Kidney Fibrosis: Origins and Interventions

Thomas Vanhove, MD,[1,2] Roel Goldschmeding, MD, PhD,[3] and Dirk Kuypers, MD, PhD[1,2]

Abstract: All causes of renal allograft injury, when severe and/or sustained, can result in chronic histological damage of which interstitial fibrosis and tubular atrophy are dominant features. Unless a specific disease process can be identified, what drives interstitial fibrosis and tubular atrophy progression in individual patients is often unclear. In general, clinicopathological factors known to predict and drive allograft fibrosis include graft quality, inflammation (whether "nonspecific" or related to a specific diagnosis), infections, such as polyomavirus-associated nephropathy, calcineurin inhibitors (CNI), and genetic factors. The incidence and severity of chronic histological damage have decreased substantially over the last 3 decades, but it is difficult to disentangle what effects individual innovations (eg, better matching and preservation techniques, lower CNI dosing, BK viremia screening) may have had. There is little evidence that CNI-sparing/minimization strategies, steroid minimization or renin-angiotensin-aldosterone system blockade result in better preservation of intermediate-term histology. Treatment of subclinical rejections has only proven beneficial to histological and functional outcome in studies in which the rate of subclinical rejection in the first 3 months was greater than 10% to 15%. Potential novel antifibrotic strategies include antagonists of transforming growth factor-β, connective tissue growth factor, several tyrosine kinase ligands (epidermal growth factor, platelet-derived growth factor, vascular endothelial growth factor), endothelin and inhibitors of chemotaxis. Although many of these drugs are mainly being developed and marketed for oncological indications and diseases, such as idiopathic pulmonary fibrosis, a number may hold promise in the treatment of diabetic nephropathy, which could eventually lead to applications in renal transplantation.

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PATHOPHYSIOLOGY

The basic mechanisms underlying renal allograft fibrosis are depicted in Figure 1. Interested readers are referred to several excellent in-depth reviews.[1-3] In essence, most of the processes that cause renal injury result in an inflammatory cascade involving macrophage activation and recruitment of immune (mainly T) cells. Under the influence of inflammatory cytokines, several cell types including macrophages, T cells and tubular epithelial cells produce profibrotic mediators such as TGF-β.[4] This results in activation of mesenchymal cells (fibroblasts, fibrocytes, and pericytes [which support the endothelium]) that then become contractile and matrix-producing myofibroblasts.[5] At the same time, a wave of epithelial dedifferentiation occurs in which injured epithelial cells lose their polarity and transporter function, reorganize their cytoskeleton into stress fibers, disrupt the tubular basement membrane and migrate into the interstitium where they synthesize increasing amounts of extracellular matrix (ECM). Whether tubular epithelial and endothelial cells undergo the complete transformation to myofibroblasts (processes known as epithelial-to-mesenchymal transition [EMT] and endothelial-to-mesenchymal transition) is not firmly established.[5] The transformation of mesenchymal and epithelial cells to myofibroblasts is characterized by de novo production of α-smooth muscle actin, vimentin, S1004A, and the translocation of E-cadherin from the cell membrane to the cytoplasm. Some of the best-characterized profibrotic mediators and molecular pathways are summarized in Figure 2.

In normal wound repair, resolution of the initial injury is followed by wound contraction, ECM degradation, cessation of inflammation and restoration of normal tissue architecture. In case of persistent allograft injury, continued fibrogenesis ultimately results in irreversibly atrophied tubuli, excessive interstitial fibrosis (IF), microvascular rarefaction and glomerulosclerosis. It must be emphasized that progressive fibrosis almost invariably indicates continuing injury. Microarray studies of human renal allografts displaying IF have confirmed early and continued upregulation of genes related to immune activation, inflammation, fibrosis and remodeling, including TGF-β, connective tissue growth factor (CTGF), mitogen-activated protein kinase, vimentin, α-smooth muscle actin, and matrix metalloproteinase-7.[6-11] However, there seems to be a "point of no return" of structural injury, beyond which fibrosis progresses on a local level regardless

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[1] *Department of Microbiology and Immunology, KU Leuven-University of Leuven, Leuven, Belgium.*

[2] *Department of Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium.*

[3] *Department of Pathology, University Medical Center Utrecht, Utrecht, the Netherlands.*

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Correspondence: Dirk R J Kuypers, MD, PhD, Department of Nephrology and Renal Transplantation, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium. (dirk.kuypers@uzleuven.be).

