophils contribute to innate immunity of AKI. The role of

these cells has been elucidated through in vivo studies using genetically altered mice. Knock-out and transgenic mice, bone marrow chimeras and adoptive transfer studies are a few in vivo methods combined with immunological methods, such as immunofluorescence microscopy and flow cytometry, that have provided new knowledge regarding the role of immune cells that support potential immune-based therapies for AKI.

Adenosine 2a receptors (A2aRs) belong to a guanine nucleotide-binding protein coupled receptor family that includes four subtypes: A1, A2a, A2b and A3Rs. Accumulating data demonstrate that selective activation of A2aRs reduces parenchymal injury in renal and nonrenal tissue including heart, liver, spinal cord, lung, and brain. We have studied A2aR agonists in animal models of AKI and have demonstrated a potent effect to block kidney IRI. Through a series of studies we have found that these agonists activate A2aRs expressed on CD4+ T cells as well as dendritic cells. Acting on these receptors, A2aR agonists attenuate immune cell activation and inflammation. Furthermore, we and others have shown that CD4+CD25+FoxP3+ T regulatory cells require A2aRs for their normal immunosuppressive function. Thus by targeting several immune cells, multiple mechanisms are activated to potently block inflammation.

Understanding the role of A2aRs in immune cells, such as dendritic cells, has led to our interest in cell-based therapy. Cell-based therapies have been studied in other disease models including cancer therapy. In our studies we induce dendritic cell tolerance through incubation of dendritic cells with A2aR agonists. Adoptive transfer of these A2aR agonist-tolerized dendritic cells attenuates IRI. The advantage of this approach is that potential side effects of this compound (or any compound) can be minimized.

Another model used is the folic acid model of CKD studied by several groups. There is marked enhancement of renal fibrosis following infusion of folic acid. Seven days following folic acid administration, we observed that both fibronectin and collagen are expressed in the kidney to a high degree. Treatment with an A2aR agonist, 1 day after the folic acid is administered, results in notable reduction in collagen expression, indicating the potential importance of targeting immune cells in AKI to prevent progressive CKD.

Another compound, FTY720, is derived from a fungus that is also the source of cyclosporine. FTY720 binds to sphingosine 1-phosphate receptors and produces pleiotropic effects, including immunosuppression. For this reason it is now approved for the treatment of multiple sclerosis. We have used FTY720 in kidney ischemia-reperfusion experiments and have shown this drug to be effective in reducing injury.

A number of other novel compounds are in development/and or clinical trials, such as AP214, an analog of α-MSH, which has been used in pig and mouse models of AKI as well as for the prevention of AKI in patients following cardiac surgery (NCT01256372, clinical trials.gov). The Keap1/Nrf2 pathway has been investigated as a drug target for bardoxolone methyl, a synthetic oleanane triterpenoid that is an antioxidant inflammation modulator. In an

animal model of cisplatin-induced nephrotoxicity, bardoxolone methyl reduced injury. This drug has been shown to increase eGFR in Phase 2 studies in patients with stage 3 and 4 CKD. Lastly alkaline phosphatase has been tested in a Phase 2 clinical trial of patients with sepsis. Treatment with alkaline phosphatase led to enhanced creatinine clearance and reduced need for dialysis. Thus novel compounds are currently in development or in clinical trials for AKI.

Discussion

Timing of administration is critical for each of the drugs discussed. For example, administering A2aR agonists within 30 minutes after injury provides maximum protection. The effectiveness of treatment is attenuated considerably if administration is 1 hour later. Delayed treatment with FTY720 results in outcomes similar to those seen with A2aR agonists. In the folic acid model, delayed treatment is also less efficacious. Thus the use of biomarkers for early detection of AKI will be critical in future clinical trials as more and more studies have shown that there is a narrow treatment window. Another issue is whether drugs should be given systemically or targeted locally. While systemic delivery may attenuate distant organ injury initiated by AKI, patients may suffer from side effects of the drugs. Atrial natriuretic peptide is one example where the systemic hypotensive effect may have limited the potential kidney benefit. Catheter based local delivery may minimize systemic effects, but this approach is limited by the potential technical complications associated with invasive procedures. Targeted delivery using immunoliposomes is currently under investigation. Remote preconditioning, in which repetitive limb ischemia may reduce AKI, is undergoing investigation in primary prevention of AKI.

There is a need to have novel compounds for well-designed Phase 2 trials. For example a Phase 2 trial could be designed to test a novel compound in patients undergoing cardiac surgery. In this setting, the timing of AKI after cardiac surgery is known, which will facilitate therapeutic interventions.

Stem Cells and Targeting Drugs

Christof Westenfelder, M.D., FACP, Professor of Medicine and Physiology, University of Utah School of Medicine; Chief, Section of Nephrology, Salt Lake City VA Medical Center, Salt Lake City, Utah

Adult Stem Cells (SCs), such as bone marrow-derived Multipotent Stromal Cells (MSCs; aka Mesenchymal Stem Cells), have been shown by various investigators to protect renal function and to stimulate the repair of rodent kidneys that were injured by IRI, cisplatin or glycerol-induced rhabdomyolysis. The mode of action of MSCs in this setting is primarily paracrine, consisting of complex anti-inflammatory, anti-apoptotic, mitogenic and vasculoprotective effects. In addition, we observed that administration of autologous or allogeneic MSCs to rats with IRI prevented the late development of chronic kidney disease (CKD) that is regularly seen in vehicle treated controls (Tögel et al., *Stem Cells Dev* 2009). We also demonstrated, using siRNA technology, that knock down of vascular endothelial growth factor (VEGF) in MSCs given to rats with AKI markedly increased mortality and resulted in

subsequent loss of microvascular density and renal function. These data identified VEGF as one of the principal paracrine mediators whereby MSCs both protect renal function and enhance the regeneration of the acutely injured kidney. Importantly, the administration of MSCs in these animal models proved to be safe both early and late after the induction of AKI. Since MSCs do not express blood group and DR antigens, they are significantly immune privileged, allowing their off-the-shelf use in allogeneic protocols.

