clinical trial,[11] in which γ-glutamyltranspeptidase and alkaline phosphatase served as biomarkers. Patients were enrolled on the basis of the diagnostic elevation of the calculated product of these biomarkers, which were normalized to urinary creatinine. The first dose of erythropoietin (EPO) was given within about 6 hours of the biomarker-confirmed insult, and the second dose at 30 hours. Controls with identical biomarker profiles were treated with vehicle. The rationale for the use of EPO included its demonstrated antiapoptotic, anti-inflammatory, and mitogenic actions in EPO receptor-expressing animal and renal cells.[13] Disappointingly, EPO failed to improve outcomes versus control subjects, indicating that either the biomarkers used or the tested treatment protocol may have been suboptimal. This is again in contrast to preclinical studies with EPO, all of which showed good renoprotective activity in otherwise healthy animals.

In conclusion, the current status of optimal biomarker and AKI therapy developments, as also illustrated by Rouse *et al.*,[6] does identify a number of important points: (1) Novel AKI biomarkers, particularly when used in combination panels, possess greater specificity and earlier diagnostic and prognostic sensitivity than serum creatinine and blood urea nitrogen, during both the injury and the recovery phases of AKI. (2) Changes in urinary profiles of these very diverse biomarkers do largely occur in a temporally parallel pattern during the injury (higher specificity) and recovery (lower specificity) phases of nephrotoxin-induced AKI. (3) Changes in urinary biomarkers illustrate distinct renal responses to injury, such as shedding of proximal tubular brush border components and cytosolic proteins/enzymes, while also identifying their individual roles in defensive and repair-supporting mechanisms that may serve as the basis for the future development of novel therapeutic approaches. (4) Initial proof of principle for both biomarkers (specificity, sensitivity) and interventions (efficacy) is most optimally tested in suitable clinical AKI models, such as open heart surgery patients and kidney transplant recipients. (5) Efficacy of novel therapies should be established in relevant preclinical animal models. (6) Effective therapies should optimally target all major components of renal injury and recovery. (7) It is hoped that select biomarkers may aid the physician in pinpointing the time when specific therapies are most effective.

DISCLOSURE
During the preparation of this Commentary, the author served as a consultant to AlloCure.

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Cellular therapies: what is still missing?

Kai Pinkernell[1]

Yeagy and colleagues present long-term data from a preclinical model of cystinosis after hematopoietic stem cell transplantation. The results suggest a therapeutic benefit independent of target tissue differentiation but dependent on the level of bone marrow chimerism. The mode of action remains mysterious, but positive effects are seen. Although the work presents a potential therapeutic option for an otherwise dismal disease, the search for the mechanism of action in cellular therapies continues.

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Yeagy and colleagues[1] present long term follow-up data in a preclinical model of cystinosis after wild-type or green fluorescent protein-labeled hematopoetic stem cell transplantation. The results suggest a therapeutic benefit of this approach, independent of differentiation of the graft into a renal cell type, but dependent on the level of bone marrow chimerism. Chimerism levels correlate with improved functional parameters as well as histopathological findings resulting in a therapeutic benefit that cannot easily be explained by target tissue differentiation

[1]*Miltenyi Biotec GmbH, Bergisch Gladbach, Germany*
Correspondence: *Kai Pinkernell, Miltenyi Biotec GmbH, Friedrich Ebert Strasse 68, 51429 Bergisch Gladbach, Germany.*
E-mail: pinkerk@hotmail.com

and takeover of function. The mechanism of action remains mysterious like in many other areas of cellular therapy, but positive effects can be detected. The work on the one hand continues to stimulate the hunt for the mechanism of action in cellular therapies but on the other hand presents a very real potential therapeutic option for an otherwise dismal disease.

The therapeutic potential of hematopoietic stem cell transplantation in this preclinical model of cystinosis was shown by this group in an earlier publication.[2] Interestingly, cells of the transplanted graft could be detected in many organs displaying an interstitial phenotype rather than organ-typical cell types. Furthermore, the prior work suggested that this level of therapeutic benefit could not be achieved solely with the use of mesenchymal stem cells, but that, rather, the full hematopoietic system needed replacement, resulting in a positive outcome. This could suggest (1) that the mesenchymal stem cells used were insufficient in number or not functional (the use of cells in passage 20 or other conditions could play a part in this), (2) that another cell type in the hematopoietic system carries the regenerative/therapeutic properties, or (3) that the interplay of several cell types is necessary for a positive outcome (the appearance of several green fluorescent protein-positive cell types in the kidney could suggest this).