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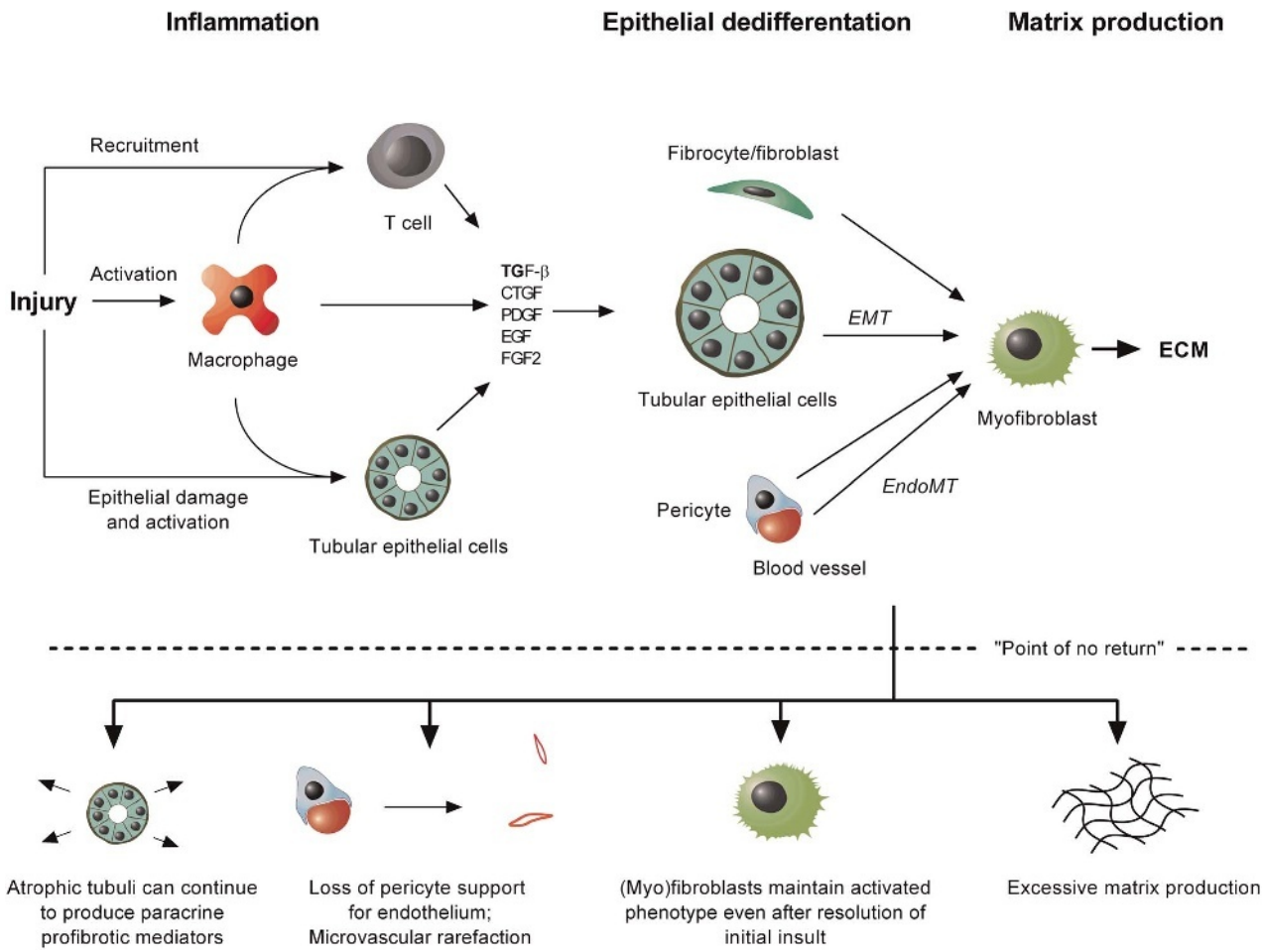

FIGURE 1. Simplified diagram of renal fibrogenesis. Most injurious stimuli result in an inflammatory cascade characterized by recruitment and activation of inflammatory cells, as well as activation of damaged epithelial cells. All of these cell types produce not only proinflammatory but also profibrotic mediators that result in consecutive waves of epithelial dedifferentiation. Resident and recruited mesenchymal cells (fibrocytes, fibroblasts, pericytes) and possibly also epithelial cells (tubular and endothelial) transdifferentiate to become contractile myofibroblasts that produce ECM. When the injury is severe and/or persistent, eventually a point of no return may be reached beyond which fibrosis progresses on a local level even after resolution of injury. EndoMT, endothelial-to-mesenchymal transition; FGF, fibroblast growth factor.

of persisting injury. This has several reasons. (A) Even if the cause of renal injury is resolved, some atrophic tubuli do not recover and continue to produce paracrine profibrotic signals[12]; (B) arteriolar narrowing and microvascular rarefaction result in chronic hypoxia, which damages tubules and is potently profibrotic[13]; (C) myofibroblasts can maintain their activated phenotype after resolution of the initial insult.[14] Importantly though, experimental (primarily nontransplant) chronic kidney disease models have revealed that fibrosis is only progressive on a local level.[12] Fibrosis does not invade normal tissue. This is reflected by the sharp demarcation that is usually observed between fibrotic and normal areas in a kidney biopsy. This injury can be in any part of the nephron, as glomerulosclerosis, tubular damage, vascular rarefaction and obliteration all affect each other and ultimately result in a conglomerate of chronic histological lesions of which IF is only one of the hallmarks.

Finally, although fibrosis does not spread throughout the graft, each allograft has a limited reserve capacity beyond which structural and functional deterioration will progress in the absence of persistent injury. This occurs if nephron loss reaches a critical point, after which compensatory hyperfiltration results in hypertensive damage to the remaining glomeruli and proteinuria can result in sustained tubular injury. It is important to keep in mind that in a patient who, for example, receives a single 50-year old kidney that has sustained the injury of ischemia-reperfusion, this threshold of critical nephron loss may be reached relatively quickly.

The Clustering and Prognostic Value of Chronic Histological Lesions

In the Banff classification of renal allograft histology, the individual chronic lesions are IF (ci), tubular atrophy (TA) (ct), arterial fibrous intimal thickening (cv), arteriolar hyalinosis (ah), mesangial matrix increase (mm) and transplant glomerulopathy (cg).[15] There is a high degree of clustering between all these lesions except transplant glomerulopathy,[16] which is a unique pathologic entity with specific prognostic implications.[17] IF and TA, in particular, almost invariably occur together[18] and are often considered as the single parameter IF/TA (previously chronic allograft nephropathy). Because the tubulointerstitium comprises 90% of kidney volume, IF/TA is generally the most prominent manifestation of structural allograft deterioration. However, grouping IF/TA with other related chronic lesions better reflects the global burden of histological damage and better predicts graft outcome, at least