"Targeting drugs," such as Atrial Natriuretic Peptide, IGF-I and erythropoietin were shown to be renoprotective in otherwise healthy animals with AKI. However, when tested in clinical trials, these drugs failed to improve outcomes in patients with AKI. Distinct from the pre-clinical results obtained in overall healthy animals, these negative clinical outcomes were likely due to the presence of important comorbidities (CKD, diabetes, older age, etc.) in study subjects, and possibly, the intrinsic limitations of these drugs to adequately target the critical pathophysiologic mechanisms of AKI. In contrast, it is increasingly recognized that MSCs have the capacity to target all critical pathways in AKI, thereby making them superior to pharmacological interventions, and they can be delivered, through infusion, to essentially any site within the body.

Mechanistically, MSCs delivered to sites of injury in the kidney or other organs, regulate their adhesion and detachment in the microvasculature, respond to injury-generated cues in the affected microenvironment by adjusting their own gene expression profiles and those of injured renal cells, through paracrine signals, to beneficial ones, together improving survival of renal cells, reducing inflammation, and supporting tubular and microvascular repair. Administered MSCs that are recruited to the injured kidney reside there for less than 72 hours. After they detach they rapidly undergo anoikis in the circulation.

We investigated whether injury to an organ, such as the kidney, would result in the organ specific generation of a biochemical signal that causes stem cell mobilization from the bone marrow, increased delivery of these cells to the kidney, and organ repair. IRI of the kidney does result in rapid renal cellular upregulation of SDF-1 (CXCL12), a chemokine that facilitates mobilization and recruitment of HSCs and other cells that express CXCR4, the SDF-1 receptor. Recruitment of a fraction of administered MSCs is also facilitated through the SDF-1/CXCR4 axis. Since MSCs express CD44, the receptor for hyaluronic acid, which is robustly upregulated after AKI, this mechanism also contributes to MSC homing to the kidney.

In summary, extensive pre-clinical studies in rats and mice with various forms of AKI have demonstrated that the administration of autologous or allogeneic MSCs protects renal function and stimulates repair through anti-apoptotic, vasculo-protective, anti-inflammatory, and anti-oxidant actions, and through complex regenerative actions, all mediated through paracrine and endocrine modes of action. Only very rare and low-level microvascular engraftment of occasional MSCs with an endothelial phenotype is observed, the significance of which is uncertain. Importantly, late development of CKD after an episode of AKI is prevented by MSC therapy of AKI, and this is significantly mediated by MSC-delivered VEGF. In addition, MSCs possess potent immune modulating actions, in part by suppression of the T cell response. In conclusion, we posit that our data and critical observations from other investigators demonstrate the therapeutic superiority of MSCs in AKI over that of clinically tested "targeting drugs."

Based on these pre-clinical observations, a Phase 1 clinical trial was conducted in which 16 adult, open-heart surgery patients who were at high risk for post-operative AKI were treated with allogeneic MSCs (Tögel and Westenfelder, *Nat Rev Nephrol* 2010). Preliminary results suggest that MSCs were safe and potentially beneficial in the prevention of AKI and secondary CKD. However, an adequately powered, randomized, double blind and placebo controlled Phase 2 trial must be conducted to test the definitive efficacy of this novel, cell-based intervention in patients with AKI.

Discussion

A significant improvement of renal outcomes and survival after AKI has been demonstrated following MSC treatment. Allogeneic MSCs were infused directly into the suprarenal aorta rather than intravenously. Following AKI, SDF-1 is rapidly up regulated throughout the kidney, including in the glomeruli, tubular cells and capillaries. This response facilitates recruitment of MSCs to the sites of injury where they temporarily reside, and where they exert complex paracrine actions that are cytoprotective and stimulate repair. When large numbers of MSCs were directly infused into the renal artery of rats with anti-Thy1-induced glomerular disease, that is, into a highly inflammatory microenvironment, these cells underwent intraglomerular differentiation into adipocytes, obviously an undesirable but not completely unexpected response. Thus, in order to avoid potentially unsafe ectopic differentiation of MSCs into mesenchymal lineages (adipocytes, osteocytes, chondrocytes), it is important that they are not administered in large numbers and into confined spaces that exhibit a heightened pro-inflammatory milieu. Finally, oncogenic transformation of MSCs is exceedingly rare. It can be prevented, as has been shown, by using cells that are cultured under low "stress" conditions and at low doubling numbers.

NIH PARTNERSHIP GUIDEPOSTS

Moderator: Dr. Kimmel

Public–Private Partnerships/Collaborative Opportunities/An NIH View

Shawnmarie Mayrand-Chung, Office of the Director, Office of Science Policy Analysis, NIH, Bethesda, MD

The NIH fosters partnerships if they are mission-consistent, have a research focus, are science-driven, and have a goal to improve public health. The NIH Public-Private Partnership Program (PPP) program, which was established in 2005 under the NIH Roadmap initiative, serves all 27 of the NIH Institutes and Centers (ICs) and serves as a point of entry for potential outside partners. In this role, the PPP, located within the Office of Science Policy Analysis within the NIH Office of the Director, acts as a "matchmaker" and resource for developing partnerships. The PPP is trans-NIH in scope and will work with individual or multiple ICs.

