Cellular Senescence in the Kidney

Marie-Helena Docherty,1 Eoin D. O’Sullivan,1,2 Joseph V. Bonventre,3 and David A. Ferenbach1,2

1Department of Renal Medicine, Royal Infirmary of Edinburgh, Edinburgh, UK; 2Centre for Inflammation Research, Queen’s Medical Research Institute, University of Edinburgh, Edinburgh, UK; and 3Renal Division and Division of Engineering in Medicine, Brigham and Women’s Hospital, Department of Medicine, Harvard Medical School, Boston, Massachusetts

ABSTRACT

Senescent cells have undergone permanent growth arrest, adopt an altered secretory phenotype, and accumulate in the kidney and other organs with ageing and injury. Senescence has diverse physiologic roles and experimental studies support its importance in nephrogenesis, successful tissue repair, and in opposing malignant transformation. However, recent murine studies have shown that depletion of chronically senescent cells extends healthy lifespan and delays age-associated disease—implicating senescence and the senescence-associated secretory phenotype as drivers of organ dysfunction. Great interest is therefore focused on the manipulation of senescence as a novel therapeutic target in kidney disease. In this review, we examine current knowledge and areas of ongoing uncertainty regarding senescence in the human kidney and experimental models. We summarize evidence supporting the role of senescence in normal kidney development and homeostasis but also senescence-induced maladaptive repair, renal fibrosis, and transplant failure. Recent studies using senescent cell manipulation and depletion as novel therapies to treat renal disease are discussed, and we explore unanswered questions for future research.


Global demographics are changing, with the proportion of individuals over the age of 65 in the United States predicted to double within the next 25 years.1 Older kidneys exhibit decreased function, altered homeostasis, and increased susceptibility to pathologies including AKI, maladaptive repair, and the subsequent development of CKD.2,3 In recent years, cellular senescence has emerged as an important driver of aging and age-related disease in multiple organs including the kidney. This review examines our current understanding of renal senescence, along with examples from other organ systems where this highlights areas of potential research and translational relevance to kidney homeostasis and disease.

WHAT IS SENESCENCE?

“Senescence” derives from the Latin “senex” meaning “old” and was originally used by biologists interchangeably with the term “aging” to describe the decline in organism function with time. A seminal description by Leonard Hayflick in 1961 of what is now recognized as replicative senescence due to progressive telomere shortening with prolonged cell culture in vitro helped shape our modern understanding of the term. Senescence is now recognized as both a response to prolonged culture or cell stress in vitro and as an important cellular stress and aging response in vivo.4 Although telomere shortening is accepted as sufficient to induce growth arrest and senescence in vitro, the key importance of telomere shortening in human aging is less well established, and is not the focus of this review.5 Although the association between aging and senescence is well recognized,6,7 only recently have experiments depleting senescent cells in murine models been shown to postpone the onset of age-related diseases and extend healthy lifespan, reigniting clinical and research interest.8–10

Initiation of Senescence

Today, the term “cellular senescence” is used to describe irreversible proliferative arrest with associated changes in chromatin organization, gene transcription, and protein secretion either in vitro or in vivo.11,12 Senescence can occur in multiple contexts across tissue and organ lifespan including physiologically appropriate “acute senescence” responses during injury, normal organogenesis, tissue homeostasis, and repair.13 In these settings senescence appears to limit fibrosis before senescent cells are removed by apoptosis or immune clearance.14 This form of senescence seems a tightly controlled, scheduled process.

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Correspondence: Dr. David A. Ferenbach, Centre for Inflammation Research, Queen’s Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh, EH16 4TJ, UK. Email: david.ferenbach@ed.ac.uk

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In contrast, chronically senescent cells accumulate in organs in response to a variety of stressors including DNA damage or critical telomere shortening leading to exposure of DNA ends,15 oncogenic mutations, metabolic stress, mitochondrial dysfunction, and inflammation.16,17 These insults trigger cell-cycle inhibition and permanent cell-cycle arrest via pathways either dependent on or independent of the DNA damage response (summarized in Figure 1). These cells persist within affected organs and are increasingly recognized as drivers of disease progression rather than bystanders.