Seeing these results in the light of the current work by the group,[1] a long-term benefit with the use of cellular therapy seems feasible, even in this type of hereditary defect, in which one would normally expect the need for a genetic correction in almost all cells of the body rather than only the hematopoietic system. Furthermore, hematopoietic stem cell transplantation does seem to permanently cure or substantially improve the functional defect in the *Ctns*$^{-/-}$ group even without any evidence of target tissue differentiation and replacement of damaged cells as described in other disease models.[3] The biggest question, then, is: How does this work? Is cystinosin somehow transferred from the interstitial cell types, identified as being from the graft, to surrounding cells (for example, the tubular epithelium in the kidney), leading to a rescue in function? The fact that a high percentage of chimerism correlates with a good functional outcome would suggest that a certain level of functional wild-type cells in the target organs is necessary, similar to a dose dependency or a threshold dose. Diffusion of cystine and metabolism in the donor cells or transport of cystinosin could ultimately be responsible for progressive clearance that is measurable in all organs. Through simple diffusion gradients, the donor cells might clear the otherwise accumulating cystine if diffusion can be postulated, or if the functional protein somehow makes its way into the deficient cells.

Even in older animals, transplantation led to a reduction in chronic kidney disease, which underlines the gradual increase in damage the genetic defect causes, suggesting a therapeutic window before irreversible changes occur that would prevent any benefit. It is also important to put this kind of chronic injury, with a gradual decrease in function, into perspective with acute disease models that were also studied. Acute kidney injury has been tested by several groups using various cellular therapy approaches, and despite the lack of evidence for permanent engraftment, a therapeutic benefit can be shown.[4]

So where will we go from here? The results presented by Yeagy *et al.*,[1] in the context of other work in the field, suggest that we are still missing something—the critical piece of the puzzle that would result in the completion of a model to explain the diverse phenomena we see. For cellular therapies (Figure 1): It is not differentiation into tubular epithelium or hepatocytes or cardiomyocytes; we rather see an improvement in function through an interstitial cell type, well in line with prior observations in acute models.[5] For cystinosis: It is not the correction of the defective gene in all cells of the body that results in a critical rescue of lysosomal function. The interstitial cell type seems capable of taking over the missing metabolic function.

Still, much work must be done to figure out exactly how to apply this in the patient setting of cystinosis. A syngeneic donor pool, such as the one available in the mouse system, is something far away from

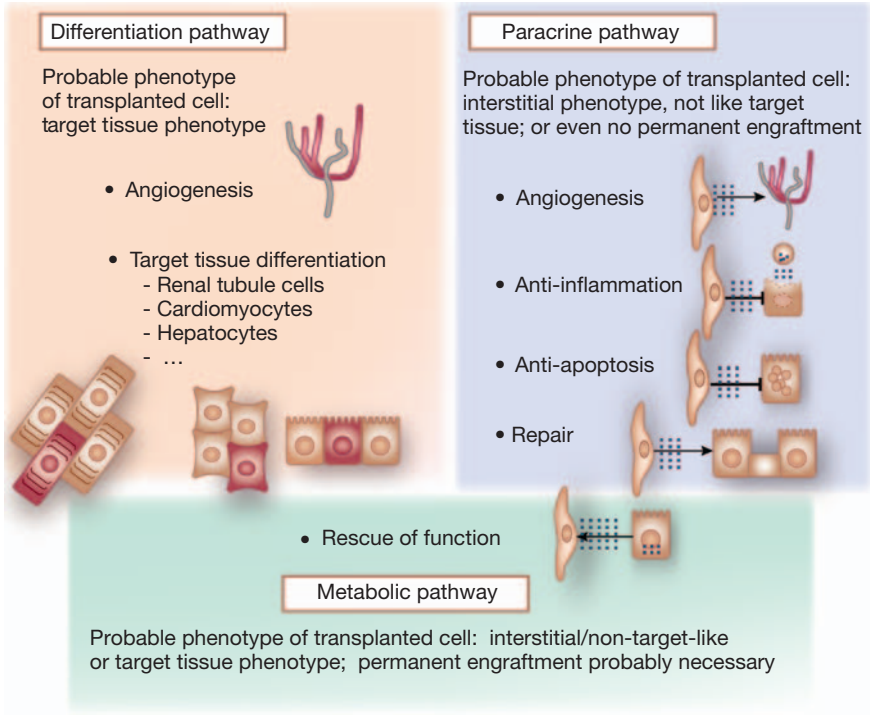

Figure 1 | Putative mechanisms of action in cellular therapies. Several mechanisms have been postulated, ranging mainly from target tissue differentiation to paracrine pathways. A critical piece is still missing that would allow an explanation of all phenomena observed. The observed events are dependent on multiple variables, including the cell type transplanted and the underlying injury (e.g., acute ischemic vs chronic metabolic).

reality in the clinic. Taking allogeneic, hematopoietic donor cells will result in therapy-related mortality and morbidity that might push the risk–benefit ratio in the wrong direction. And last but not least, gene therapy approaches have their own set of issues—for example, vector safety, which becomes especially apparent when stem cells have to be transduced.