Partnerships must align with scientific priorities of the ICs and are designed to leverage NIH resources. This allows novel synergies, new efficiencies, and the opportunity to embrace common goals by working together. By leveraging NIH appropriations with partner resources these PPPs allow the NIH to conduct additional research which otherwise could not be accomplished.

As long as the goals and practices of the parties are aligned, partnerships can be formed with few limitations. However, NIH will never be able to delegate their federal authority. Partners may include other Federal Agencies ("public-public" partnerships), public and patient advocates, professional societies and industry. The U.S. Congress authorized the Foundation for the National Institutes of Health (FNIH) to provide a mechanism for the NIH to develop partnerships. The Foundation can raise funds from other organizations, for example industry, non-profit organizations, etc. to develop collaborative research programs that align with the mission of the NIH. Some examples of these programs are The Biomarkers Consortium (BC) and the Alzheimer's Disease Neuroimaging Initiative (ADNI).

The alignment of common missions and goals among partners has been found to be the easiest part of developing partnerships. What is more difficult is the alignment of cultures, including differences in timelines and the concept of time, differences in internal processes and decision points, and different vocabularies. Details that must be addressed before the partnership is established are: intellectual property, data sharing, resource sharing, publication, transparency of process, inclusiveness and fairness of access, decisions on who can commit resources and what resources can be committed, and conflicts of interest. Additional considerations that must be addressed include:

- Who in the organizations will hold the decision-making power?
- Who is affected and bound by those decisions?
- How long does it take to come to a decision?
- How is the project progress surveyed and what decision points result from surveillance?
- Who can decide to enter or pull out?

Although PPPs can be challenging to develop, the NIH believes that this partnership model provides a way to improve the scientific process and support the outside scientific community, while at the same time providing new avenues for research that may not be possible within the NIH. It is important that partnerships support rigorous science, ensure the highest standards of practice, scientific impartiality, fairness, and inclusiveness, promote the generation of public resources and translate discovery to benefits in public health.

Discussion

The time it takes to establish a partnership depends on how prepared the partners are at the onset of the partnership discussion. It can take as little as 3 months or up to a year.

The NIDDK participates in the Acute Safety Biomarker partnership which the Biomarker Consortium (BC) is managing. One of the hallmarks of the BC partnership model is that no decisions can be made by the BC without each founding partner, including NIH, FDA, and PhARMA, having input and approval.

A hurdle for the NIH and NIDDK is to support research that is difficult to conduct under the current NIH requirements. An example is the need to conduct clinical trials using combination therapies. This presents challenges because combination agents may involve more than one industry partner, and some may resist comparisons between their therapy and those from a competing company. The PPP may serve as an ideal vehicle to bring these multiple companies together in a "safe haven." Additionally, the FNIH has been successful when the partner wants to have a collaborative effort but wants to have more input. This becomes somewhat of an advisory position for the partner, with NIH becoming an "honest broker" in the process. The Foundation has gained the trust of partners through its work with the Biomarkers Consortium and the Alzheimer's Disease Neuroimaging Initiative. Currently, there is no element of commercialization within the BC, but there is a mandate to improve the public health. Although these different perspectives often conflict, the Intellectual Property clauses can help provide direction on the use of ideas and products within a partnership. Partnerships also offer an opportunity for translational activities, which may necessitate a change in the mission and mandate.

Intellectual Property—An NIDDK View

Anna Amar, Senior Technology Transfer Advisor, NIDDK, NIH, Bethesda, MD

Industry should want to work with the NIH because our mission is to improve the public health. NIH wants to help others succeed so the public will have new options for health needs, and the NIH has many resources to share with industry. The NIH is not in business to make a profit and is not in the manufacturing business, which are the goals and missions of the private sector.

NIDDK has an extramural component that funds clinical trials, usually through Cooperative Agreements, which are collaborative grant mechanisms which have substantial NIDDK staff involvement. The goal of these grants is to support and stimulate the clinical area of interest by working in partnership with the grantee. Recipient investigators have primary responsibility for: planning and directing the research, and for patient recruitment, as well as following regulatory and NIH requirements that are defined in the terms and conditions of the award.

There are a number of transactional agreement mechanisms that can be used to partner with the NIDDK including a:

- Confidentiality Agreement (CDA) -- an agreement to cover exploratory discussions and the exchange of proprietary information between the NIDDK and potential collaborators,
- Clinical Trial Agreement (CTA) -- that is used to establish terms and conditions for use of proprietary drugs or devices and sharing of research data and publications with the collaborator; and
- Cooperative Research and Development Agreement (CRADA) -- which permits the NIDDK to accept funding for the collaboration from an industry collaborator.

Considerations in the agreements for intellectual property (IP) offer protection for both sides which cover:

- **New inventions** (where ownership follows inventorship, although by law the government retains internal use rights), and federal march-in rights that protect the taxpayers' investment. It should be noted, though, that the NIH has never made use of march-in-rights.

- **The Bayh-Dole Act** must be taken into consideration, as this provision allows grant recipient institutions to retain ownership of inventions their employees create under U.S. federal funding mechanisms. This means that the NIH cannot require clinical networks to assign their inventions; however, grantees typically sign a separate agreement directly with the collaborator to license or assign any IP related to the trial. Usually the sites are universities that do not have additional funds to patent an improvement to a technology that already is well protected, so sites are open to the idea of negotiating assignment or licensing options upfront.