Most senescence-inducing stimuli result in the induction of the cyclin-dependent kinase inhibitors p16\(^{ink4a}\) and/or p21\(^{cip1}\) which enhance checkpoint activity, inducing cell-cycle arrest at the G1/S cell-cycle checkpoint with induction of the senescent phenotype.11,12 Studies of experimental renal disease in mice lacking p16\(^{ink4a}\) and/or p21\(^{cip1}\) show increased cellular proliferation but also increased mortality after ischemia/reperfusion injury, suggesting that close regulation of the cell cycle is necessary for postinjury repair.18,19 Work with several experimental models of fibrotic kidney disease identified that accumulation of cells arrested at the later G2/M checkpoint (via inhibition of cdc25c/cdc2 by p53 or activation of the Chk1 and Chk2 proteins) not only results in senescence but triggers a pro-fibrotic secretory phenotype.20–22 Inhibiting this checkpoint using JNK, histone deacetylase, or p53 inhibitors results in reduced numbers of G2/M arrested cells and reduced fibrosis in a model of unilateral renal ischemia.20 Delineating the differences and commonality between G1/S and G2/M arrested cells will be of interest in the future. Inhibition of movement of the cell through G1/S may, for example, reduce the number of cells that are arrested at G2/M.

**Defining and Detecting Senescent Cells**

Whereas the physical and functional alterations seen with senescence in vitro are well characterized, identifying and characterizing senescent cells in vivo remains challenging.23 This is due in part to the lack of any single feature uniquely identifying senescence, and difficulties in assaying multiple attributes in individual cells within a tissue.11,12,14 Important markers include senescence-associated β-galactosidase (SA-β-gal), p16\(^{ink4a}\), and p21\(^{cip1}\); however, the presence/absence of one or more of these factors is insufficient to confirm senescence. The Van Deursen group has proposed that quantifying tissue senescence should comprise three approaches: staining of cell-cycle arrest markers, quantifying the effects of senescence-associated protein release, and excluding coexisting cellular proliferation.12 The generation of transgenic mice expressing genes driven by promoters

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**Figure 1.** Pathways to cellular senescence in eukaryotic cells. Multiple discrete cellular insults act via distinct signaling mechanisms to induce cell-cycle arrest in the kidney at either the G1/S (via inhibition of cdk2 and/or cdk4/6) or G2/M checkpoints (via Chk1/2 activation or cdc2/25c inhibition). Inactivation of oncogenes and spindle/epigenetic/nucleolar stress trigger activation of the cyclin-dependent kinase inhibitor p16\(^{ink4a}\). Telomere shortening, DNA damage, mitogen or oncogene activation, and hypoxia/reoxygenation also result in G1/S cell-cycle arrest, via a pathway dependent on p53 and p21\(^{cip1}\) activation. In contrast to this, developmental senescence appears to induce p21\(^{cip1}\) by pathways mediated by TGFβ/P13K and independent of p53. ATM/ATR/ARF, Ataxia–Telangiectasia Mutated/Ataxia Telangiectasia and Rad3-related protein/p14 Alternate Reading Frame (human).
cloned from the Cdkn2a locus which encodes p16
ink4a (INK-ATTAC, p16 [LUC], and p16–3MR mice) represents a major contribution to this field.\textsuperscript{24–26} Recent advances in single-cell sequencing should facilitate the identification and characterization of senescence on an individual cell basis for the first time.

**The Senescence-Associated Secretory Phenotype**

Despite exiting the cell cycle, senescent cells remain metabolically active, releasing a modified secretome into local tissues which can significantly affect neighboring cells and ultimately tissue function. This secretion of cytokines, chemokines, growth factors, and proteases is termed the senescence-associated secretory phenotype (SASP).\textsuperscript{27–29} SASP components include IL-1B, IL-6, IL-8, TGFβ1, and WNT16B and these can act in a paracrine and autocrine fashion leading to persistence and propagation of senescence within tissues.\textsuperscript{14,30–33}

Proinflammatory cytokines and chemokines are relatively conserved SASP components across multiple organ injuries.\textsuperscript{27} However, SASP composition may differ depending on the mode of senescence induction and biologic context. Although direct evidence in the kidney is lacking, studies in other organ systems provide a framework for future studies in experimental renal disease. In repair of cutaneous wounds in vivo, the secretion of CYR61 (CCN1) is reported to be a key stimulus of fibroblast senescence, whereas SASP-derived PDGF-AA is required for successful wound healing.\textsuperscript{24,34} In contrast, immune clearance of oncogene-induced senescent cells in the liver is promoted by CCL2 and chronically senescent cells are reported to induce IL-6, plasminogen activator inhibitor 1 (PAI-1), and other diverse factors.\textsuperscript{10,35} The composition of the SASP reflects both the origin of the cell and the initiating stimuli, often initiated by NF-κB and p38 mitogen-activated protein kinase signaling.\textsuperscript{36}