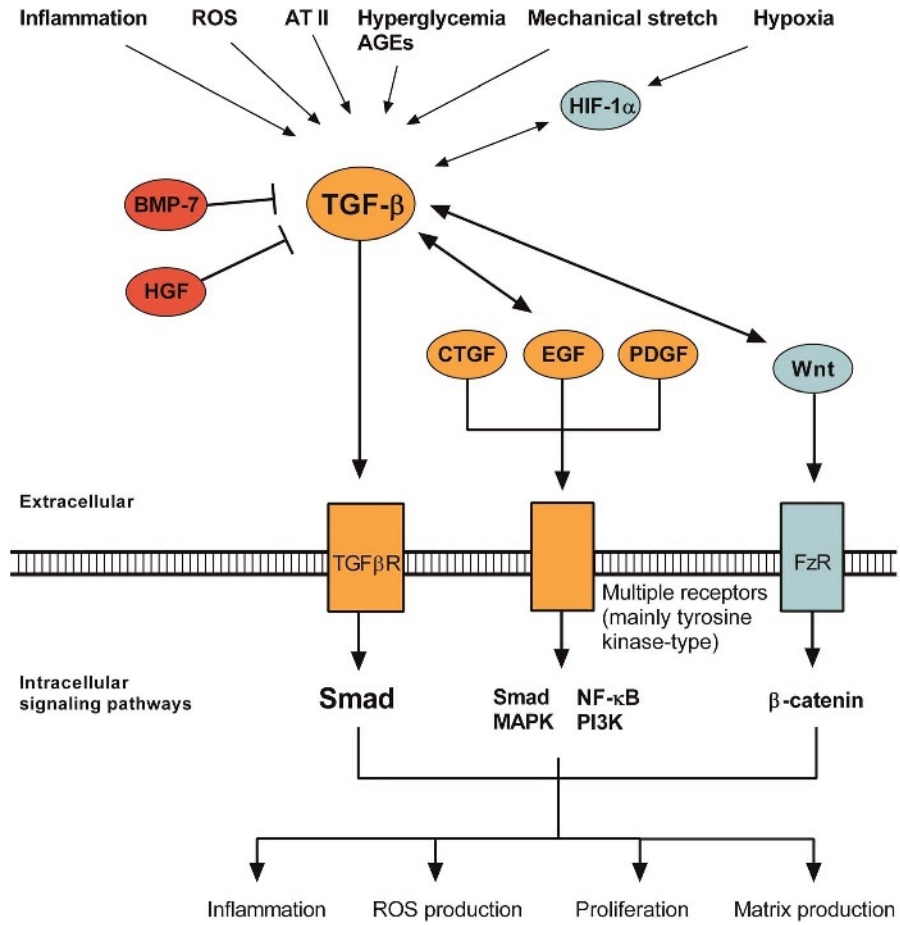

FIGURE 2. Canonical mediators and molecular pathways in renal fibrosis. Many injurious stimuli converge on the TGF-β pathway, which has context-dependent pleiotropic effects and interacts with several related pathways. AGEs, advanced glycation end products; BMP-7, bone morphogenetic protein 7; FzR, frizzled receptor; HGF, hepatocyte growth factor; HIF, hypoxia-inducible factor; ROS, reactive oxygen species.

in indication biopsies.[16] This review uses the terms fibrosis and IF/TA virtually interchangeably as they both indicate an accumulation of chronic histological damage that is not specific to any type of renal injury.

IF/TA cannot be considered a disease but only the final common end point of countless disease processes and, by itself, it is hardly ever actionable. However, it may become actionable (regardless of or, preferably, in addition to disease-specific therapy) in the near future as targeted antifibrotic therapies are being developed. This is discussed in the second part of this review. In addition, IF/TA still has major prognostic implications. IF is the strongest histological predictor of graft outcome in native kidney glomerulonephritis.[19] Similarly, IF/TA of the renal allograft (assessed using the Banff scoring system or by computerized quantification of IF) associates with worse renal function[20,21] and predicts future functional decline as well as graft survival.[21-25] IF/TA implies a worse prognosis independent of the underlying diagnosis (eg, T cell-mediated rejection and antibody-mediated rejection [AMR]).[26,27] Consequently, even though identifying specific disease processes in a renal biopsy is a priority for prognostic and therapeutic reasons, IF/TA has additional value as a proxy for outcome and can help qualify/quantify the impact of factors that are detrimental to the graft. It is important to note that only moderate to severe fibrosis is predictive of outcome in most studies whereas mild fibrosis, even with extended follow-up, is not.[28-31]

The "Natural Evolution" of Allograft Histology

Much of the studies mentioned in this review are based on protocol or "surveillance" biopsy programs. These involve performing kidney biopsies at fixed time points in renal recipients with stable renal function and have provided much information regarding the natural evolution of graft histology.[32-34] It must be noted, however, that these studies are inherently biased to some extent, with an overrepresentation of low-risk patients. Particularly at later time points, many patients are no longer biopsied because they have lost their graft, have developed medical comorbidities, refuse or are lost to follow-up. Additionally, few centers perform protocol biopsies later than 2 years after transplantation, so our knowledge of the evolution of graft histology beyond that point is relatively limited.

Regardless of these limitations, most protocol biopsy programs consistently report the same basic trend. Fibrosis develops in many patients during the first year after transplantation, but is generally mild.[21,25,32,33,35-37] Most of this fibrosis is accumulated in the first 3 months, after which the rate of progression slows significantly. It is likely that this early accumulation of fibrosis mainly results from self-limiting

inflammation related to implantation stress.[38,39] Nevertheless, it will often continue to progress. In the seminal study by Nankivell et al[32] (performed in kidney-pancreas recipients treated with relatively high-dose cyclosporine), moderate-severe IF/TA was present in 66% of patients by 5 years and 90% by 10 years after transplantation. More recent studies report significantly lower rates of fibrosis at all time points with only limited progression beyond 1 year in most patients, at least in the intermediate term.[34,40] The prevalence of moderate-severe fibrosis at 5 years was only 17% in a large analysis of predominantly living-donor, tacrolimus (Tac) treated single kidney recipients from the Mayo clinic.[34] Several aspects of patient care have evolved in parallel over the last 2 decades, which makes it difficult to disentangle what effect individual innovations may have had on reducing the accumulation of histological damage. These possibly beneficial changes include better preservation techniques, lower dosing of calcineurin inhibitors (CNIs), CNI minimization strategies and the use of Tac rather than cyclosporine. On the other hand, these factors may have been partly offset by increasing use of kidneys of suboptimal quality (expanded-criteria donor, donation after cardiac death [DCD]) in many regions of the world. The next section discusses the available evidence regarding the factors that drive fibrosis in the renal allograft.

