

## Renal Medicine 3

### Kidney regeneration

Ariela Benigni, Marina Morigi, Giuseppe Remuzzi

*Lancet* 2010; 375: 1310–17

This is the third in a **Series** of three papers about renal medicine

See **Editorial** page 1226

See **Comment** page 1227

See **Perspectives** page 1245

Mario Negri Institute for Pharmacological Research, Bergamo, Italy (A Benigni PhD, M Morigi PhD, G Remuzzi MD); and Unit of Nephrology and Dialysis, Azienda Ospedaliera Ospedali Riuniti di Bergamo, Italy (G Remuzzi)

Correspondence to: Dr Ariela Benigni, Mario Negri Institute for Pharmacological Research, Via Gavazzeni 11, 24125 Bergamo, Italy [ariela.benigni@marionegri.it](mailto:ariela.benigni@marionegri.it)

Neonephrogenesis, the capacity to regenerate renal tissue, is a distinctive feature of fish but not usually of mammals. However, evidence exists for kidney repair in response to insulting agents for animals and human beings. Studies have therefore been designed in the past few years to clarify the cellular and molecular basis of renal repair, with the aim to investigate the potential regenerative capacity of animal and human kidneys. Three main questions are being addressed by this research: whether terminally differentiated cells in adult animal kidneys have regenerative capacity; whether multipotent progenitor cells exist in kidneys; and whether renal repair can be favoured or accelerated by cells of extrarenal origin migrating to the kidney in response to injury. In this Review, we describe evidence of cellular and molecular pathways related to renal repair and regeneration, and review data from animal and human studies that show that the kidney might have regenerative capacity.

#### Introduction

Regeneration of part of the body has been reported in some plant and animal species,<sup>1</sup> and has been a highly conserved process throughout evolution. In pioneer studies<sup>2</sup> in 1744, Abraham Trembley showed that simple animals such as the polyp were capable of regeneration. A few decades later, Spallanzani<sup>3</sup> and Bonnet<sup>4</sup> showed that regeneration was shared by metazoans, including earthworms, snails, and salamanders. Members of both the plant and animal kingdoms survive various insults because of regeneration strategies, such as preservation of stem-cell niches in adult life or induction of stem-cell potential in differentiated cells. Findings from recent studies<sup>5</sup> suggested that regenerative reprogramming of an entire organ in plants does not need transition to a stereotypical stem-cell environment. Data from studies of the axolotl, a salamander that is endemic to Mexico, suggest that a similar process might occur in animals. During limb regeneration, adult tissue of the salamander near the plane of amputation is converted into a zone of undifferentiated progenitor cells, called the blastema, that reforms the different tissues of the limb.<sup>6</sup> Cells from the salamander blastema undergo reprogramming events that allow the cells to re-enter embryonic programmes of tissue formation, without complete dedifferentiation to a pluripotent state.<sup>6</sup> By tracking limb tissue with a green fluorescent protein, tissue of the axolotl has been shown to produce progenitor cells that retain a strong memory of their

tissue origin, which restricts their differentiation potential accordingly.<sup>6</sup>

The weak immune systems of amphibians allows expression of embryonic antigens, which might have been lost in mammals with development of a competent immune system.<sup>7</sup> In mammals, tissue regeneration after injury is a restricted process, apart from in the liver and appendage tissue in a specific mouse strain.<sup>8,9</sup> Cells responsible for regeneration are terminally differentiated resident cells or organ-specific progenitor or stem cells that are identified in tissues with high proliferative capacity, such as the skin, but also in the CNS and heart—organs previously thought to have no regenerative properties.<sup>7,10</sup> For the kidney, which has a restricted intrinsic ability to regenerate,<sup>11</sup> the search for specific renal progenitor or stem cells is in progress. The panel presents definitions of progenitor and stem-cell populations and processes of cell differentiation.

#### Kidney regeneration in the evolutionary context

Neonephrogenesis throughout life is a common feature of elasmobranchs and teleosts.<sup>12</sup> In adult elasmobranchs, new glomerular-like structures are formed in an unusual site, the neonephrogenic zone, which resembles the embryonic kidney. This zone contains mesenchymal stem cells that are capable of neonephrogenesis because they presumably derive from a pool of embryonic stem-cell-like cells and essential structures of the embryonic mammal metanephros. Renal cells from the neonephrogenic zone of tissue respond to part reduction of renal mass with formation of new nephrons.<sup>13</sup> In elasmobranch fish that have undergone partial nephrectomy, cell proliferation assessed as incorporation of the nucleotide label bromodeoxyuridine (BrdU), which inserts into cell DNA during DNA synthesis, was greatly enhanced in the nephrogenic zone both in the remnant tissue and contralateral kidney, although it was virtually absent in mature kidney tissue.<sup>13</sup> Mesenchymal cell aggregates and the frequency of developmental nephron stages both increased in the nephrogenic zone. In these

#### Search strategy and selection criteria

We searched PubMed for reports published in English in the past 10 years with the terms “stem cells”, “progenitors”, “repair”, “regeneration”, and “regression of lesions”. These searches were cross-referenced with the keyword “kidney”, “chronic kidney disease”, or “acute kidney disease”. Additional references were identified from reviews, original research articles, and scientific meetings.

animals, neonephrogenesis seems to act as a key mechanism to sustain physiological renal growth and possibly to repair the kidney after damage. Neonephrogenesis does not happen in mammals. In rodents, the kidney continues to develop for a few days after birth,<sup>14</sup> whereas in human beings, development of functional units stops at the end of gestation.<sup>15</sup> Resection of kidney tissue does not elicit regenerative responses in mammals, possibly because, unlike fish, mammals do not have nephrogenic zones.