The SASP provides an important mechanism through which senescent cells affect organ function via induction of paracrine senescence. Recent research shows that transplanting senescent preadipocytes intraperitoneally into young mice results in a 50-fold increase in total body senescent cell number compared with controls.\textsuperscript{8} Furthermore, administration of serum from patients with chronic obstructive pulmonary disease (COPD) induces senescence in bronchial epithelial cells in vitro, suggesting that circulating factors may promote systemic senescence.\textsuperscript{37}

**PHYSIOLOGIC FUNCTIONS OF SENESCENT CELLS**

**Embryogenesis/Development**

Developmental senescence is a highly ordered and conserved cellular process observed in multiple species including tetrapods and fish.\textsuperscript{38} The mesonephros and endolymphatic sac of the human embryo contain senescent cells which affect tissue remodeling and cell balance.\textsuperscript{13} Murine studies provide evidence that TGFβ and PI3K signaling activate p21cip1-induced senescence in vitro.\textsuperscript{13,39} Studies in mice show induction of SA-β-gal in the mesonephros simultaneous with falling proliferation markers immediately before involution of mesonephric tubules.\textsuperscript{13} Further studies show that growth arrest requires p21cip1 induction independent of p53, with mice lacking p21cip1 exhibiting delayed mesonephric regression but developing functional kidneys—with increased apoptosis restoring appropriate cell number.\textsuperscript{13}

**Postinjury Repair**

Acute senescence is known to be important in successful scar formation, with murine studies showing that senescent cell depletion after skin wounding in vivo results in delayed wound closure—although eventual healing still occurs.\textsuperscript{24} Induction of fibroblast senescence controls the fibrotic pathways required for normal healing,\textsuperscript{34} whereas activation of hepatic stellate cell senescence limits liver fibrosis in a murine injury model.\textsuperscript{40} Interestingly, p16
ink4a knockout mice exposed to experimental renal injury show increased epithelial proliferation and functional recovery after ischemia/reperfusion injury but worsened fibrosis in unilateral ureteric obstruction models, suggesting that senescent cells are required at specific timepoints and in specific populations in response to different injuries for optimal repair.\textsuperscript{19,41}

**Cancer Defense**

Senescence induction by oncogene activation is recognized as an important physiologic mechanism preventing neoplastic transformation. Although data in human renal cancer are limited, one study showed that lowered expression of senescence markers in human renal cell carcinoma associates with worsening prognosis, whereas the antiproliferative effects of calcitriol on human renal cancer cells in vitro have been attributed to induction of senescence.\textsuperscript{42,43} Oncogene activation in murine hepatocytes drives secretion of chemokines and cytokines resulting in immune-mediated clearance of these premalignant cells. Im paired removal of senescent hepatocytes results in subsequent development of hepatocellular carcinoma\textsuperscript{35} introducing the concept of “senescent surveillance” as a defensive process, protecting against malignant transformation by inducing cell-cycle arrest. The components of the SASP act to reinforce the growth arrest in neighboring cells, protecting an area of tissue from cancerous change.\textsuperscript{13,39} Animal models where senescence induction is blocked demonstrate increased susceptibility to several cancers.\textsuperscript{44,45}

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**SENCENCE IN RENAL DISEASE**

Tubular epithelial cells are frequently implicated in renal senescence, although other cell types can exhibit positive staining for markers of cell-cycle arrest. In a series of human renal transplant biopsies, all biopsy specimens showed p16
ink4a staining in the nuclei of distal tubules and collecting duct but staining was also present in podocytes, parietal epithelium of glomeruli, vascular smooth muscle cells, and interstitial cells. Furthermore, a series of patients with glomerular disease showed p16
ink4a staining in a subset of glomerular, tubular, and interstitial cell nuclei; however, senescent tubular epithelial cells were...
the key difference between disease and control kidneys, present in 80% of cases compared with 21% in normal kidneys. Additionally, a biopsy series of patients with IgA nephropathy demonstrated increased p21cip1 and p16ink4a protein expression which was confined to tubules. Experimental data support the importance of the tubular epithelium. p16ink4a knockout mice have increased senescence and subsequent fibrosis in tubular and interstitial cells after unilateral ureteric obstruction, most prominently in the collecting duct. After ischemia reperfusion injury, nuclear p21cip1 was localized to both distal and proximal nephron, including collecting ducts, with no glomerular staining found. In Baker et al’s seminal 2016 study of ablation of senescent cells in INK-ATTAC transgenic mice demonstrated senescence occurring in proximal tubules with increasing age. The importance of the epithelial cell is supported by in vitro data which demonstrate that a variety of injuries can induce renal proximal tubular epithelial cells into a senescent phenotype.