This is what most of us will love about the science part: seeing how the puzzle will be solved. In the meantime, we should think hard about how to help our patients. Ensuring safety and guided by an acceptable risk–benefit ratio, cellular therapies will have to be developed at the bench and the bedside, and the paper by Yeagy *et al.*[1] is a great example of a very involved experimental system adding pieces to the puzzle to make a difference.

DISCLOSURE
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Hexokinase: a novel sugar kinase coupled to renal epithelial cell survival

R. Brooks Robey[1,2]

Hexokinases have emerged as novel mediators of the antiapoptotic effects of growth factors in a wide variety of cells. These effects have been attributed to highly regulated direct physical and functional interactions with mitochondria. The demonstration that mitochondrial hexokinases can prevent apoptogenic 'Bax attack' in proximal tubule cells suggests a need to reexamine the specific contributions of hexokinases and glucose metabolism in this nephron segment and elsewhere within the kidney.

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Hexokinases (HKs) catalyze the first committed step of glucose (Glc) metabolism, namely, the phosphorylation of Glc to yield glucose-6-phosphate. This reaction maintains the concentration gradient permitting facilitated Glc entry into cells and also initiates all major pathways of Glc utilization.[1] As such, this rate-controlling step is ideally positioned to influence the magnitude and direction of Glc flux within cells. The major HKI and HKII isoforms also exhibit a novel capacity for specific physical and functional interaction with mitochondria that directly couples and coordinates oxidative phosphorylation with cytosolic Glc flux.[1,2] Mitochondrial HKs have recently emerged as major mediators of the antiapoptotic effects of growth factors.[1,3,4] Competition with apoptogenic Bax and Bak for common mitochondrial binding sites and direct metabolic coupling with oxidative phosphorylation have both been implicated in these effects. When examined in cells with intact mitochondria, partial or complete Glc dependence has been observed, suggesting specific metabolic requirements.[1] Gall *et al.*[5] (this issue) now report the ability of mitochondrial HK to mitigate Bax-induced apoptosis in an adenosine triphosphate (ATP) depletion model of proximal tubule cell injury, suggesting that metabolism *per se* is not an absolute prerequisite for Bax antagonism.

Under normal conditions, proximal tubules exhibit a lower relative glycolytic capacity than other nephron segments and a heavy reliance on non-Glc energy substrates.[6] Although it does not follow that proximal tubule cells do not—or cannot—utilize Glc, these observations have led to the common misperception that Glc metabolism is unimportant in this nephron segment. There is, however, considerable evidence to the contrary. All mammalian cells can utilize Glc,[2] including proximal tubule cells.[6–9] Although less than that observed in distal nephron segments, the total Glc-phosphorylating capacity of freshly isolated proximal tubules[10,11] is not much lower than that reported for normal muscle or adipose—tissues largely responsible for systemic Glc disposal. This suggests a substantial cellular capacity for Glc utilization, with the caveat that only a fraction of this capacity is probably required to support the demands of even the most Glc-avid cell types.[2] The corresponding ability to oxidize Glc[11] is similarly comparable to that observed in adipocytes. As the only energy substrate that can be effectively utilized in the absence of O_2, Glc has particular relevance to ischemic or hypoxic conditions,[1] so it is of considerable interest that proximal tubule glycolytic activity increases *in vivo* following brief ischemia.[7] Similar changes can prevent ATP depletion, cellular injury, and transport dysfunction in proximal tubule preparations subjected to brief hypoxia or chemical anoxia.[12]

[1]*Veterans Affairs Medical Center, White River Junction, Vermont, USA and* [2]*Dartmouth Medical School, Hanover, New Hampshire, USA*
Correspondence: *R. Brooks Robey, WRJVA R&D Svc (405/151), 215 N Main Street, White River Junction, Vermont 05009-0001, USA. E-mail: R.Brooks.Robey@Dartmouth.edu*