### Kidney repair from a cell biology perspective Do terminally differentiated cells have regenerative potential?

In a physiological setting, the mammalian kidney has a low cell turnover, whereas after injury increased cell proliferation is the driving event behind tissue repair. Studies<sup>16–18</sup> have been undertaken to establish which cell type promotes renal repair in animals and whether reparative programmes depend on proliferation and dedifferentiation of resident cells, specialised renal progenitor cells, or both. Terminally differentiated resident tubular epithelial cells that survive a given damage might proliferate and generate identical cells or dedifferentiate and subsequently re-enter the cell cycle. Tubular cell dedifferentiation, which recapitulates certain aspects of renal development, was shown by the finding that tubular cells acquire an immature mesenchymal phenotype with re-expression of vimentin<sup>16</sup> and Pax2<sup>17</sup> in post-ischæmic recovery. By genetic fate-mapping techniques, nephron repair after ischaemic reperfusion injury is predominantly accomplished by intrinsic, surviving tubular epithelial cells<sup>18</sup> without a contribution from any extratubular renal progenitor or stem cells. However, this study did not clarify whether intratubular stem cells or adult epithelial cells with different regenerative potential participate in the process of kidney repair.<sup>18</sup>

Very recent evidence has been provided that activated renal macrophages contribute to kidney tissue regeneration through induction of Wnt-7b, a protein associated with kidney tubule development, which initiates tubule repair and regeneration after injury.<sup>19</sup> A great support to nephron regeneration through re-epithelialisation of the proximal tubules derives from the contribution of distal tubular epithelium.<sup>20</sup> Data show that distal tubular cells release reparative and prosurvival factors.<sup>20</sup> These factors are key for repair of proximal tubular cells (which are most sensitive to ischaemia) that express receptors to many reparative and prosurvival factors, but do not synthesise them.<sup>20</sup>

Findings from a recent in-vitro study<sup>21</sup> suggested that murine glomerular parietal epithelial cells undergo spontaneous epithelial-mesenchymal transition to generate cells expressing both epithelial and mesenchymal markers. When these cells were implanted under the renal capsule of uninephrectomised mice, they

#### Panel: Definitions

##### Adult stem cells or progenitor cells

Present in adult tissues; have clonogenic, self-renewing ability and give rise to terminally differentiated cells of the tissue of origin and to other different lineages

##### Pluripotent stem cells

Capable of giving rise to all cells of the three germ layers of the embryo

##### Multipotent stem cells

Have the capacity to differentiate into cells of the tissue of origin and can generate cells of different lineages

##### Transdifferentiation

A process whereby stem cells jump the lineage barrier and differentiate into cells other than their own tissue

##### Dedifferentiation

A process whereby the phenotype of cells becomes less mature than previously

##### Niche

A specific microenvironment where stem cells can reside and produce progenitor cells while self-renewing

##### Fate mapping

Technique to trace a cell population fate

differentiated into immature glomeruli, suggesting that epithelial-mesenchymal transition could confer plasticity to terminally differentiated glomerular parietal epithelial cells, giving rise to functionally active progenitors.<sup>21</sup>

#### Is there a candidate renal stem cell?

Answering this question needs an understanding of the molecular basis of the final stage of kidney development in mammals—development of the metanephros. This structure derives from the inductive interaction between the metanephric mesenchyme and adjacent ureteric bud, and gives rise to the adult kidney.<sup>22</sup> Metanephric mesenchymal cells can generate all the different types of nephron epithelia, such as Bowman's capsule, podocytes, and the proximal and distal tubules (apart from collecting ducts), suggesting that these cells are renal epithelial stem cells.<sup>23,24</sup> The transcriptional regulator Six2 has been indicated as a key factor for maintenance of a progenitor cell population in the metanephric mesenchyme throughout kidney development.<sup>25</sup> Experiments with transgenic-reporter cell lines provided evidence that Six2-expressing cap mesenchymal cells represent the multipotent, self-renewing nephron progenitor population that gives rise to all epithelial cells of the main nephron body.<sup>26</sup> Moreover, at the 2009 American Society of Nephrology meeting in San Diego, Kobayashi and colleagues<sup>27</sup> reported identification of another subset of self-renewing progenitor cells expressing the transcriptional regulator forkhead box domain 1 (Foxd+1)

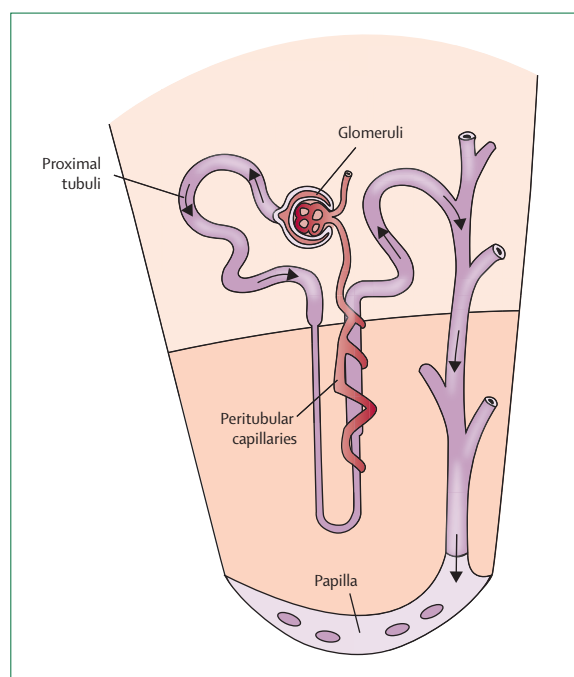


Figure 1: Potential niches for renal progenitor or stem cells in postnatal kidney

in the cortical stroma, contributing to the medullary interstitium, mesangium, and pericytes. The transcription factor Pax2 seems to be essential for different developmental fates of Six2-positive cap mesenchyme and the Foxd+1 cortical stroma, because in its absence cap mesenchymal cells transdifferentiate into medullary interstitial cells.

Results of studies<sup>28,29</sup> in which rat or human metanephroi were transplanted into the renal subcapsular space or abdominal cavity of normal rats and mice showed that metanephroi were able to engraft and differentiate into functional mature nephrons, reinforcing the notion that metanephroi are committed to form renal cells. Furthermore, metanephroi transferred alone or with syngeneic mesenchymal stem cells in rats that are genetically programmed to develop proteinuria and glomerulosclerosis mature into new nephrons and create a proregenerative environment.<sup>30</sup> Metanephric mesenchymal cells are pluripotent because *in vitro* they are able to generate not only epithelia but also other cell types such as myofibroblasts, smooth muscle cells, and cells expressing endothelial markers.<sup>31</sup> Therefore, a cell population expressing the phenotype for metanephric mesenchymal epithelial precursor cells would be a good renal stem-cell candidate.

#### Evidence that multipotent progenitor cells exist in adult kidney

The search for renal stem cells in the adult kidney has followed two pathways so far. One is based on identification of cells expressing stem-cell markers, and

the other on identification of cells in the kidney that share functional properties of stem cells. Figure 1 shows potential niches for progenitor or stem cells described in postnatal kidneys.