- **The Freedom of Information Act (FOIA)**, which may require the Government to release information to outside entities (5 USC § 552), has an exemption for information that concerns trade secrets and commercial or financial information that is privileged or confidential. It is important that industry partners mark such information accordingly. Note that CRADA-related data are protected by law for 5 years [15 USC § 3710a(c)(7)(A)]. If information can be shown to be patentable, it is protected until a patent is filed (35 USC § 205).

- **Confidentiality** may be maintained and protected if the information provided to the NIH is marked. Businesses should provide an oral summary for disclosures for maximum protection. Investigators are not authorized to sign agreements that bind their institute, although they can acknowledge the agreement. All Federal employees are bound by 18 USC § 1905, which provides criminal penalties for disclosing confidential information.

- **Data and publications** have their own considerations in partnerships. There are ownership versus access and usage issues with data. The NIH can agree to provide collaborators full access to, and use of, results from released locked data sets from joint clinical trials, and send regulatory data to the FDA. In addition, although the NIH mission is to disseminate information to the public (for example through published articles), the NIH can withhold publication for a reasonable time while patent

applications are filed, and the parties can agree to a review of disclosures before public release and to the removal of any collaborator confidential information.

In summary, the NIH has much offer at very little cost, and is interested in public health, not financial gain or competition. This is an advantage that the NIH can bring to a partnership. However, the Government must work within the restrictions of law, regulation, and policy. Much can be gained from a bit of flexibility when partnering with the NIH.

Discussion

Most programs at the NIH are offered to both industry and academia, but academia is less likely to be able to take a drug through the pre-clinical and clinical studies needed to submit an investigative new drug application to the FDA. This is why collaborative efforts between academia (new idea), government (translational) and industry (final testing and manufacture) are advantageous.

One of the critical challenges for partnering with the NIH is how to meet the needs of the biotechnology industry. This is an issue for translational research because biotechnology is critical to this field. The NIH sometimes has had limited success in this arena because the amount of time needed to develop the partnership is too lengthy for biotechnology. The 3-6 month time period required to develop a partnership is problematic for biotechnology because that industry must promote the data immediately to obtain funding to develop the technologies quickly. There are IP and data protection issues, and the needs are different in this realm. However, if the biotechnology company understands NIH policy and is willing to work within federal requirements, the time required to set up a collaboration, or to release data from an existing collaboration can be considerably reduced.

Venture capitalists usually are not scientists and are primarily focused on ensuring that the product they are backing can move quickly to the marketplace. For clinical trials, they want confirmation that the trial can be conducted with little delay. This presents challenges for Phase 2 trials, which are midstream in the process to obtain FDA approval and begin marketing the drug to consumers. However, once again, clinical trial agreements at NIH have been put into place within as little as a month in cases where the collaborator can work within the NIH template with only minimal changes.

DRUGS FOR CLINICAL TRIALS IN AKI—INDUSTRY ROUND TABLE

Moderator: Dr. Paul Kimmel

AM-Pharma BV

Jacques Arend, Ph.D., Vice President, Department of Clinical Development, AM-Pharma B.V., The Netherlands

AM-Pharma BV is a small drug development company based in the Netherlands that has conducted clinical trials on intravenous (IV) alkaline phosphatase (AP) for the treatment of AKI, and oral treatment for ulcerative colitis (UC) and necrotizing enterocolitis (NE). AP is an endogenous enzyme that acts via the detoxification of pro-inflammatory molecules (lipopolysaccharide [LPS] and extracellular ATP). Endogenous levels of AP are reduced in AKI, UC, and NE. Exogenous AP has anti-inflammatory effects in these indications. The mechanism of action of AP in AKI occurs in the extracellular ATP pathway to deactivate LPS.

Two Phase-2 randomized, double-blind prospective clinical trials (APSEP and APREN) in 72 patients with AKI caused by sepsis tested the effects of IV administration of bovine AP with 28-day follow up. The primary endpoint of the trial was kidney function improvement, measured by RRT requirement, SCr and renal creatinine clearance. AKI was defined according to the AKIN criteria. Results indicated that IV administration of bovine AP improves kidney function in patients with AKI, including faster improvement and normalization of creatinine clearance with a reduced need for or shorter duration of dialysis. In addition, biomarkers KIM-1, IL-18, CRP, LBP**,** and GSTA1-1 were improved. The biomarkers N-GAL, PCT, SCr, and cystatin-C were not improved significantly. Treatment improved outcomes, based on shorter ICU stays and a reduced need for mechanical ventilation. In addition, the treatment was considered safe based on the low number of negative side effects.

Bovine AP does have disadvantages, including a risk of bovine spongiform encephalitis, regulatory hurdles, logistical supply problems, clinical limitations, and limited patent protection. Based on the number of disadvantages, AM-Pharma BV is designing both a pre-clinical Phase 1 trial of recombinant AP, with results expected in the final quarter of 2012, and a Phase 2 dose finding trial with results expected in early 2014.

Studies such as these can be problematic because of differing guidelines of diagnosis and entry of patients with sepsis, different RRT modalities in the United States and Europe, and differing guidelines for the start/stop point for RRT, ICU, and hospital discharge criteria, standards of care, and definitions of AKI.

Discussion

The use of creatinine clearance in the non-steady state is a concern in this study. This is something that needs to be considered in subsequent studies but there is no solution at this time. There also are issues with time of collection for creatinine clearance.

Brookdale Pharmaceuticals
Randy Milby, M.B.A., Chief Operating Officer, Brookdale Pharmaceuticals, Old Greenwich, CT

Brookdale Pharmaceuticals is developing therapeutics for endothelial disease and dysfunction (kidney, heart, and peripheral vascular); identifying drugs that reduce oxidative stress and damage the vascular endothelium in vessels that supply blood to the brain, heart, kidney, and periphery; and identifying and developing easy-to-ingest formulations of current medications that are standards in cardio-renal care.