Quality of the Graft

Several factors determine the intrinsic quality of the graft and the damage it sustains as a result of ischemia-reperfusion injury. These include donor age, donor type (living vs deceased; donation after brain death (DBD) vs DCD, cold ischemia time and warm ischemia time. In the context of fibrosis, donor age is arguably the most important factor. Donor age is a key predictor of graft outcome,[41] not only because it is a strong determinant of graft quality at implantation.[42] High donor age is also independently and consistently associated with accelerated progression of chronic histologic damage (mainly IF/TA and chronic vasculopathy) as well as functional decline in the years after transplantation.[25,32,37,43-45] The fact that older kidneys deteriorate faster regardless of baseline histology is likely to be partly explained by an age-related loss in renal regenerative capacity.[46] Delayed graft function (DGF), a proxy for suboptimal graft quality and/or significant ischemia-reperfusion injury, consistently associates with higher degrees of IF/TA early after transplantation.[38,47-50] Surgical anastomosis time has been shown to predict IF/TA independently of its effect on DGF.[51] The individual effects of cold ischemia time and donor type (living vs deceased) on early and long-term IF/TA, however, are not as consistent among studies.[20,21,33,37,52]

Inflammation

There is no doubt that inflammation in a renal allograft is a potent and, arguably, the most studied predictor of subsequent allograft fibrosis. Protocol biopsy studies performed on patients transplanted in the late 1980s and 1990s identified early biopsy-proven acute rejection (BPAR) as a risk factor for IF/TA.[32,35,53,54] In this review, the term BPAR refers to clinical acute rejections (ie, accompanied by graft dysfunction). Subclinical rejections (SCR) are defined as histologic acute rejection in the absence of graft dysfunction. Borderline rejections do not meet the Banff criteria for acute rejection and can be either clinical or subclinical. Rush et al were among the first to report that early SCR, too, was independently predictive of IF/TA.[55] Several groups have since reported accelerated progression of IF/TA related to BPAR,[56] any rejection of at least Banff t2i2 severity (clinical or subclinical),[21,57,58] any rejection of at least Banff t1i1 severity (ie, including borderline rejections [Banff '97 criteria])[59] and even just higher Banff i score on a 1-year protocol biopsy.[60] Early acute rejections and (subclinical) inflammation also predict future development of de novo donor-specific antibodies (dnDSA) and chronic AMR, which are powerful predictors of graft loss.[59,61-63] Not all groups have confirmed this.[64] Two trends are noteworthy. First, just as the incidence and severity of BPARs has strongly decreased since the late 1980s, so has SCR become less frequent (reviewed by Mehta et al).[65] Three-month SCR rates were often around 25% to 40% in earlier studies,[36,57] whereas recently, they are generally < 10%.[37,45,50,66] There is convincing evidence that SCR rates are lowered by Tac (compared with cyclosporine [CsA])[24,32,63,64,67] and adequately dosed mycophenolate mofetil (MMF).[68,69] Use of induction therapy, however, has not been proven to lower the risk of SCR. In fact, the opposite has been reported in a trial of low-risk pediatric patients.[70] Second, there are indications that SCR has become a better predictor of IF/TA progression than BPAR. With modern immunosuppression, most early BPARs are mild, steroid-responsive and have no negative impact on graft outcome if renal function recovers after treatment[71] or no histological damage is sustained,[59] though they may still predispose to later dnDSA development.[59] Some recent studies have reported that subclinical inflammation had a larger effect on IF/TA progression[37,45,50,72] and dnDSA development[63] than BPAR. The underlying reasons for these observations are not completely clear, but the fact that BPARs are systematically treated while borderline rejections and subclinical mild inflammation, in many centers, are not, could be a factor. However, steroids frequently do not result in complete resolution of subclinical inflammation[73] and whether treating it has long-term benefits is still a matter of debate (see below).

There is evidence that the presence of inflammation, even below the threshold for borderline rejection, discriminates between fibrosis that is inactive scar tissue and fibrosis that reflects an underlying progressive (albeit poorly defined) process. Studies performed in the Mayo clinic demonstrated that, in low-risk renal recipients, fibrosis plus interstitial inflammation predicted poor graft survival but mild fibrosis without inflammation did not.[31,74,75] The same has been reported for "IF/TA + SCR."[76] Persistence of low-grade inflammation in repeated protocol biopsies might be particularly detrimental.[77,78]

MAKING SENSE OF NONSPECIFIC INFLAMMATION

The goal of any histological evaluation of the renal allograft, whether for cause or per protocol, should be to identify specific diagnoses. By the time the graft fails, this is generally possible,[26,79] but early (particularly protocol) biopsies will often demonstrate nonspecific IF/TA and relatively low-grade, nonspecific tubulitis and interstitial inflammation. The biological nature and long-term impact of these infiltrates is unclear: even though many are probably actual mild rejections or at least manifestations of alloimmune injury, it is

likely that some of them are merely resorptive inflammatory responses. A key future challenge will be differentiating between types of inflammation, that is, separating harmful alloimmune activation from resorptive, nondetrimental or even beneficial (tolerogenic) immune activity.

First, every effort must be made to exclude a specific disease process by combining histological, clinical and laboratory information. For example, subclinical AMR (suggested by presence of DSA, glomerulitis, peritubular capillaritis, and/or C4d deposition) is more potently profibrotic and carries a worse prognosis[80] in comparison with nonspecific subclinical inflammation.[81] In late for-cause biopsies (>1 year), the detrimental effect of inflammation very often results from its association with progressive diseases such as AMR and recurrent glomerulonephritis.[82]

Second, severe inflammation is strongly suggestive of detrimental alloimmune injury. For example, acute rejection ≥ Banff grade IIA (arteritis or "v" score > 0) seems mostly incompatible with stable renal function as it is very rare in protocol biopsies.[45,57,78] In less severe cases, however, light microscopy might not provide an accurate estimate of the severity of inflammation. Microarray studies have revealed significant upregulation of genes related to immunity, inflammation, remodeling and fibrosis in allografts indication biopsies displaying IF/TA,[8,11,83] biopsies with IF/TA but no histological inflammation[83,84] and even in completely normal protocol biopsies.[84] Many of these immune-related gene sets are shared with acute rejection,[83] indicating the presence of significant inflammatory and fibrotic activity even in the absence of clear histological inflammation. Microarray studies have also revealed qualitative and quantitative differences in gene expression between BPAR and SCR,[85] between borderline rejections with and without graft dysfunction,[44] and between early biopsies that later developed IF/TA or did not.[11,86] In the future, gene expression analysis could be a promising strategy to differentiate between detrimental and harmless infiltrates and, by extension, determine which should be treated. Microarray studies have already been used to identify molecular signatures that predicted graft loss in indication biopsies[87] and IF/TA progression in early protocol biopsies[88] better than traditional clinicopathological risk factors.