Stem-cell markers include CD133 and CD24; CD24 is also regarded as a surface molecule that is characteristic of uninduced metanephric mesenchyme during kidney development. Cells expressing CD133 have been isolated from the tubular fraction of the healthy human renal cortex. These cells were able to differentiate *in vitro* towards renal epithelium and endothelium.<sup>32</sup> When injected into mice with glycerol-induced acute kidney injury, they enhanced recovery from tubular damage, possibly by integrating into the proximal and distal tubules.<sup>32</sup>

Multipotent progenitor cells expressing CD133 and CD24 were also isolated from Bowman's capsules in adult human glomeruli.<sup>33</sup> Three parietal epithelial cell populations with a precise location in the Bowman's capsule have been described:<sup>33</sup> renal progenitor cells at the urinary pole expressing CD133 and CD24 in the absence of podocyte markers, including nestin, complement receptor 1, and podocalyxin (PDX); transitional cells positive for CD133, CD24, and PDX localised between the urinary and vascular pole; and differentiated podocytes (negative for CD133 and CD24, and positive for PDX) at the vascular pole.<sup>33</sup> Clonally expanded progenitor cells positive for CD133 and CD24 were multipotent, generated podocytes and tubular cells *in vitro*,<sup>33</sup> and contributed to tubular-cell regeneration after injection into mice with acute renal failure.<sup>34</sup> Lineage tracing of parietal epithelial cells in transgenic mice<sup>35</sup> showed that parietal epithelial cells migrated into the glomerular tuft and became podocytes, suggesting that these cells are responsible for podocyte renewal.

The perivascular site has also been proposed as a potential stem-cell niche, common to many organs including the kidney<sup>36,37</sup> and harbouring a reserve of progenitor cells expressing markers of both pericytes and mesenchymal stem cells that are able to migrate in response to focal injury and promote tissue repair.<sup>38</sup> Organ-specific stem cells can be recognised on the basis of their characteristically slow cycling time *in vivo*. Slow-cycling cells can be identified experimentally by their retention of the proliferation marker BrdU. After a pulse of BrdU, only slow-cycling cells retain a concentration of label high enough to detect them over a long period.<sup>39,40</sup> With this technique, the existence of label-retaining cells in proximal, distal, and collecting tubules of normal rat kidneys has been reported.<sup>41</sup> Further studies<sup>42</sup> showed the presence of these cells in the interstitium that were capable of re-expressing the mesenchymal cell markers vimentin and e-cadherin after ureteral obstruction.

Renal papillae have also been proposed as a niche for kidney stem cells<sup>43</sup> because of the presence of large numbers of BrdU-retaining cells in the papillary interstitium in rats and mice. BrdU-retaining cells

	Intervention	Effect of treatment
Puromycin aminonucleoside nephropathy <sup>60</sup>	ACE inhibitor	Reduction of proteinuria and early FSGS or H lesions
Subtotal nephrectomy <sup>61</sup>	ACE inhibitor	Preservation of structure and function of glomeruli with early sclerosis; high-dose imparts additional benefit to glomerular structure
Genetic model of progressive renal injury <sup>62</sup>	ACE inhibitor	Progressive reduction of protein excretion, correlated with baseline proteinuria; stabilisation of glomerulosclerosis
Experimental model of ageing <sup>63</sup>	ARB	Regression of glomerulosclerosis and aorta wall thickness ratio; reduction of proteinuria, collagen content, apoptosis, and PAI1
Genetic model of progressive renal injury <sup>64</sup>	ACE inhibitor+ARB	Normalisation of proteinuria and inflammatory cell infiltration; improvement of glomerular ultrafiltration coefficient, glomerular and tubular structural changes
Subtotal nephrectomy <sup>65</sup>	ACE inhibitor	Regression of glomerulosclerosis, tubulointerstitial, and vascular damage; reduction of glomerular volume, mesangial and endothelial cell number
NO-deficiency induced hypertension <sup>66</sup>	ARB	Normalisation of renal functional and histological parameters; decrease in collagen I, IV, and TGFβ gene and protein expression.
5 of 6 nephrectomy <sup>67</sup>	ARB	Dose-dependent renal protection; a very high dose is needed for maximum protection: arrest of renal inflammation, glomerular or interstitial injury, and albuminuria
5 of 6 nephrectomy <sup>68</sup>	High-dose ACE inhibitor or ARB, or both	Regression of glomerulosclerosis in 62% of ARB-treated and 57% of ACE-inhibitor-treated rats; reduction of TIMP-1 and PAI1; no changes in TGFβ1 and MMP2/9 expression
Genetic model of progressive renal injury <sup>69</sup>	High-dose ACE inhibitor	Reduction of 35% of total sclerotic volume, increase of 40% of normal capillary tissue; normalisation of TGFβ and α-SMA overexpression; stabilisation of interstitial changes
Accelerated type 1 diabetes <sup>70</sup>	ARB	Regression of mesangial expansion and severity of synechiae (adhesions of glomerular tufts to Bowman's capsule)
Genetic model of progressive renal injury	High-dose ACE inhibitor	Regression of functional and structural changes; restoration of glomerular capillary by increasing tuft podocyte, parietal podocyte, and proliferating Wilms'-tumour-1-positive cell numbers

ACE=angiotensin-converting-enzyme. ARB=angiotensin-II type 1 receptor blocker. FSGS or H=focal and segmental glomerular sclerosis or hyalinosis. TGFβ=transforming growth factor β. TIMP-1=tissue inhibitor of metalloproteinase-1. MMP=metalloproteinase. PAI1=plasminogen activator inhibitor 1. NO=nitric oxide. αSMA=α-smooth-muscle actin.

**Table: Kidney repair by angiotensin-II blockers in animal models**

were identified<sup>43</sup> to be quiescent throughout life of the animal. During recovery from transient renal ischaemia, they entered the cell cycle and disappeared from papillae, an event that could not be ascribed to cell apoptosis. In-vitro papillary cells displayed a plastic phenotype, forming sphere-like structures in a similar way to other stem cells. Single-cell clones of papillary cells coexpressed mesenchymal and epithelial proteins and gave rise to myofibroblasts and cells expressing neuronal markers.<sup>43</sup> Subsequent experiments have confirmed that kidney papillae contain a population of cells with characteristics of adult stem cells, such as retention of BrdU. Papillary BrdU-retaining cells generated new cells in adult kidneys after migration to the upper papilla, where they associated with chains of proliferating cells, similar to those identified with adult stem cells of the skin, brain, and gonads.<sup>44</sup>

#### Evidence that bone-marrow-derived stem cells contribute to kidney repair

Adult stem cells derived from bone marrow might contribute to turnover and regeneration of several compartments of the kidney. These stem cells can give rise to all types of blood cells but they also differentiate into many other cell lines.<sup>45</sup> The bone marrow contains at least two populations of stem cells, haemopoietic and mesenchymal stem cells, with mesenchymal stem cells providing stromal support for haemopoietic stem cells.