Brookdale is investigating NGAL as a therapeutic agent, not as a biomarker. Two independent groups are responsible for identifying NGAL as a sensitive marker for AKI and a protective agent in AKI. Dual actions of NGAL are as a bacteriostatic compound, as a siderophore:iron binding protein, and as a growth factor in multiple cell types including in developing and mature renal epithelia, where its kidney-protective properties are seen (Kai et al., *J Am Soc Nephrol* 2007). The therapeutic value of NGAL has been investigated in mouse models to demonstrate that it protects the kidney from AKI and accelerates recovery (Mishra et al., *J Am Soc Nephrol* 2004), and that NGAL may represent a novel therapeutic intervention in ischemic AKI (Mori et al., *J Clin Invest* 2005). In a transitional study between the mouse and the human, NGAL expression was shown to increase epidermal growth factor receptor (EGFR) activation, correlate with lesion progression in mouse and human, act as a target of EGFR signaling, and mediate the proliferative effect of EGFR (Viau et al., *J Clin Invest* 2010).

Additional questions regarding NGAL need to be addressed, including pre-clinical animal studies to verify NGAL's tissue-protective effect, especially in mice with comorbidities such as CVD and diabetes. In human studies, there is a need to develop NGAL for the treatment of CI AKI in the cardiac catheterization laboratory in patients with risk factors for CI AKI, and to test NGAL in other acute nephropathies, such as cardiac surgery-associated AKI, kidney transplant-associated AKI and AKI in critically ill patients.

Two drugs currently are in development for renal disease. BRK-002 (Phoseaz) is an easy-to-ingest oral phosphate binder designed to improve compliance in the ESRD population with high pill burdens. It will have multiple dosing levels for convenience, and is being targeted for the CKD dialysis and pre-dialysis populations. BRK-003 (OsteoD), is a compliance-designed Vitamin D2 analogue for CKD patients with metabolic bone disease or renal osteodystrophy. OsteoD will accompany Phoseaz to ensure bone health for the ESRD/CKD populations.

Discussion

As in any drug development process, there are barriers. Foremost is having funding to set up pre-clinical studies and to have a clear understanding on details on how to get to Phase 1 trials. At this point, none of the drugs are ready for human trials.

As for what disease entity to target for future trials in humans, a small first step might be to focus on CI AKI, but there is a need for more animal data before a decision is made about human trials.

Interest in conducting studies of combination therapies would be problematic without partners.

CorMedix

Mark Houser, M.D., Chief Medical Officer, CorMedix, Inc., Bridgewater, NJ

CorMedix is a small cardio-renal company based in New Jersey. Its primary focus at this time is testing the hypothesis that labile iron has a role in renal disease and CVD. There is strong evidence that CKD is a regional iron overload disorder, a strong biologic plausibility for the importance of labile iron in the pathogenesis of AKI, and accumulating evidence suggesting that it is important in the pathogenesis of CVD.

The labile iron pool (LIP) is generally defined as chemical forms of iron that can participate in redox-cycling, can generate damaging radicals, and can be scavenged by iron chelators. The LIP is associated with low-molecular-weight chelates such as citrates and phosphates, and accounts for less than 3% of total cellular iron. The generation of cellular radicals is directly related to the size of the cellular LIP, and increased labile iron can occur in the face of iron deficiency. There is evidence that LIP is seen in specific disease states (e.g., iron overload, IRI, and AKI), and that the size of the LIP correlates with injury. The protective effects seen with the use of iron chelators establishes the cause-effect relationship. In various models of AKI, including ischemia, gentamicin-induced, cisplatin-induced, and myoglobinuria, iron chelators appear to have a protective effect.

The therapeutic agent being developed is called Deferiprone (DFP), the first commercially-available iron chelator. DFP is enterally absorbed, easily penetrates cells and subcellular organelles, chelates mitochondrial iron, and can translocate iron from multiple cell compartments and transfer it to transferrin and pre-erythroid cells. DFP also raises transferrin saturation without generating redox-active iron, is not nephrotoxic, is effective in iron overload disorders, and has a predictable safety profile.

A Phase 2 proof-of-concept study was started in June 2010 with a primary endpoint of evidence of protection from AKI as assessed by novel renal biomarkers. An agreement with the FDA already has been negotiated for a Phase 3 trial. The primary endpoint will be composite, comprised of renal and CV events, including death, MI, dialysis, TIA/stroke, heart failure, and hospitalization.

Successful outcomes in AKI research and drug development require the validation of biomarkers for the diagnosis of AKI, the prediction of renal events and outcomes, the prediction of associated CV events and outcomes in the context of AKI, and the prediction of other important clinical events and outcomes. Access to observational or clinical trial data that will allow more accurate assessments of event rates for outcomes of interest also is needed. Barriers include validated, acceptable endpoints (e.g., surrogates and renal, CV endpoints), cost, poor predictive value of pre-clinical models, and a lack of detailed accurate event-rate data for clinical outcomes.

Potential solutions to the barriers include the ease of determination of safety issues, risk/benefit issues, and the ability to have an easier time gaining approval of outcomes based on surrogates. Each of these is based on the short-term nature of preventive or therapeutic strategies. In addition, these types of studies lend themselves to potential Bayesian statistical designs.