Third, what renal compartments are inflamed? In the Banff classification, interstitial inflammation is scored in unscarred areas (i-Banff), but not in scarred areas (i-IF/TA), subcapsular cortex or adventitia around large vessels because these are not considered specific for acute rejection (the diagnosis of which was the original raison d'être for the Banff classification).[15] There have, however, been reports that i-IF/TA is an independent predictor of IF/TA progression in 3-month protocol biopsies[89] and graft loss[90] in late for-cause biopsies. Total-i (inflammation in all scarred and unscarred cortical tissue) has also been reported to be a better predictor of graft survival than i-Banff.[91] Total-i in 6-week protocol biopsies was not predictive of IF/TA progression by 1 year,[92] although this does not necessarily contradict the previous findings, as inflammation at 6 weeks likely mainly reflects the injury response after implantation stress.[39] It must be noted that i-IF/TA is very highly correlated with i-Banff[90] and with the severity of IF/TA,[82,93] that is, severely fibrotic areas contain more inflammatory cells. This is in agreement with the concept of fibrosis as a process that, beyond a certain threshold of severity, becomes self-perpetuating on a local level. Mannon et al[90] noted that i-IF/TA in the absence of i-Banff almost never occurred, whereas others found that every protocol biopsy with fibrosis and i-Banff also had a total-i score > 0.[74] Additional studies at different time points after transplantation will need to examine the precise prognostic value i-IF/TA after correction for i-Banff and fibrosis (which not all studies performed).

Fourth, what types of immune cells are present? In routine evaluation of renal allograft pathology, no differentiation is made between the various mononuclear cells (T cells, B cells, natural killer cells, dendritic cells and monocytes/macrophages) so they all contribute to the scoring of inflammation. Even though T cells are generally the most abundant infiltrating cells, histologically similar infiltrates may be composed of very different types of immune cells. In general, severe acute rejections are more heterogeneous[15,94,95] and infiltrates with a higher proportion (or activity) of immune cells that are not "regular" T cells seem to portend a worse prognosis. There is convincing evidence regarding the negative prognostic value of natural killer cells (due to a strong association with AMR),[96] dendritic cells[97,98] and macrophages.[99-101] Macrophage infiltration/activation has been reported to be more pronounced in severe compared with mild and subclinical acute rejections[99,102] and to correlate with tubular dysfunction, chronic histological damage as well as with concurrent and future renal dysfunction,[99-101] although this is not a consistent finding.[103] The prognostic relevance of high B- and plasma cell infiltration in adults is unclear.[103-106] Their presence and activity are strongly related to post transplant time,[107-109] which may confound their relationship with graft outcome: late (often nonadherence) rejections often have a humoral component and have a worse prognosis compared with early rejections.[110] The prognostic value of the T cell subsets of FOXP3 expressing regulatory T (Treg) cells in infiltrates is also unclear. Treg cells are crucial for containing inflammation, maintaining self- and donor-specific tolerance and play a central role in most tolerance inducing regimens in rodent models of allogeneic transplantation.[111] However, FOXP3 may also be transiently expressed by activated CD4 cells that have no suppressor activity.[112] Some reports have indicated that FOXP3 expression mainly mirrors the general degree of inflammation and FOXP3 expression in acute rejections carries no prognostic benefit.[113,114] Others have reported that high urinary FOXP3 mRNA during acute rejections predicted better outcome,[115] that there were proportionally more Treg cells in subclinical versus clinical rejection (compatible with successful damage control)[116,117] and that their presence predicted better long-term renal outcome.[117-121] Most studies showing a benefit of Treg cells used immunohistochemistry to quantify their presence as it has been argued that mRNA may be too sensitive.[117] Treg cells are also more common in mammalian target of rapamycin inhibitor–based regimens, which may partly confound their association with better estimated glomerular filtration rate (eGFR).[118,120] Finally, not only the infiltrate but also properties of the graft microenvironment play a role. For example, tubular cells overexpressing protease inhibitor 9 may be better protected against the action of cytotoxic T cells.[122]

Infections

Polyomavirus-associated nephropathy (PVAN), when untreated, results in rapid accumulation of IF/TA.[123] Even with adequate reduction of immunosuppression, a history of PVAN has been associated with higher degrees of IF/TA in subsequent protocol biopsies[21,50,75] and increases the risk of graft loss.[26] When PVAN is diagnosed early through BK viremia screening and/or protocol biopsy programs, however, IF/TA is typically less severe at diagnosis.[124] Furthermore, prompt reduction of immunosuppression in case of BK viremia seems to prevent further accumulation of IF/TA in sequential biopsies[125] and is associated with excellent intermediate-term outcomes.[126] In summary, the chronic lesions present at the time of PVAN diagnosis are irreversible, but early detection and correct management likely prevent further structural and functional decline.

Other viral infections including human herpesvirus 6/7, Epstein-Barr virus and particularly cytomegalovirus (CMV) have been associated with more severe IF/TA in concurrent [127] and subsequent biopsies,[128] as well as accelerated eGFR decline.[49,129] However, causality has not been firmly established as CMV viremia is also strongly related to poor intrinsic graft quality.[130] In a large retrospective study, patients who developed a CMV infection already had higher IF/TA on early protocol biopsies that were often performed before the CMV infection.[131] Furthermore, occurrence of CMV did not predict future IF/TA progression or graft loss.

Immunosuppressive Therapy

CNIs have acute nephrotoxic effects, primarily resulting from hemodynamic alterations (vasoconstriction of the afferent arterioles) and reversible tubular dysfunction.[132] Long-term exposure to CNIs, on the other hand, leads to irreversible damage to all compartments of the kidney.[133,134] CNIs mediate this chronic nephrotoxicity through a variety of mechanisms: chronic vasoconstriction and arteriolar narrowing result in persistent local hypoxia; stimulation of reactive oxygen species production[135,136] and chronic renin-angiotensin-aldosterone system (RAAS) stimulation, which has profibrotic effects.[137] CNIs also seem to have direct cytotoxic effects on tubular cells, induce EMT-like changes[138,139] and stimulate TGF-β production.[140,141] Although the nephrotoxic effects of CNIs are well established, the morphological changes typically associated with their long-term use (IF/TA, *de novo* arteriolar hyalinosis, glomerular capsular fibrosis, glomerulosclerosis and tubular microcalcifications) are all nonspecific.[132] As a result, how much the toxic and direct profibrotic effects of CNIs contribute to IF/TA accumulation remains unclear. Evidence regarding the effect of various CNI sparing/avoiding regimens on graft histology is discussed under "Therapeutic strategies to minimize progression of fibrosis".