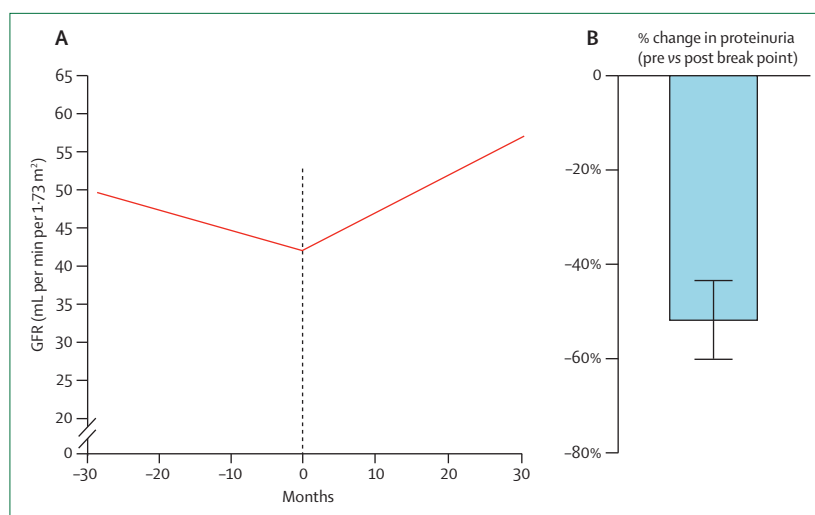
Bone marrow cells are known to migrate to the kidney and participate in normal tubular epithelial cell turnover and repair after acute kidney injury.<sup>46</sup> Evidence of the

kidney engraftment capacity of cells derived from male bone marrow is based on the presence of cells positive for the Y chromosome with epithelial cell markers in the tubules from kidneys of female recipient mice.<sup>47,48</sup> Of special interest is the finding<sup>47</sup> that in male patients receiving a female donor kidney the presence of Y-chromosome-positive cells was reported after acute renal injury, and accounted only for 1% of the tubular cells. Although a rare event, presence of male tubular cells in female grafts possibly shows that extrarenal cells participate in regeneration of tubules after injury.<sup>47,49</sup> Others researchers, however, have been unable to reproduce these findings.<sup>50</sup> In different animal models, bone-marrow-derived cells differentiated into mesangial cells,<sup>51</sup> podocytes,<sup>47</sup> and endothelial cells of glomerular capillaries.<sup>52</sup>

#### Kidney repair from an animal and human perspective

##### Evidence of kidney repair in animals

An ischaemic or toxic acute renal injury results in cell necrosis, apoptosis, and detachment of cells, leading to denudation of tubular basement membrane.<sup>53</sup> The repair process is accomplished by migration of new cells into the region, reconstituting a functional tubular epithelium.<sup>54</sup> Improved understanding of the mechanisms of kidney repair has stimulated researchers to clarify whether supplementary cells injected into an acutely damaged kidney might aid repair and regeneration of injured tissue, thus accelerating and augmenting the ongoing natural healing process.<sup>55–58</sup> Adult stem cells, either derived from bone marrow<sup>56–58</sup> or of renal origin,<sup>32,34</sup>



**Figure 2: Time-dependent changes in glomerular filtration rate (A) and proteinuria (B) in patients with proteinuric kidney disease on continued ramipril therapy**

Time-dependent changes in glomerular filtration rate (GFR) in ten patients with proteinuric kidney disease during 5-year continued ramipril therapy.<sup>64</sup> Breakpoint (dashed line) signals shift from an initial phase of progressive renal function loss to a second phase of renal function improvement. No changes in blood pressure control, ramipril doses, concomitant drugs, and diet and sodium intake were introduced during the observation period.

might participate in cellular repair and tissue remodelling after acute renal injury.

However, the question is whether chronic kidney damage, which has many different causes, has a complex pathophysiology, and is associated with different segments of the nephron can be repaired. Trying to unravel the process of repair of chronic lesions needs some understanding of functional adaptations of remaining nephrons to loss of units that happens in many diseases independent of the initial insult. Intraglomerular hypertension, hyperfiltration, and the consequent loss of size-selective properties of the glomerular barrier are determinants of the initial process of adaptation. These factors, however, lead to further renal damage, largely mediated by excessive concentrations of ultrafiltered proteins accumulating in the Bowman's space and lumen of tubules where they activate inflammatory and apoptotic pathways, fuelling progressive renal damage.<sup>59</sup>

Drugs that restrict glomerular hypertension and protein trafficking restrict falls in renal function and promote kidney repair. Among them, angiotensin-converting-enzyme inhibitors and angiotensin-II type 1 receptor blockers, are most effective. Importantly, animal models of non-diabetic and diabetic nephropathy have shown that treatment with angiotensin-converting-enzyme inhibitors, angiotensin-II type 1 receptor blockers, or their combination prevents progressive renal damage and promotes regression of glomerulosclerosis and development of vascular lesions.<sup>60-70</sup> Three-dimensional reconstruction of the glomerular capillary tuft<sup>69</sup> has been important in showing the extent to which sclerosis volume was effectively reduced by treatment, a feature that was underestimated by the widely used two-dimensional

imaging methods. In rats with advanced nephropathy, giving a high-dose angiotensin-converting-enzyme inhibitor reduced the volume of sclerosis in most glomeruli while substantially increasing the volume occupied by capillaries,<sup>69</sup> suggesting remodelling of the glomerular architecture and regeneration of the capillary network.<sup>65,71,72</sup> The table shows the evidence obtained from animal studies, drawing attention to the importance of angiotensin-II antagonism in induction of regression of renal disease.