Possible collaborations between the NIH, FDA, academia, and industry can be facilitated to evaluate novel compounds in pre-clinical models. This provides an opportunity for those unaccustomed to interacting with basic research scientists. Other areas for collaboration are for protocol development and design, data access needed for event rate assumptions, sharing of expertise required for novel statistical designs and development of an AKI study consortia to reduce the time and cost of drug development. This also would include collaboration to develop a clinical data registry for AKI outcomes.

Discussion

The endpoints in the DFP trial appear to be distant from CI AKI, and are removed from the small changes in SCr and creatinine clearance usually seen in these types of trials. CorMedix chose these outcomes as part of the FDA agreement. From the literature, the best hard endpoint is re-hospitalization, but there is some concern about how this endpoint would assessed in a trial. Clinical outcomes are required by the FDA and need to be causally related. Biomarkers may not be accepted in the near future.

The dosing strategy begins before the angiographic procedure and the drug is administered for a week thereafter.

The size of the trial was the determining factor in choosing CI AKI as the population to study because it would afford more patients than use of other factors. The Phase 2 study has 60 total subjects (30 in each arm) with 8 days of dosing. The Phase 3 study is planned to have between 600 to 1,000 subjects depending on power calculations.

Genzyme Corporation
Steve Ledbetter, Ph.D., Group Vice President, Renal Portfolio Department, Genzyme Corporation, Framingham, MA

Genzyme Corporation is a large biotechnology company that focuses on many disease entities and technologies for use in scientific research. For AKI, the company approaches drug development by assessing the need for the drug, the regulatory pathway to gain approval, and the pivotal study needed in Phase 3 to show measureable outcomes. The major barrier to AKI studies for prevention is the large number of subjects, thousands to tens of thousands, needed for such studies. This diminishes enthusiasm for these types of studies in the commercial sector, which would need some assistance and advice to conduct these studies. The lack of a clear definition of the cause or description of the injury that leads to AKI explains some of the low enthusiasm for prevention studies.

Genzyme has a great deal of experience working with molecules that are effective in modulating the inflammatory response. An example is SDF-1, mentioned in a previous presentation (Westenfelder), which has been very effective in animal studies.

Therapeutic interventions have gained a lot of attention. One example is a transforming growth factor (TGF)-beta (TGF-β) antagonist being investigated in a Phase 2 renal study. Animal studies were successful in showing the TGF-β antagonist reduced the development of proteinuria and glomerular injury in rats.

Collaboration is a critical part of developing a research agenda for AKI.

Discussion

There are significant barriers for testing combination therapies, even in animals, and industry has very little experience in this area. The first step would be to have a clear understanding of what outcome you are seeking before starting this process.

In the renal domain, industry has some flexibility in developing trials by leveraging resources with other companies and the NIH, but the FDA still has problems with this approach. Designing trials is difficult without endpoints that are accepted by the FDA. For example, if the FDA will not accept changes in SCr as a legitimate endpoint or outcome, many of the trials being planned will not be able to move forward. The FDA needs to hear a scientific case for using surrogates or biomarkers as endpoints in Phase 2 trials. There is some leeway for industry to use these as exploratory endpoints, but this must be discussed with the FDA. Once robust Phase 2 data are collected, it then will be important to have hard endpoints for a Phase 3 trial.

If a TGF-β antagonist is to be used for AKI, administration would need to occur within days of diagnosis. This is known from clinical data, but its use in other organ systems may be different.

A discussion will be held during the panel session on the level of confidence participants have regarding the use of transient changes in SCr to predict long-term outcomes. Epidemiological studies seem to suggest that a steady rise in SCr, rather than a transient change, is responsible for long-term outcomes.

PLC Medical Systems

Mark Tauscher, President and Chief Executive Officer, PLC Medical Systems, Inc., Franklin, MA

PLC Medical is a small start-up company that developed RenalGuard Therapy® for CI AKI prevention, which includes the maintenance of high urine output (using furosemide), to prevent contrast agents from clogging tubules, and limit toxin exposure in kidneys. PLC is unique in that it has developed a device for automated matched fluid replacement in real-time to reduce side effects associated with over- or under-hydration. The RenalGuard Therapy® system has been tested in two single-center trials in Italy and has demonstrated significant reductions in CI AKI rates in at-risk patients. The system was tested in a 23-patient, Phase 2 U.S. safety trial, which established the safety profile of the device and therapy, and a 406-patient, Phase 3 pivotal trial to begin soon has been approved by the FDA. The trial design goals of the Phase 3 trial are to definitively establish the efficacy of RenalGuard Therapy®, to secure FDA approval, and to provide solid evidence to drive market adoption. The primary endpoint of the trial is based on SCr with 90- and 180-day follow up on clinical outcomes. PLC medical is using the old SCr standard of 25% or 5% absolute rise because when the trial was designed, the newer standard of 3% by AKIN was not yet published.

An issue that must be addressed is whether the device will qualify for reimbursement as an accepted standard of care. Also, it is unclear how much treatment effect is needed for the device to be adopted. CI AKI research has been moving rapidly over the past decade, with bicarbonate and NAC treatments being conducted, although neither has shown conclusive benefits in CI AKI. PLC is struggling to decide what factors to include in the Phase 3 trial because of the issue of the changing landscape in CI AKI prevention and changing recommendations. Biomarkers do not have enough validation to be included in clinical trials by industry, although this may be an area that presents an opportunity for partnerships with those interested in including biomarkers in Phase 2 or 3 trials.