Genetic Factors

Genetically determined variation in genes relevant to alloimmunity, injury response, drug metabolism and fibrosis could have an influence on graft prognosis. For example, 1 study reported better long-term renal allograft outcome in recipients carrying the complement C3 slow/slow allotypes who received a fast/slow or fast/fast kidney.[142] In the context of allograft fibrosis, genetic polymorphisms in 2 genes are specifically worth mentioning: *ABCB1* (also *MDR1*, coding for P-glycoprotein) and *CAV1* (coding for caveolin-1). P-glycoprotein is a wide-substrate efflux pump that is present on many epithelia including intestine, bile ducts and kidney tubules. It limits the intestinal absorption and facilitates renal elimination of various compounds including toxins and xenobiotics like CNIs. Early studies identified a link between reduced expression of P-glycoprotein at the apical side of tubular cells and increased CNI nephrotoxicity in rat models[143] and human renal allografts,[144,145] presumably resulting from higher intracellular concentrations of CNIs and possibly their metabolites. On the other hand, animal models suggest that P-glycoprotein deficiency protects against renal injury, which may be related to its antiapoptotic properties.[146] The net effect of loss-of-function *ABCB1* SNPs (particularly *C3435T*[147]) on long-term renal function and histology remains unclear, as later studies have reported conflicting results.[37,148-152] Specifically, Moore et al found that the wild-type donor CC genotype was associated with an increased risk of graft failure in a large cohort of (mainly CsA-treated) renal recipients. Bloch et al similarly reported that the loss-of-function T genotype independently predicted less fibrosis and less evidence of EMT on 3-month protocol biopsies in 140 Tac-treated patients. In contrast, in a protocol biopsy study of 252 Tac-treated patients by Naesens et al, a combined donor-recipient TT genotype independently predicted more severe IF/TA and worse renal function in the first 3 years after transplantation.

Caveolin-1, the primary structural component of caveolae, has antifibrotic properties related to its role in internalizing the TGF-β receptor.[153] Presence of the donor AA genotype for the rs4730751 single nucleotide polymorphism (SNP) in CAV1 independently predicted graft failure in 2 large cohorts of renal recipients.[154] No protocol biopsies were performed, but 155/184 of failed grafts had a late for-cause biopsy. The incidence of IF/TA was higher in the AA group (59% vs 26%).

Therapeutic Strategies to Minimize Progression of Fibrosis

Because of the multifactorial etiology and nonspecific nature of fibrosis, all interventions aimed at minimizing damage to the allograft (such as controlling hypertension and hyperglycemia, optimal matching, reducing ischemia time and early recognition of PVAN) can be considered "antifibrotic" We focus on a few strategies that potentially interfere directly with major fibrotic pathways.

CNI SPARING/AVOIDING REGIMENS

As mentioned earlier, retrospective reports indicate that IF/TA severity is lower with modern (low-dose) Tac-based regimens compared with older (high-dose) CsA-based regimens, but other differences between the transplant eras could contribute to these observations (eg, screening for PVAN, better matching and preservation techniques, differences in induction regimens). Where possible, we will focus on the best available evidence: randomized controlled trials (RCTs) comparing different immunosuppressive regimens in which protocol biopsies were performed.

Belatacept

In the BENEFIT study of belatacept versus CsA, both belatacept arms had better renal function and lower prevalence

of (mainly mild) IF/TA on 1-year protocol biopsies (20-29%) compared with the CsA arm (44%).[155] Microarray analysis of a small subgroup of 1-year biopsies from the BENEFIT and BENEFIT-EXT showed that the CsA group was enriched for gene sets associated with fibrosis and chronic allograft injury.[156] Comparative data with Tac in that regard are currently lacking.

CsA versus Tac

An RCT of CsA versus Tac (with high CNI target trough levels and use of azathioprine only for DCD kidneys) reported more fibrosis in the CsA group at 1 year, despite similar rates of acute rejection.[157] However, 2 RCTs of Tac versus CsA using modern regimens (lower CNI dose, basiliximab induction, MMF and steroids) could not confirm this.[24,64]

Mammalian Target of Rapamycin Inhibitors

Two RCTs have shown that, in renal recipients treated with CsA-sirolimus(SRL)-steroids, stopping CsA at month 3 results in less IF/TA and better renal function at 1 and 3 years.[158,159] However, this may be related to the fact that SRL aggravates the nephrotoxicity of CsA by increasing local tissue concentrations, an effect that is less pronounced for the combination Tac-SRL.[160,161] A third study showed that Tac-SRL and CsA-SRL resulted in lower rates of clinical and SCR, as well as significantly less IF/TA accumulation over 5 years compared with Tac-MMF or CsA-MMF.[162] Early steroid withdrawal in all patients, high CNI target trough levels in MMF-treated patients and high rates of DGF limit the generalizability of these findings. There is no consistent evidence that substituting SRL for a CNI or using Tac-SRL combinations is beneficial for the intermediate-term histological evolution of the graft.[6,163-165] Finally, for Tac-MMF versus SRL-MMF, 1 trial reported higher rates of subclinical inflammation and moderate-severe IF/TA in the Tac-MMF group at 2 years (steroids were avoided),[166] although a second study found no difference in 1-year IF/TA.[167]

Steroid Minimization

Early steroid withdrawal and steroid avoidance have been linked with an increased risk of mild rejections in CsA-based regimens,[168] which could theoretically accelerate IF/TA progression. This has, however, not been assessed with protocol biopsy studies. For Tac-based regimens, early steroid withdrawal/avoidance is not associated with differences in the incidence of clinical rejection, SCR or the accumulation of IF/TA.[43,169,170]

CNI Exposure

The Symphony study established that the combination of low-dose Tac, MMF, steroids and daclizumab resulted in lower rates of acute rejection, better graft survival and better graft function at 1 and 3 years after transplantation compared with low-dose CsA, standard-dose CsA or low-dose SRL-based immunosuppression.[171,172] The impact of CNI exposure on graft histology is not as clear. Studies have reported both high and low CsA exposure to be an independent predictor of IF/TA accumulation by 1 to 2 years.[53,173,174] Similarly, Tac exposure is not consistently related to progression of IF/TA. Results of retrospective studies vary from no association[30,45] to more IF/TA with low trough levels[56] and less IF/TA with low trough levels.[175] We recently found that high Tac exposure did not predict IF/TA or progression of chronicity score on 2-year protocol biopsies.[45] Rather, high intrapatient variability in Tac trough levels was an independent predictor of increase in chronicity score, which has also been noted for CsA.[174,176] This seems logical, as strongly fluctuating trough levels can lead to periods of overexposure (which might be profibrotic) as well as underexposure (which can result in surges in alloimmune activation that are also profibrotic). Intrapatient variability is strongly related to nonadherence, which itself has also been shown to predict IF/TA.[177] We can conclude that there is insufficient evidence to suggest that, within the range of Tac trough levels commonly used today, any particular trough level target is associated with better preservation of renal histology in the short term. More generally, no immunosuppressive regimen has demonstrated better evolution of histology compared with the current standard-of-care, Tac-based triple therapy.