Mechanisms that have been proposed to explain the effects of angiotensin-II antagonism on regression of glomerulosclerosis mainly relate to the control of extracellular matrix deposition. They include reduction of the expression of plasminogen activator inhibitor 1, an inhibitor of matrix degradation,<sup>63</sup> decreased expression of collagen I and IV and transforming growth factor  $\beta$ ,<sup>69</sup> and increased metalloproteinase activity.<sup>66</sup> Researchers<sup>65</sup> have also suggested that angiotensin-II antagonism can modulate survival and repair of the glomerular cells themselves. Angiotensin-converting-enzyme inhibition can halt mesangial cell hyperplasia, induce glomerular endothelial cell remodelling, and increase glomerular podocyte numbers in rats with advanced nephropathy.<sup>73</sup>

The issue of whether stem cells can participate in reparative processes of chronic kidney damage has also been addressed. In mice with Alport syndrome, bone-marrow-cell transplantation exerts renoprotective effects by improving renal function and keeping glomerular scarring to a minimum through replacement of defective podocytes.<sup>74</sup> By contrast, in doxorubicin-induced nephrosis, bone-marrow-derived cells act as a source of  $\alpha$ -smooth-muscle actin-positive myofibroblasts, thus contributing to excess extracellular matrix protein production in kidney fibrosis.<sup>75</sup> Regeneration of the kidney from autologous stem cells has so far been unsuccessful.<sup>76-78</sup>

### Evidence of kidney repair in human beings

Acute kidney injury in human beings is a protean syndrome of varying severity mainly due to ischaemic or chemical injury, occurring in up to 5% of patients in hospital,<sup>79</sup> and is a major cause of morbidity and mortality.<sup>80</sup> The capacity of the kidney to regenerate functional tissue after an episode of acute injury is a major determinant of outcome for patients with acute kidney injury. No specific therapy improves the rate or effectiveness of the repair process after acute renal injury.<sup>81,82</sup>

By contrast with acute injury, renal outcomes are mostly unfavourable in patients with progressive kidney disease, showing the limited capacity of the kidney to repair chronic damage. However, pharmacological interventions are available that seem to interfere with the inexorable tendency of patients with chronic renal disease to progress to end-organ damage and need renal replacement therapy. Wilmer and coworkers<sup>83</sup> reported that 8-year angiotensin-converting-enzyme inhibitor

therapy stabilised kidney function in six patients with type 1 diabetes, and that nephrotic syndrome otherwise predicted progression to end-stage kidney disease in months. Findings from the Ramipril Efficacy in Nephropathy (REIN) study<sup>84</sup> showed that ramipril compared with non-angiotensin-converting-enzyme inhibitor therapy halved the rate of renal function loss, as assessed by a series of measurements of glomerular filtration rate with gold-standard techniques, in patients with non-diabetic chronic nephropathies.<sup>85–87</sup> Renoprotection was a function of time, and in patients on continued ramipril therapy for at least 5 years, the rate of decreases in glomerular filtration rate progressively improved to about 1 mL per min per 1.73 m<sup>2</sup> per year.<sup>87</sup> This rate was ten-fold less than in controls with similar renal dysfunction, and approximated the physiological, age-related loss of glomerular filtration rate with time that is reported in patients with no evidence of renal disease.<sup>88</sup> In ten of these patients, a two-phase model describing changes in glomerular filtration rate over time identified a breakpoint signalling the shift from an initial phase of progressive function loss (figure 2). Findings<sup>69</sup> that the increase in glomerular filtration rate was sustained over time suggested the possibility of a concomitant improvement in the underlying pathology, with regression of glomerulosclerosis combined to some degree of kidney repair and regeneration. Improvement of glomerular filtration rate that was associated with further proteinuria reduction across the breakpoint can be taken to suggest that antiproteinuric effects of therapy with angiotensin-converting-enzyme inhibitors might be permissive for kidney repair and regeneration (figure 2).

The potential for human kidneys to repair has been shown in eight patients with long-lasting type 1 diabetes, whose typical lesions of diabetic glomerulopathy ameliorated during 10 years of normoglycaemia, which was achieved by pancreas transplantation.<sup>89</sup> The observed changes—reduced thickness of the glomerular and tubular basement membranes and mesangial matrix, and the disappearance of Kimmelstiel-Wilson nodular lesions—represented substantial remodelling of the glomerular architecture. This finding lends support to evidence from animal models<sup>90</sup> that histological changes in diabetic nephropathy can regress.

Independent of interventions, years of renoprotective therapy are needed before clinically appreciable improvements of renal disease can be achieved—identified only in a few patients. Different strategies have been suggested to improve these outcomes, including use of angiotensin-converting-enzyme inhibitors at doses higher than those recommended for blood pressure control and in combination with an angiotensin-II receptor blocker and a diuretic.<sup>91</sup> This integrated intervention stabilised the glomerular filtration rate in a woman aged 22 years with rapidly worsening renal function who was given standard therapy with

antihypertensive doses of an angiotensin-converting-enzyme inhibitor.<sup>92</sup> This approach was eventually formalised in an intervention protocol,<sup>91</sup> comparing it with conventional angiotensin-converting enzyme-inhibitor therapy in a matched-cohort study of 112 patients with severe chronic kidney disease.<sup>91</sup> During the 7-year follow-up, only two of 56 patients given the integrated protocol progressed to end-stage kidney disease compared with 17 of 56 reference patients. Moreover, the glomerular filtration rate stabilised in 26 patients<sup>91</sup>—a finding thought to be indicative of remission.<sup>93</sup> This study was the first formal evidence that progressive loss of kidney function can be prevented in patients with severe chronic kidney disease, and underlines the possibility of kidney repair in this population.

### Future challenges and directions

Findings that renal regeneration is common in fish but not in mammals provide opportunities for comparative studies to identify cells with renoregenerative potential that will offer fresh insights into the difficulty of renal regeneration and repair in animals and human beings. Characterisation of gene expression profiles of such cells and elucidation of corresponding signalling molecules will be important to clarify whether neonephrogenesis can theoretically be re-established in mammal adult life. Newly created transgenic mice with cell lineage tracing can be then used to assess the extent to which theoretical knowledge gained from cell biology and gene expression studies can be implemented *in vivo*.

Studies in man will improve the understanding of the genetics governing progression and regression of chronic kidney disease and genes associated with favourable outcomes. Enhanced understanding of mechanisms of action of already available drugs with renoprotective capacity will pave the way to unravel novel pathways that are possibly relevant to renal repair. Together, insights from human genetics and mechanistic studies on renoprotection will contribute to the design of molecules targeted to genes relevant to the pathophysiology of regeneration, with the goal of kidney regeneration instead of dialysis or renal transplantation.

#### Contributors

AB selected the relevant articles, developed the framework for the review, and wrote the report. MM undertook the literature search, selected the relevant reports, and reviewed the different versions of this report. GR established the key issues under the different subheadings, interpreted data from the evidence search, and wrote the report. All authors reviewed the final version and obtained funding.

#### Conflicts of interest

We declare that we have no conflicts of interest.