Clinical follow up and collaborations are two areas that need to be discussed regarding CI AKI trials. Follow up provides hard clinical endpoints but lengthens the trial and adds significant costs. This is an issue for the private sector. Collaborations may be more positive because trials for CI AKI involve cardiologists concerned with AKI issues and nephrologists, so there is no clearly defined area of study. A set of standards and definitions from NIH that can be used by the private sector, which has little interest or funding to develop such standards, is needed. The lack of a centralized consensus driving the clinical trial operation (IRBs, contracts, and practice) or the lack of consensus driving the challenges (defining standard of care, inclusion and exclusion criteria, and endpoint definition) are impeding progress in CI AKI and other areas of study.

Discussion

The experimental design of the Phase 3 study includes two arms (RenalGuard Therapy® and control) randomized on the day of the procedure. The hope is that in the treatment arm, urine output will rise.

The system is being evaluated as a device for premarket approval (PMA). It is being reviewed by the renal group at the FDA, which may differ from a review by the FDA cardiovascular group.

The RenalGuard Therapy® can be used in overdose treatment, such as for aspirin, which hospital staff have such a difficult time treating. Throughout the development process, many other ideas have been put forth for the use of the system, but because the device is undergoing PMA review, any other use for the device would require a separate PMA. This is holding up alternative uses regardless of the logical use of the device for other conditions.

Quark Pharmaceuticals

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Quark Pharmaceuticals is a clinical-stage pharmaceutical company that focuses on the discovery and development of novel RNAi-based therapeutics, which includes microRNA (miRNA) and small interfering RNA (siRNA). QPI-1002, also designated I5NP, is a synthetic siRNA that temporarily inhibits *p53* expression in early development. I5NP is rapidly filtered at the glomerulus and actively taken up by proximal tubular epithelial cells. It is the first siRNA to be systematically administered in humans and is administered, undiluted in phosphate-buffered saline. The target of I5NP siRNA is the temporary downregulation of the acute *p53* surveillance pathway (Christophorou et al., *Nature* 2006; Efeyan et al., *Nature* 2006; Berns et al., *Nature* 2006).

Pre-clinical studies have shown siRNA accumulates in proximal tubular epithelial cells following IV bolus administration, and that p53-targeted siRNA protects rat kidneys from IRI (Molitoris et al., *J Am Soc Nephrol* 2009).

The challenges in study design for investigating AKI in cardiothoracic surgical patients include the definition of AKI, the background event rate, sample size and power, enrollment and overall flu, as well as identifying endpoints that pass regulatory review.

Given the relatively low rate at which AKI has been reported to occur among patients undergoing cardiac surgery under cardiopulmonary bypass, no matter how liberally this outcome is defined there will likely always exist the need to select for an enriched study population, that is, one at increased risk for development of AKI. This is necessary in order to provide a sufficiently high background event rate from which it is possible to demonstrate a statistically significant treatment effect, if one truly exists, in a study that can also be adequately powered with a reasonable number of patients. If a risk score threshold is used to predict the requirement

for post-operative acute dialysis, the progressively larger numbers of patients that would have to be screened to identify the subset at progressively increasing risk of AKI who would qualify for such a trial, would be prohibitive. One example is the Cleveland Clinic (CC) risk score algorithm (Thakar et al., *J Am Soc Nephrol* 2005), which was shown to predict the need for acute dialysis following cardiac surgery on the basis of a set of 12 pre-operative risk factors, including gender, prior cardiac surgery, diabetes mellitus, and the presence and severity of pre-existing chronic kidney disease. Using this algorithm, the number of patients requiring acute dialysis following cardiac surgery, as a percentage of the overall population studied, was shown to decrease progressively at increasingly higher Risk Score thresholds from 0 to 13.

The challenges associated with efforts to develop definitions of AKI, and to enrich AKI risk among patient populations, are ones that have come from disparities in guidelines and physician practices. For example, use of a smaller change from baseline renal function to characterize AKI is likely to increase the number of patients in a clinical trial who meet the endpoint so defined, but such studies may obscure identification of a treatment effect due to the presence of a greater amount of background "noise" affecting renal function outcomes than when the AKI endpoint is characterized by a more ridigly defined endpoint. Methods to enrich the patient population may also include the use of biomarkers shown to predict AKI risk, other background event rates, or selection of other, surrogate endpoints for clinically-relevant outcomes (Mehta et al., *Circulation* 2006; Thakar et al., *J Am Soc Nephrol* 2005; Kuitunen et al., *Ann Thorac Surg* 2006; Paganini et al., EVOLVE study abstract 2010). Each has been used in a proof-of-concept Phase 1 study of the prophylaxis of AKI in patients undergoing non-emergent cardiac surgery. Approximately 12% of patients screened for the study were found to be eligible.

Quark designed a Phase 1 safety trial using the CCF risk score criteria, and is considering a RIFLE-/AKIN-based AKI definition for Phase 2 in addition to event rate enrichment using a pre-operative AKI risk threshold for eligibility in combination with the early post-operative requirement to detect the presence of at least threshold levels of selected biomarkers in body fluids. An adaptive design is being considered in association with interim blinded reviews to assess the predictive value of presumptive AKI biomarker(s).

Discussion

The cost of synthesizing siRNA is considerable, and should be taken into account when a company decides to bring an siRNA into clinical development. The cost/benefit ratio will have to be favorable enough to justify the cost of the clinical development program and drug synthesis.

Renal uptake of "naked" siRNAs—those not complexed to a carrier molecule—generally occurs rapidly, via glomerular filtration, such that little of the compound has been shown to distribute extra-renally, even in 5/6 nephrectomized rats.

The use of biomarkers shown to be predictive of AKI risk, in studies such as those described for cardiac surgery, has yet to be definitively validated. However, to function as an enrichment tool in selection of the population to be studied, the post-surgical window during which results

must become available is limited for a p53 inhibitor such as I5NP, which is administered approximately 4 hours after discontinuation of cardiopulmonary bypass.