BLOCKADE OF THE RAAS

The RAAS has potent profibrotic effects, in part through renin-, angiotensin II- and aldosterone-mediated activation of the TGF-β system.[178] In renal transplantation, however, it is not well established whether RAAS blockade slows the progression of fibrosis or improves graft or patient survival. Large retrospective analyses provided conflicting results regarding the effect of angiotensin converting enzyme inhibitors (ACE-I) or angiotensin receptor blockers (ARB) on graft loss and mortality,[179-182] and a recent RCT in proteinuric renal recipients showed no benefit of ramipril on a combined endpoint of doubling of serum creatinine, end-stage renal disease (ESRD) or death after 4 years of treatment.[183]

With regard to histology, Rush et al performed a post-hoc analysis of an RCT in which renal recipients were randomized to standard care or a protocol biopsy program, with treatment of SCR only in the protocol biopsy arm. Use of ACE-I or ARB at any time in a subgroup of patients independently predicted less IF/TA progression between months 6 and 24.[33] In nonalbuminuric, nontransplanted type I diabetics randomized to losartan, enalapril or placebo, there was no functional renal benefit and no difference in the increase in interstitial fractional volume at 5 years.[184] Finally, an RCT of losartan versus placebo in renal recipients demonstrated that the odds ratio for doubling of fraction of renal cortical volume occupied by interstitium between baseline and 5 years was 0.39 for losartan, although the effect was only borderline significant ($P = 0.08$).[185] To summarize: although ACE-I and ARB have antifibrotic properties in addition to reducing proteinuria and hyperfiltration, whether this translates into long-term structural and functional benefits in renal transplantation has yet to be established. The available prospective studies showed no convincing benefit, possibly because they mainly included low-risk patients and did not always correct for other profibrotic drivers such as inflammation.

TREATMENT OF SCR

Most transplant centers that perform protocol biopsies treat subclinical acute rejections with high-dose steroids, as they would a clinical acute rejection. The approach to subclinical borderline rejection is more variable: often left

untreated, decided case-by-case in some centers and treated almost systematically in others. Indeed, the belief that treating SCR has long-term benefits is arguably the strongest clinical argument for having a protocol biopsy program in the first place. The evidence, however, is not ironclad. A seminal study by Rush et al randomized renal recipients to undergo repeat protocol biopsies (with treatment of SCR) or no biopsies except at months 6 and 12 (blinded).[36] Patients were treated with high-dose CsA-azathioprine-steroids; almost none received induction therapy. The incidence of SCR was 30% to 40% in the first 2 months, and the biopsy group had lower IF/TA at 6 months, less late acute rejections, better renal function and better graft survival at 2 years. However, a follow-up study with very similar design in low-risk renal recipients treated with Tac-MMF-steroids (no induction), showed no benefit of treatment with regards 6-month IF/TA or renal function.[48] This was attributed to the very low overall incidence of SCR (4.6%). A retrospective analysis of patients treated with basiliximab-CNI-MMF-steroids showed that untreated SCR on 6-month protocol biopsies (incidence 7.4%) had no effect on the severity of 1-year fibrosis.[42] Finally, another prospective study randomized renal recipients, almost all without previous induction therapy, to undergo protocol biopsies at 1 and 3 months. Treatment of SCR (incidence 12-17.3%) was associated with better renal function at 1 year.[186] Borderline rejections had a similar outcome to no rejection. In summary, treating SCR has only proven beneficial to short-term structural and functional outcome in studies that did not use induction therapy, with an incidence of SCR greater than 10% to 15% in the first 3 months after transplantation. Long-term follow-up data are not available. Given the extensive evidence regarding the detrimental effect of SCR, we believe treatment is justifiable. However, the effect is not likely to be dramatic, and proving that a "SCR treatment" strategy is beneficial to a low-risk population (Tac-based triple therapy and systematic induction) with a low incidence of SCR would likely require an exceedingly large RCT. Given that the rate of serious complications from protocol biopsies is <0.5%[187] and the rate of SCR in our center is 8% to 10% at 3 months, we perform protocol biopsies as we believe that the possible benefits of performing (treating early inflammation) outweigh the risks in our particular setting. Additionally, SCR is not the only actionable finding in protocol biopsies, as patients without significant inflammation at 3 months are weaned off steroids. There is, however, no evidence to support the latter strategy. There may also be a "window of opportunity" for protocol biopsies between 1 and 4 months, as much subclinical

TABLE 1.