#### Acknowledgments

We thank Irene van der Meer, Piero Ruggenenti, and Paola Rizzo for their assistance; Antonella Piccinelli for her work on figures and tables; and Manuela Passera for her assistance with preparation of the report. This report was supported by grants from the European Community STAR-T REK (FP7-HEALTH-2007-1.4-8) and from Fondazione Cariplo (Milano, Italy; grant 2007-5549).

## References

- 1 Birnbaum KD, Sanchez Alvarado A. Slicing across kingdoms: regeneration in plants and animals. *Cell* 2008; **132**: 697–710.
- 2 Trembley A. Mémoires pour servir à l'histoire d'un genre de polypes d'eau douce, à bras en forme de cornes. (In French) Notes for describing a particular type of polyp living in fresh water. Leiden: Jean and Herman Verbeek, 1744.
- 3 Spallanzani L. Prodomo di un'opera da imprimeri sopra la riproduzione animale (An essay on animal reproduction). (In Italian) London: T Becket & de Hondt, 1769.
- 4 Bonnet C. Oeuvres d'histoire naturelle et de philosophie de Charles Bonnet. (In French) Work on natural history and philosophy by Charles Bonnet. Neuchatel, Switzerland: Samuel Fauche, 1779.
- 5 Sena G, Wang X, Liu HY, Hofhuis H, Birnbaum KD. Organ regeneration does not require a functional stem cell niche in plants. *Nature* 2009; **457**: 1150–53.
- 6 Kragl M, Knapp D, Nacu E, et al. Cells keep a memory of their tissue origin during axolotl limb regeneration. *Nature* 2009; **460**: 60–65.
- 7 Mathur A, Martin JF. Stem cells and repair of the heart. *Lancet* 2004; **364**: 183–92.
- 8 Michalopoulos GK. Liver regeneration: alternative epithelial pathways. *Int J Biochem Cell Biol* 2009; published online Sept 27. DOI:10.1016/j.biocel.2009.09.014.
- 9 Bedebaeva K, Snyder A, Gourevitch D, et al. Lack of p21 expression links cell cycle control and appendage regeneration in mice. *Proc Natl Acad Sci USA* 2010; DOI:10.1073/pnas.1000830107.
- 10 Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U, Frisen J. Identification of a neural stem cell in the adult mammalian central nervous system. *Cell* 1999; **96**: 25–34.
- 11 Hopkins C, Li J, Rae F, Little MH. Stem cell options for kidney disease. *J Pathol* 2009; **217**: 265–81.
- 12 Haller H, de Groot K, Bahlmann F, Elger M, Fliser D. Stem cells and progenitor cells in renal disease. *Kidney Int* 2005; **68**: 1932–36.
- 13 Elger M, Hentschel H, Litteral J, et al. Nephrogenesis is induced by partial nephrectomy in the elasmobranch *Leucoraja erinacea*. *J Am Soc Nephrol* 2003; **14**: 1506–18.
- 14 Hartman HA, Lai HL, Patterson LT. Cessation of renal morphogenesis in mice. *Dev Biol* 2007; **310**: 379–87.
- 15 Kuure S, Vuolteenaho R, Vainio S. Kidney morphogenesis: cellular and molecular regulation. *Mech Dev* 2000; **92**: 31–45.
- 16 Witzgall R, Brown D, Schwarz C, Bonventre JV. Localization of proliferating cell nuclear antigen, vimentin, c-Fos, and clusterin in the postischemic kidney. Evidence for a heterogenous genetic response among nephron segments, and a large pool of mitotically active and dedifferentiated cells. *J Clin Invest* 1994; **93**: 2175–88.
- 17 Imgrund M, Grone E, Grone HJ, et al. Re-expression of the developmental gene Pax-2 during experimental acute tubular necrosis in mice 1. *Kidney Int* 1999; **56**: 1423–31.
- 18 Humphreys BD, Valerius MT, Kobayashi A, et al. Intrinsic epithelial cells repair the kidney after injury. *Cell Stem Cell* 2008; **2**: 284–91.
- 19 Lin SL, Li B, Rao S, et al. Macrophage Wnt7b is critical for kidney repair and regeneration. *Proc Natl Acad Sci* 2010; **107**: 4194–99.
- 20 Gobe GC, Johnson DW. Distal tubular epithelial cells of the kidney: Potential support for proximal tubular cell survival after renal injury. *Int J Biochem Cell Biol* 2007; **39**: 1551–61.
- 21 Swetha G, Chandra V, Phadnis S, Bhande R. Glomerular parietal epithelial cells of adult murine kidney undergo EMT to generate cells with traits of renal progenitors. *J Cell Mol Med* 2009; published online Oct 16. DOI:10.1111/j.1582-4934.2009.00937.x.
- 22 Dressler GR. The cellular basis of kidney development. *Annu Rev Cell Dev Biol* 2006; **22**: 509–29.
- 23 Herzlinger D, Koseki C, Mikawa T, al-Awqati Q. Metanephric mesenchyme contains multipotent stem cells whose fate is restricted after induction. *Development* 1992; **114**: 565–72.