CKD
Senescent cells are known to be present at increased levels in many renal diseases (Figure 2), and it appears likely that senescence plays a role in the maladaptive repair which contributes to many cases of progressive renal fibrosis after injury (Figure 3). Senescence, as indicated by p16ink4a-expressing cells, increases in patients with glomerular disease as compared with age-matched controls. In IgA nephropathy, shorter telomere lengths are found in mononuclear cells extracted from urinary sediment collections versus healthy controls, and a significant inverse correlation is shown between telomere length and serum creatinine. Recent bioinformatic analysis of both murine experimental renal injury and human renal transplantation identifies significant upregulation of Cdkn1a (p21cip1) in the aftermath of injury and in human transplanted kidneys undergoing AKI-to-CKD transition. Finally, senescence may be a factor in progression of diabetic nephropathy (DN). Levels of p16ink4a and SA-β-gal staining, decreases tubular cell proliferation, and attenuates disease progression in a murine model of DN. Treatment with the cyclin-dependent kinase (CDK) inhibitor roscovitine restores p21cip1 levels, increases SA-β-gal staining, decreases tubular cell proliferation, and attenuates disease progression in a murine model of ADPKD. This suggests that a failure to induce senescence in ADPKD may contribute to cystic progression.

Autosomal Dominant Polycystic Kidney Disease
In contrast to the above, autosomal dominant polycystic kidney disease (ADPKD) is an example of senescence attenuating disease progression. Levels of p21cip1 are reduced in kidneys from human patients with ADPKD and in animal models of PKD. Treatment with the cyclin-dependent kinase (CDK) inhibitor roscovitine restores p21cip1 levels, decreases SA-β-gal staining, decreases tubular cell proliferation, and attenuates disease progression in a murine model of ADPKD. This suggests that a failure to induce senescence in ADPKD may contribute to cystic progression.

Kidney Transplantation
Human studies have correlated levels of pre-transplant senescent cells in the kidney with the subsequent development of interstitial fibrosis, tubular atrophy, and
Supporting this, elevated mRNA expression of p21cip1 and p16ink4a in pretransplant biopsy specimens correlates with poor outcomes in both experimental animals and man.58,59 In the setting of renal transplantation, immune activation and reduced immune clearance due to immunosuppression may contribute to senescent cell accumulation.60,61 The interaction between senescent cells and the post-transplant immune milieu is likely complex, because allograft rejection is associated with increased senescence and glomerular, tubular, and interstitial cells from rejected grafts express elevated levels of p16ink4a and p27kip1, correlating with the grade of chronic allograft nephropathy.62

Immunosenescence and Senescent Cell Clearance
Although accumulation of senescent cells with advancing age and with renal disease is well documented, whether this reflects (1) increased senescence induction, (2) altered SASP release preventing immune clearance, or (3) a primary failure within the immune system itself remains largely unexplored. Although no direct studies exist on the role of the immune system in senescent cell clearance from the adult kidney, SASP release is immunostimulatory35 and studies within the murine liver, uterus, and endometrium have reported key roles for monocytes, macrophages, CD4+ T lymphocytes, and natural killer cells in the physiologic clearance of senescent cells.35,67,68 Studies using senolytic agents show that senescent cell ablation results in reduced levels of inflammation in mice26 and in cells removed from inflamed human joints.69 Complementary work has shown that, after irradiation, the generation of senescent hematopoietic stem cells results in immune dysfunction, with selective depletion of these cells improving both stem cell and overall immune function in mice.70 Whether senescence within the kidney or the immune system interrupts the physiologic clearance of senescent cells and promotes renal disease remains an important unanswered question.