Thrasos Innovation, Inc.
Richard Andrews, M.S., President and Chief Executive Officer, Thrasos Innovation, Inc., Montreal, Canada

Thrasos Innovation, Inc. was established to design, develop, and commercialize proprietary compounds that selectively activate receptors of the Bone Morphogenetic Proteins (BMP) pathway without generating bone growth, and deliver clinically significant protection, repair, and regeneration of critical organs and cells in both acute and chronic indications. The portfolio of proprietary compounds that activate specific receptors of the BMP pathway include 11 compounds with defined activity and more than 300 patent-protected sequences. The BMP pathway is important because it regulates/generates bone growth, differentiation and apoptosis. Receptors in the pathway include Type 1 (activin-like kinases [ALK]) and Type II (BMPR-II, ActR-II/IIB). BMP signaling occurs through the SMADs and requires Type I and Type II activation.

Thrasos has IP-protected 100 million compound variants in the first core family and is expanding into a new compound family. The main concern of the company is to design and conduct Phase 2 trials that will generate enough information to move to a Phase 3 trial. This is always the concern of agent developers.

Compounds with demonstrated activity include THR-184 addressing AKI (anti-apoptotic, anti-inflammatory actions) and THR-123 addressing CKD (evidence suggesting inhibition and reversal of fibrosis). Properties of the compounds include agonist activity, defined mechanisms of action, protease resistance, and being selective for targeted BMP receptors. Pre-clinical efficacy has included established performance in multiple rat and mouse models of AKI and CKD, and consistent performance in all studies and all models. Safety and dosage regimens have been investigated in pre-clinical studies and the compounds have acceptable profiles.

The clinical development strategy is straightforward. A Phase 1 safety trial in normal human volunteers, and a Phase-2 early intervention trial in high-risk cardiac surgery patients showing biomarker evidence of AKI. The study will have 2 or 3 arms depending on available funding. The Phase 2 study will include a run-in portion to demonstrate protocol feasibility.

Of the compounds tested, AA184 (THR-184) has been successful in pre- and/or post-insult studies. Challenges and concerns, however, remain, including the challenge of completing a successful cost-effective Phase 2 trial that gives investors, regulators, partners and clinicians confidence that a Phase 3 trial will be successful. There is still a need for refined definition of endpoints, timelines, and associated clinical outcomes before a Phase 2 trial can be successfully completed. A critical need is an in-place network of strong clinical sites with sufficient infrastructure to sustain ongoing trials (PIs and clinical coordinators).

Discussion

It may be of interest to have all the companies that presented at the workshop get together to see if they could design a Phase 3 trial based on what each has learned in their individual Phase 2 trials. The data could be pooled. Although this would be unprecedented in basic research in the private sector, it may be able to be done with the proper legal agreements and NIH involvement. Businesses tend to want to retain discretion over their business decisions and keep confidential information to themselves, but the businesses also want to take care of these patients as does the NIH. The community of businesses working in the field of AKI is very small, which means that more dialogue on this concept is warranted.

Industry Round Table - Panel Discussion

There are areas in which private businesses would be able to collaborate with other businesses and the NIH. Specifically, the area of biomarkers is ideal for collaboration, but it would be desirable for biomarker companies to have a seat at the table throughout the process. The biomarker companies could help with definitions and expected outcomes. There is little confidential information in the biomarker field, because the biomarker is a tool that anyone can use. If biomarkers are used as an endpoint, then there may be some resistance to allowing that to be used by other companies. In early trials not subject to FDA blinding rules, biomarkers could be shared. Once the biomarker is to be used in a randomized clinical trial (RCT), it becomes more difficult to share everything until the trial has been completed and the results are known.

Other areas for discussion are the sharing of data from clinical trials and samples collected and banked. Samples have the advantage of containing raw biomarkers that may not have been tested in the trial when they were collected. Company representatives said that if there was a way to do this without violating confidentiality issues, it may be possible. It certainly is something that business and the NIH could discuss further.

NIDDK is already working with pharmaceutical companies in the manner being discussed for AKI. An example is the Chronic Renal Insufficiency Cohort (CRIC) study. These types of collaborations are ongoing in many other studies and could be used as models for developing collaborations for AKI. However, AKI does have unique challenges in finding enough patients because of different inclusion criteria.

The Critical Path Consortium (CPC), composed of 16 pharmaceutical companies, has been meeting to identify relevant safety-related biomarkers. Individual companies validate the biomarkers through the use of standardized assays. The Consortium has been taking samples from various clinical trials to check for these biomarkers. The hope is that members will bring forth the data generated from these trials and be able to relate the data to more long-term outcome findings. At this point, the FDA is requesting more data from the CPC. Successful collaborations have been developed and have demonstrated openness to sharing data.

Biomarkers are also used outside the domain of drug development. Diagnostic biomarkers are far more likely to get through the FDA approval process if they will only be used to identify patients with certain conditions. For example, a diagnostic biomarker could be used

to identify patients that will respond to specific therapies, but they do not have to be based on outcome data for the disease. This is currently being done in cancer treatments and diagnosis. It is when the biomarker is related to a specific outcome or efficacy criterion that the bar is significantly raised at the FDA.

Children with AKI should be considered - but only very carefully for Phase 2 trials because they have fewer comorbidities. There are significant challenges related to both the regulatory process and ethics in such populations but there may be advantages to such studies. There are few children who suffer from AKI, and consequently the FDA views it as a predominantly adult disease. There is no information unique to children that would direct treatment in a different manner in children than in adults.