- 24 Boyle S, Misfeldt A, Chandler KJ, et al. Fate mapping using Cited1-CreERT2 mice demonstrates that the cap mesenchyme contains self-renewing progenitor cells and gives rise exclusively to nephronic epithelia. *Dev Biol* 2008; **313**: 234–45.
- 25 Self M, Lagutin OV, Bowling B, et al. Six2 is required for suppression of nephrogenesis and progenitor renewal in the developing kidney. *EMBO J* 2006; **25**: 5214–28.
- 26 Kobayashi A, Valerius MT, Mugford JW, et al. Six2 defines and regulates a multipotent self-renewing nephron progenitor population throughout mammalian kidney development. *Cell Stem Cell* 2008; **3**: 169–81.
- 27 Kobayashi A, Valerius MT, Mugford JW, et al. Pax2 maintains a self-renewing nephron progenitor population by repressing interstitial cell fates during mammalian kidney development. *J Am Soc Nephrol* 2009; **20**: (abstracts) 103A.
- 28 Rogers SA, Lowell JA, Hammerman NA, Hammerman MR. Transplantation of developing metanephroi into adult rats. *Kidney Int* 1998; **54**: 27–37.
- 29 Dekel B, Burakova T, Arditti FD, et al. Human and porcine early kidney precursors as a new source for transplantation. *Nat Med* 2003; **9**: 53–60.
- 30 Imberti B, Corna D, Xinaris C, et al. Transplanted embryonic kidneys develop and contribute to kidney regeneration in a rat model of chronic renal disease. *J Am Soc Nephrol* 2009; **20**: (abstracts) 103A.
- 31 Oliver JA, Barasch J, Yang J, Herzlinger D, Al-Awqati Q. Metanephric mesenchyme contains embryonic renal stem cells. *Am J Physiol Renal Physiol* 2002; **283**: F799–809.
- 32 Bussolati B, Bruno S, Grange C, et al. Isolation of renal progenitor cells from adult human kidney. *Am J Pathol* 2005; **166**: 545–55.
- 33 Ronconi E, Sagrinati C, Angelotti ML, et al. Regeneration of glomerular podocytes by human renal progenitors. *J Am Soc Nephrol* 2009; **20**: 322–32.
- 34 Sagrinati C, Netti GS, Mazzinghi B, et al. Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. *J Am Soc Nephrol* 2006; **17**: 2443–56.
- 35 Appel D, Kershaw DB, Smeets B, et al. Recruitment of podocytes from glomerular parietal epithelial cells. *J Am Soc Nephrol* 2009; **20**: 333–43.
- 36 da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci* 2006; **119**: 2204–13.
- 37 Crisan M, Yap S, Casteilla L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 2008; **3**: 301–13.
- 38 Bruno S, Bussolati B, Grange C, et al. Isolation and characterization of resident mesenchymal stem cells in human glomeruli. *Stem Cells Dev* 2009; **18**: 867–80.
- 39 Braun KM, Niemann C, Jensen UB, Sundberg JP, Silva-Vargas V, Watt FM. Manipulation of stem cell proliferation and lineage commitment: visualisation of label-retaining cells in whole mounts of mouse epidermis. *Development* 2003; **130**: 5241–55.
- 40 Yan L, Han Y, He Y, et al. Cell tracing techniques in stem cell transplantation. *Stem Cell Rev* 2007; **3**: 265–69.
- 41 Maeshima A, Yamashita S, Nojima Y. Identification of renal progenitor-like tubular cells that participate in the regeneration processes of the kidney. *J Am Soc Nephrol* 2003; **14**: 3138–46.
- 42 Yamashita S, Maeshima A, Nojima Y. Involvement of renal progenitor tubular cells in epithelial-to-mesenchymal transition in fibrotic rat kidneys. *J Am Soc Nephrol* 2005; **16**: 2044–51.
- 43 Oliver JA, Maarouf O, Cheema FH, Martens TP, Al-Awqati Q. The renal papilla is a niche for adult kidney stem cells. *J Clin Invest* 2004; **114**: 795–804.
- 44 Oliver JA, Klinakis A, Cheema FH, et al. Proliferation and migration of label-retaining cells of the kidney papilla. *J Am Soc Nephrol* 2009; **20**: 2315–27.
- 45 Wagers AJ, Weissman IL. Plasticity of adult stem cells. *Cell* 2004; **116**: 639–48.
- 46 De Broe ME. Tubular regeneration and the role of bone marrow cells: 'stem cell therapy'—a panacea? *Nephrol Dial Transplant* 2005; **20**: 2318–20.
- 47 Poulosom R, Forbes SJ, Hodivala-Dilke K, et al. Bone marrow contributes to renal parenchymal turnover and regeneration. *J Pathol* 2001; **195**: 229–35.
- 48 Fang TC, Alison MR, Cook HT, Jeffery R, Wright NA, Poulosom R. Proliferation of bone marrow-derived cells contributes to regeneration after folic acid-induced acute tubular injury. *J Am Soc Nephrol* 2005; **16**: 1723–32.
- 49 Gupta S, Verfaillie C, Chmielewski D, Kim Y, Rosenberg ME. A role for extrarenal cells in the regeneration following acute renal failure. *Kidney Int* 2002; **62**: 1285–90.