THERAPEUTIC TARGETING OF SENESCENCE IN THE KIDNEY

Animal Models of Senescent Cell Deficiency or Depletion in the Kidney
Our understanding of senescent cell accumulation and clearance in renal disease remains incomplete. Important lessons have been learned using wild-type and transgenic mice to test the effect of defective senescent cell induction on renal development and disease.13,18,19,41,53,54 Recent seminal studies demonstrate the safety and efficacy of senescent cell depletion in vivo, using transgenic mice with selective sensitivity to pharmacologic agents,26,70 or wild-type mice treated with...
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Transgenic and genetic knockout mice have been used to study the effect of (1) deficiencies in the induction of senescence or (2) depletion of established senescent cells. Several of these models are summarized in this table, with description of the experimental model of renal disease used, the alteration in senescence induction used, and any alterations in renal disease outcomes. TTD/TTD, trichothiodystrophy/trichothiodystrophy; GCV, ganciclovir; WT, wild-type; KO, knock-out; UUO, unilateral ureteric obstruction; IRI, ischemia-reperfusion injury; Tx, Transplant.
drugs targeting antiapoptotic pathways upregulated in senescent cells, \[^{8,70,71}\](summarized in Table 1). These studies provide evidence that senescent cell depletion increases healthy lifespan by postponing the onset of several age-associated pathologies. Importantly, such benefits have not come at the expense of increased cancer incidence, indicating that depletion of chronically senescent cells differs from models lacking the ability of p16ink4a-expressing cells to participate in senescence in response to DNA damage.\[^{26}\] Accordingly, there is major interest in the potential to manipulate senescent cell number or behavior to delay the onset and progression of numerous age-related diseases, including those of the kidney (Figure 4).

**Nonpharmacologic Approaches to Reduce Senescent Cell Accumulation**

Increased levels of physical activity and weight loss represent two nonpharmacologic approaches to reduce senescent cell generation. In mice exposed to a high-fat diet, regimes of increased exercise reduce expression of senescence markers and SASP release in adipose tissue.\[^{72}\] Studies examining circulating leukocytes in both mice and track and field athletes show that exercise associates with reduced markers of senescence.\[^{73}\] In mice undergoing controlled weight gain, animals switching to reduced calorie intake reduce senescent cell number in white adipose tissue.\[^{74}\]

**Senolytics**

The INK-ATTAC and p16–3MR transgenic mice use components of the Cdkn2a promoter (responsible for p16ink4a expression) to confer selective sensitivity of p16ink4a–expressing cells to pharmacologic depletion.\[^{74,75,76}\] Depletion of chronically senescent cells from both strains in aged or irradiated animals benefits multiple organs including the kidney.\[^{26,70}\] Although informative, the results of studies based exclusively upon such transgenes may not fully recapitulate the spectra of senescent cells seen \textit{in vivo}, unless all such cells express p16ink4a, which seems unlikely.

Although the field of senolysis is in its infancy, several agents have generated encouraging results in rodent models. One such agent is ABT-263 (Navitoclax), a Bcl-2/wxl inhibitor which targets a dependence on the Bcl-2 pathway in senescent cells, resulting in widespread senescent cell apoptosis. Recent studies have used this drug successfully to target irradiation-induced senescence \textit{via} the c-Jun/Bcl-xl/p21^{CIP1} pathway.\[^{70,75,76}\] With ABT-263 currently in phase 1 and 2 clinical trials for both hematologic and solid organ malignancies there is potential for early clinical translation in nonmalignant conditions.\[^{77,78}\]

Another combination includes dasatinib (a tyrosine kinase inhibitor) and quercetin (a flavonoid) discovered using a bioinformatics-driven approach by Zhu et al.\[^{79}\] in 2015. Cultured senescent and nonsenescent cells were exposed to compounds known to target senescent cell apoptotic pathways. Dasatinib and quercetin eliminate senescent but not nonsenescent cells in rodent and human cell cultures, freshly isolated human tissue explants, old mice, and mice with accelerated ageing.\[^{8,79,80}\] Treating with dasatinib and quercetin alleviated the physical dysfunction and frailty associated with increased senescence. Mice lived longer and performed better in all measures of frailty tested.\[^{8}\]

Other groups used an assay of SA-β-gal expression to guide identification of drugs capable of selectively targeting
Senescence—leading to the identification of the HSP90 inhibitor 17DMAG as a compound capable of increasing healthy lifespan and reducing p16ink4a-positive cell number in a progeroid mouse model. Similarly, the increased expression of SA-β-gal present within diverse senescent cells has recently been harnessed to allow selective activation of cytotoxic compounds in vivo. This approach was used to target senescent cells effectively in two models of cancer and fibrosis and represents an exciting avenue for further clinical translation.