Studies of renal antifibrotic interventions in humans

Setting	Molecular target	Intervention	Study type	Results	Ref
FSGS	TGF-β	Pirfenidone	Open label, nonrandomized	25% slower decline in eGFR	190
	TGF-β	Fresolimumab (antibody)	Phase I	Well tolerated. No studies regarding efficacy	191
	CTGF	FG-3019 (antibody)	Phase I	Early termination	192
Diabetic nephropathy	TGF-β	Pirfenidone	Phase III	Improvement in eGFR after 1 year (only in 1200 mg/d arm) vs decrease in placebo group. No difference in proteinuria.	193
	TGF-β	LY2382770 (antibody)	Phase II	Terminated early: lack of efficacy on eGFR	194
	CTGF	FG-3019 (antibody)	Phase I	Reduction in albuminuria	195
	CTGF	FG-3019 (antibody)	Phase II	Early termination	196
	Oxidative stress	GKT137831(NOX1/4 inhibitor)	Phase II	Completed, not yet published	197
	Oxidative stress	Pyridoxamine (inhibits oxidative stress and AGE formation)	Phase III	Ongoing	198
	Chemotaxis	CCX140-B (CCR2 blocker)	Phase II	Reduction in albuminuria	199
	Chemotaxis	Bindarit (inhibitor of CCL2, -7, and -8)	Phase II	Completed, not yet published	200
	Prostaglandins	Pentoxifylline	Multiple studies	Modest reductions in albuminuria and rate of eGFR decline. RCT powered to detect difference in serum creatinine doubling or ESRD occurrence is ongoing.[201]	202,203
	Prostaglandins	PF-00489791 (PDE5 inhibitor)	Phase III	Reduction in albuminuria	204
	Prostaglandins	CTP-499 (multispecific PDE inhibitor)	Phase I	Well tolerated. No data on efficacy.	205
	Prostaglandins	Beraprost (oral prostacyclin analogue)	Phase II	No difference in slope of 1/SCr after 28 weeks	206
	Endothelin	Avosentan (endothelin A receptor antagonist)	Phase III	Early termination: reduction in proteinuria but increased mortality	207
	Endothelin	Atrasentan (endothelin A receptor antagonist)	Phase III	Ongoing	208
	JAK pathway	Baricitinib (JAK1 and -2 inhibitor)	Phase III	Completed, not yet published	209
Prevention of cardiac surgery-related acute kidney injury	BMP	Thr-184	Phase II	Completed, not yet published	210

Pirfenidone has multiple mechanisms, 1 of which is blocking the TGF-β promoter; Pentoxifylline is a nonspecific PDE inhibitor, inhibits Smad3/4 and CTGF and has anti-inflammatory properties.
AGE, advanced glycation end product; BMP, bone morphogenetic protein; CCR, C-C chemokine receptor type 2; FSGS, focal segmental glomerulosclerosis; JAK, Janus kinase; NOX, NADPH oxidase; PDE: phosphodiesterase.

inflammation will have subsided spontaneously by 6 months (which does not mean it cannot have damaged the graft during that period). There are no data on whether subclinical borderline rejections should be treated. Even if it is assumed that many borderline rejections are predominantly on the mild end of a continuum of alloimmune injury, the potential benefit of treating them is likely to be even smaller.

VITAMIN D SUPPLEMENTATION

Chronic kidney disease models suggest that vitamin D may protect against inflammation, EMT and fibrosis.[188] However, in a retrospective analysis comparing 64 renal recipients receiving vitamin D with historic controls that did not take vitamin D, there was no difference in evolution of eGFR or IF/TA between months 3 and 12 after transplantation.[189] It is clear that a prospective study with longer histologic follow-up would be needed to definitively settle this issue.

POTENTIAL FUTURE STRATEGIES

Novel antifibrotic interventions tested in human renal disease are summarized in Table 1. Many more molecules have shown promise in preclinical models; these are reviewed in detail elsewhere.[211-214] The preclinical pipeline does not seem to be a problem, and neither is there a lack of interest from industry in antifibrotic drugs per se. Rather, bringing specific antifibrotic interventions to the clinic has proved very challenging, even in prototypical fibrotic disease states such as idiopathic pulmonary fibrosis (IPF) and systemic sclerosis, where these drugs are potentially lifesaving.[215,216] This could have several reasons. Many profibrotic molecules also have beneficial effects, depending on the context. A typical example is the anti-inflammatory and antineoplastic effects of TGF-β, which could limit the benefits of systemic TGF-β antagonism.[217,218] Downstream mediators of TGF-β, such as CTGF and the tyrosine kinase ligands (epidermal growth factor [EGF], platelet-derived growth factor [PDGF], vascular endothelial growth factor), are less pleiotropic but these pathways are at least partly redundant.[219] Blocking several profibrotic pathways in parallel may be the best way to avoid escape phenomena, but could increase the probability of adverse effects. Finally, a crucial question in any fibrotic disease, and renal allograft pathology in particular, is how much can be gained from halting maladaptive tissue repair mechanisms and excessive matrix deposition if there is continuing underlying epithelial injury and inflammation. On the other hand, there are numerous factors that directly stimulate TGF-β (eg, CNIs, angiotensin II, hyperglycemia) and they might respond well to specific targeting of this pathway.

Apart from a pilot study of renal recipients with chronic allograft nephropathy in which pentoxifylline reduced proteinuria in some patients,[220] none of these drugs have been tested in renal transplantation. It is likely that most renal antifibrotic therapies will first be tested in diabetic nephropathy, as this is a large market with a homogeneous and strongly TGF-β-dependent disease process.[221] The pleiotropic molecule pentoxifylline seems to reduce albuminuria and eGFR decline in diabetic nephropathy, although the results of a RCT powered to detect differences in ESRD occurrence and serum creatinine doubling are awaited.[201] Pirfenidone, which is licensed for IPF, could be promising for diabetic nephropathy[193] but no large RCT has currently been undertaken.

Overall, there are very few studies of novel antifibrotic drugs being performed in the setting of renal disease. Studies using the anti-CTGF monoclonal antibody FG-3019 for focal segmental glomerulosclerosis (FSGS)[192] and diabetic nephropathy[196] were terminated early. The focus seems to have shifted to other nonrenal indications including IPF[222] and oncology,[223,224] which is true for several other pipeline drugs. Particularly promising are several tyrosine kinase inhibitors (including EGF and PDGF antagonists), which are very well established antifibrotic strategies in preclinical models[211] and have been studied extensively in human oncology, but have also never been tested in human (nonmalignant) renal disease. This may be partly related to the fact that side effect profiles acceptable in an oncological context will often be perceived as problematic for long-term use in transplant recipients. The multi-target tyrosine kinase inhibitor nintedanib is modestly effective and licensed for IPF,[215] but to our knowledge, no trials of nintedanib or any other tyrosine kinase inhibitor are currently being performed in renal disease.

CONCLUDING REMARKS

Like all organs, the renal allograft responds to a wide variety of injurious stimuli by highly conserved and stereotypical injury-response mechanisms that result in a limited number of chronic histological lesions. IF/TA is usually a dominant feature of chronic damage and carries major prognostic implications, but it is particularly nonspecific. The difficulty in tracing these generic histological features back to their underlying disease processes remains a key challenge in transplantation. As our knowledge regarding specific disease processes and the maladaptive tissue repair mechanisms that exacerbate their detrimental effects expands, novel therapeutic strategies are likely to emerge that prolong graft survival and improve quality of life.

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