- 50 Duffield JS, Park KM, Hsiao LL, et al. Restoration of tubular epithelial cells during repair of the postischemic kidney occurs independently of bone marrow-derived stem cells. *J Clin Invest* 2005; **115**: 1743–55.
- 51 Ito T, Suzuki A, Imai E, Okabe M, Hori M. Bone marrow is a reservoir of repopulating mesangial cells during glomerular remodeling. *J Am Soc Nephrol* 2001; **12**: 2625–35.
- 52 Li B, Morioka T, Uchiyama M, Oite T. Bone marrow cell infusion ameliorates progressive glomerulosclerosis in an experimental rat model. *Kidney Int* 2006; **69**: 323–30.
- 53 Bonventre JV, Weinberg JM. Recent advances in the pathophysiology of ischemic acute renal failure. *J Am Soc Nephrol* 2003; **14**: 2199–210.
- 54 Bonventre JV. Dedifferentiation and proliferation of surviving epithelial cells in acute renal failure. *J Am Soc Nephrol* 2003; **14** (suppl 1): 55–61.
- 55 Brodie JC, Humes HD. Stem cell approaches for the treatment of renal failure. *Pharmacol Rev* 2005; **57**: 299–313.
- 56 Herrera MB, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G. Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. *Int J Mol Med* 2004; **14**: 1035–41.
- 57 Morigi M, Imberti B, Zoja C, et al. Mesenchymal stem cells are renoprotective, helping to repair the kidney and improve function in acute renal failure. *J Am Soc Nephrol* 2004; **15**: 1794–804.
- 58 Bi B, Schmitt R, Israилоva M, Nishio H, Cantley LG. Stromal cells protect against acute tubular injury via an endocrine effect. *J Am Soc Nephrol* 2007; **18**: 2486–96.
- 59 Perico N, Benigni A, Remuzzi G. Present and future drug treatments for chronic kidney diseases: evolving targets in renoprotection. *Nat Rev Drug Discov* 2008; **7**: 936–53.
- 60 Marinides GN, Groggel GC, Cohen AH, Border WA. Enalapril and low protein reverse chronic puromycin aminonucleoside nephropathy. *Kidney Int* 1990; **37**: 749–57.
- 61 Ikoma M, Kawamura T, Kakinuma Y, Fogo A, Ichikawa I. Cause of variable therapeutic efficiency of angiotensin converting enzyme inhibitor on glomerular lesions. *Kidney Int* 1991; **40**: 195–202.
- 62 Remuzzi A, Fassi A, Bertani T, Perico N, Remuzzi G. ACE inhibition induces regression of proteinuria and halts progression of renal damage in a genetic model of progressive nephropathy. *Am J Kidney Dis* 1999; **34**: 626–32.
- 63 Ma LJ, Nakamura S, Whitsitt JS, Marcantoni C, Davidson JM, Fogo AB. Regression of sclerosis in aging by an angiotensin inhibition-induced decrease in PAI-1. *Kidney Int* 2000; **58**: 2425–36.
- 64 Remuzzi A, Gagliardini E, Donadoni C, et al. Effect of angiotensin II antagonism on the regression of kidney disease in the rat. *Kidney Int* 2002; **62**: 885–94.
- 65 Adamczak M, Gross ML, Krttil J, Koch A, Tyralla K, Amann K, Ritz E. Reversal of glomerulosclerosis after high-dose enalapril treatment in subtotal nephrectomized rats. *J Am Soc Nephrol* 2003; **14**: 2833–42.
- 66 Boffa JJ, Lu Y, Placier S, Stefanski A, Dussault JC, Chatziantoniou C. Regression of renal vascular and glomerular fibrosis: role of angiotensin II receptor antagonism and matrix metalloproteinases. *J Am Soc Nephrol* 2003; **14**: 1132–44.
- 67 Fujihara CK, Velho M, Malheiros DM, Zatz R. An extremely high dose of losartan affords superior renoprotection in the remnant model. *Kidney Int* 2005; **67**: 1913–24.
- 68 Ma LJ, Nakamura S, Aldigier JC, et al. Regression of glomerulosclerosis with high-dose angiotensin inhibition is linked to decreased plasminogen activator inhibitor-1. *J Am Soc Nephrol* 2005; **16**: 966–76.
- 69 Remuzzi A, Gagliardini E, Sangalli F, et al. ACE inhibition reduces glomerulosclerosis and regenerates glomerular tissue in a model of progressive renal disease. *Kidney Int* 2006; **69**: 1124–30.
- 70 Teles F, Machado FG, Ventura BH, et al. Regression of glomerular injury by losartan in experimental diabetic nephropathy. *Kidney Int* 2009; **75**: 72–79.
- 71 Adamczak M, Gross ML, Amann K, Ritz E. Reversal of glomerular lesions involves coordinated restructuring of glomerular microvasculature. *J Am Soc Nephrol* 2004; **15**: 3063–72.
- 72 Fogo AB. New capillary growth: a contributor to regression of sclerosis? *Curr Opin Nephrol Hypertens* 2005; **14**: 201–03.
- 73 Macconi D, Sangalli F, Bonomelli M, et al. Podocyte repopulation contributes to regression of glomerular injury induced by ACE inhibition. *Am J Pathol* 2009; **174**: 797–807.
- 74 Prodromidi EI, Poulson R, Jeffery R, et al. Bone marrow-derived cells contribute to podocyte regeneration and amelioration of renal disease in a mouse model of Alport syndrome. *Stem Cells* 2006; **24**: 2448–55.
- 75 Li J, Deane JA, Campanale NV, Bertram JF, Ricardo SD. The contribution of bone marrow-derived cells to the development of renal interstitial fibrosis. *Stem Cells* 2007; **25**: 697–706.
- 76 Choi S, Park M, Kim J, Hwang S, Park S, Lee Y. The role of mesenchymal stem cells in the functional improvement of chronic renal failure. *Stem Cells Dev* 2009; **18**: 521–29.
- 77 Ninichuk V, Gross O, Segerer S, et al. Multipotent mesenchymal stem cells reduce interstitial fibrosis but do not delay progression of chronic kidney disease in collagen4A3-deficient mice. *Kidney Int* 2006; **70**: 121–29.
- 78 Magnasco A, Corselli M, Bertelli R, et al. Mesenchymal stem cells protective effect in adriamycin model of nephropathy. *Cell Transplant* 2008; **17**: 1157–67.
- 79 Nash K, Hafeez A, Hou S. Hospital-acquired renal insufficiency. *Am J Kidney Dis* 2002; **39**: 930–36.
- 80 Schrier RW, Wang W, Poole B, Mitra A. Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. *J Clin Invest* 2004; **114**: 5–14.
- 81 Abuelo JG. Normotensive ischemic acute renal failure. *N Engl J Med* 2007; **357**: 797–805.
- 82 Kellum JA. Acute kidney injury. *Crit Care Med* 2008; **36** (suppl): 141–45.
- 83 Wilmer WA, Hebert LA, Lewis EJ, et al. Remission of nephrotic syndrome in type 1 diabetes: long-term follow-up of patients in the Captopril Study. *Am J Kidney Dis* 1999; **34**: 308–14.
- 84 The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. *Lancet* 1997; **349**: 1857–63.
- 85 Gaspari F, Perico N, Matalone M, et al. Precision of plasma clearance of iothexol for estimation of GFR in patients with renal disease. *J Am Soc Nephrol* 1998; **9**: 310–13.
- 86 Ruggenenti P, Perna A, Gherardi G, Gaspari F, Benini R, Remuzzi G. Renal function and requirement for dialysis in chronic nephropathy patients on long-term ramipril: REIN follow-up trial. Gruppo Italiano di Studi Epidemiologici in Nefrologia (GISEN). Ramipril Efficacy in Nephropathy. *Lancet* 1998; **352**: 1252–56.
- 87 Ruggenenti P, Perna A, Benini R, et al. In chronic nephropathies prolonged ACE inhibition can induce remission: dynamics of time-dependent changes in GFR. Investigators of the GISEN Group. Gruppo Italiano Studi Epidemiologici in Nefrologia. *J Am Soc Nephrol* 1999; **10**: 997–1006.
- 88 Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. *J Am Geriatr Soc* 1985; **33**: 278–85.
- 89 Fioretto P, Steffes MW, Sutherland DE, Goetz FC, Mauer M. Reversal of lesions of diabetic nephropathy after pancreas transplantation. *N Engl J Med* 1998; **339**: 69–75.
- 90 Mauer SM, Steffes MW, Sutherland DE, Najarian S, Michael AF, Brown DM. Studies of the rate of regression of the glomerular lesions in diabetic rats treated with pancreatic islet transplantation. *Diabetes* 1975; **24**: 280–85.
- 91 Ruggenenti P, Peticucci E, Cravedi P, et al. Role of remission clinics in the longitudinal treatment of CKD. *J Am Soc Nephrol* 2008; **19**: 1213–24.
- 92 Ruggenenti P, Brenner BM, Remuzzi G. Remission achieved in chronic nephropathy by a multidrug approach targeted at urinary protein excretion. *Nephron* 2001; **88**: 254–59.
- 93 Ruggenenti P, Schieppati A, Remuzzi G. Progression, remission, regression of chronic renal diseases. *Lancet* 2001; **357**: 1601–08.