Important work by Baar et al. identified the FOXO4 protein as an important regulator of p53 activity in senescent cells, and, by designing an interfering peptide “FOXO4-DRI,” demonstrated that selective apoptosis could be achieved in both aging- and chemotherapy-induced senescent cells, with resultant improvement in organismal fitness, including protection of baseline renal structure and function.

**Senostatics**

Senostatics are drugs that inhibit elements of the senescent phenotype without removing the cells. Drugs familiar to nephrologists are being re-examined for this purpose, including metformin and sirolimus (rapamycin), both of which activate autophagy and improve mitochondrial complex I function and can extend lifespan. mTOR signaling is known to be an important pathway in some forms of senescence and furthermore there is some experimental evidence that sirolimus itself inhibits the senescent cell phenotype. Given the previously mentioned role of senescent cells in wound healing, it is interesting to speculate whether a hitherto-unrecognized effect on senescence inhibition may contribute to the impaired wound healing associated with clinical sirolimus use, although this link remains unproven.

Other candidate drugs include inhibitors of TfkB kinase, NF-κB, and the Janus kinase pathway. Such approaches inhibit proinflammatory signaling pathways, such as NFκB or p38 mitogen-activated protein kinase pathways, disrupting SASP production.

A recent study undertook an RNAi-based approach to identify transcriptional factors mediating the SASP profile of senescent cells. Using this approach, PTRP1 was identified as a factor mediating the inflammatory effects of the SASP with blockade inhibiting the proinflammatory and tumorigenic properties of senescent cells in the liver—a good example of basic science identifying novel and druggable targets for translation.

**THE FUTURE**

Fundamental questions remain surrounding the pathways regulating senescence, their roles in tissue aging and dysfunction, and their potential therapeutic use (Figure 5).

**How Can Senescent Cell Load Be Determined In Vivo?**

Senescent cell burden in the kidney may be a useful predictor of renal outcomes. Current means of detection are not sufficiently specific and are often impractical because stained tissue is required. Whether increased senescent cell number on renal biopsy predicts progression and functional decline better than existing markers remains untested. Prospective studies...
following patients with differing numbers of senescent cells will be required to answer this question. Noninvasive serum or urine biomarkers of senescence would help researchers associate senescent burden with clinical phenotypes, inform basic science research, and build a more complete understanding of their role in disease.

Are Senescence Cells Important in Recovery? It is likely that optimal recovery post renal injury requires the induction of senescent cells at key timepoints, and a delicate balance is needed to promote adaptive rather than maladaptive repair. The specific identity and roles of these cells in renal repair versus development of fibrosis remain unknown.

Why Do Senescent Cells Accumulate with Age and Disease? Whether the accumulation seen in multiple organs, including the kidney, with age reflects increased production or reduced immune clearance remains poorly understood. Intriguing studies from the Fogo laboratory show that murine renal fibrosis is reduced in the aftermath of a young bone marrow transplant when compared with an older donor. The potential for therapies targeting immune system aging and function to affect renal disease merits further study.

Is Depletion of Senescent Cells Safe in Man? Although agents are being developed to deplete senescent cells, their efficacy and safety in humans remain unproven. Whether minimizing development of senescent cells or reducing total senescent cell burden will have clinical relevance for elderly patients is unknown. It is encouraging that interventions started in relatively elderly mice still positively affect aging pathologies. Four clinical trials are currently underway testing senolytic therapies in man, including one (NCT02848131) testing dasatinib and quercetin as senolytics in patients with diabetes with CKD.

Research into senescence will continue to shed light on these ubiquitous yet mysterious players in the aging and damaged kidney. With better understanding of their role in damage and repair it may become possible to target them therapeutically, without compromising their essential regulatory functions in healing and cancer suppression.

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DISCLOSURES
J.V.B. is coinventor on KIM-1 patents that are assigned to Partners HealthCare and licensed to Johnson & Johnson, Sekisui, Novartis, Biogen Idec, R&D, and Astute. He is a consultant for Astellas, Novartis, Roche, and Sekisui regarding the safety and efficacy of therapeutics or diagnostics for AKI. He holds equity in MediBeacon, Sentient, and Thrasos, and has grant support from Boehringer Ingelheim and Roche. All other authors report no conflicts of interest.

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