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## TWEAK and the progression of renal disease: clinical translation

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### ABSTRACT

Tumour necrosis factor-like weak inducer of apoptosis (TWEAK) activates the fibroblast growth factor-inducible-14 (Fn14) receptor. TWEAK has actions on intrinsic kidney cells and on inflammatory cells of potential pathophysiological relevance. The effects of TWEAK in tubular cells have been explored in most detail. In cultured murine tubular cells TWEAK induces the expression of inflammatory cytokines, downregulates the expression of Klotho, is mitogenic, and in the presence of sensitizing agents promotes apoptosis. Similar actions were observed on glomerular mesangial cells. *In vivo* TWEAK actions on healthy kidneys mimic cell culture observations.

Increased expression of TWEAK and Fn14 was reported in human and experimental acute and chronic kidney injury. The role of TWEAK/Fn14 in kidney injury has been demonstrated in non-inflammatory compensatory renal growth, acute kidney injury and chronic kidney disease of immune and non-immune origin, including hyperlipidaemic nephropathy, lupus nephritis (LN) and anti-GBM nephritis. The nephroprotective effect of TWEAK or Fn14 targeting in immune-mediated kidney injury is the result of protection from TWEAK-induced injury of renal

intrinsic cells, not from interference with the immune response.

A phase I dose-ranging clinical trial demonstrated the safety of anti-TWEAK antibodies in humans. A phase II randomized placebo-controlled clinical trial exploring the efficacy, safety and tolerability of neutralizing anti-TWEAK antibodies as a tissue protection strategy in LN is ongoing. The eventual success of this trial may expand the range of kidney diseases in which TWEAK targeting should be explored.

**Keywords:** apoptosis, clinical trials, fibrosis, inflammation, necroptosis, proteinuria

### INTRODUCTION

Tumour necrosis factor-like weak inducer of apoptosis [TWEAK, Apo3L, tumour necrosis factor superfamily (TNFSF)12] is a cytokine that belongs to the TNFSF that activates Fibroblast growth factor-inducible-14 (Fn14, TWEAK receptor, TNFRSF12A, CD266), a TNF receptor superfamily (TNFRSF) protein [1–4]. In recent years, evidence has accumulated supporting a role for TWEAK activation of intrinsic renal cell Fn14 receptors in the pathogenesis of acute and chronic kidney injury, glomerular and tubulointerstitial damage and non-immune and

immune-mediated kidney disease. This has led to the launch of a clinical trial of neutralizing anti-TWEAK antibodies in human lupus nephritis (LN). We now review the pathophysiology of TWEAK/Fn14 signalling and the preclinical and clinical data on the involvement of this system in kidney disease (Figure 1).

## TWEAK AND ITS RECEPTOR

The human TWEAK gene encodes a type II transmembrane glycoprotein of 249 amino acids. The C-terminal extracellular TNF homology domain participates in self-trimerization and receptor binding [5], and contains two furin cleavage sites. Proteolytic processing of membrane TWEAK generates soluble TWEAK (156-amino acids) [5, 6]. The N-terminal cytoplasmic domain has a potential protein kinase C phosphorylation site, and a furin recognition site, suggesting that full-length TWEAK can be processed intracellularly [5, 7, 8]. Moreover, TWEAK has nuclear localization sequences and may localize to nuclei, but the biological significance is unknown [8]. TWEAK is broadly expressed. In the kidneys, both resident kidney cells and infiltrating leucocytes express TWEAK [1, 9–11].

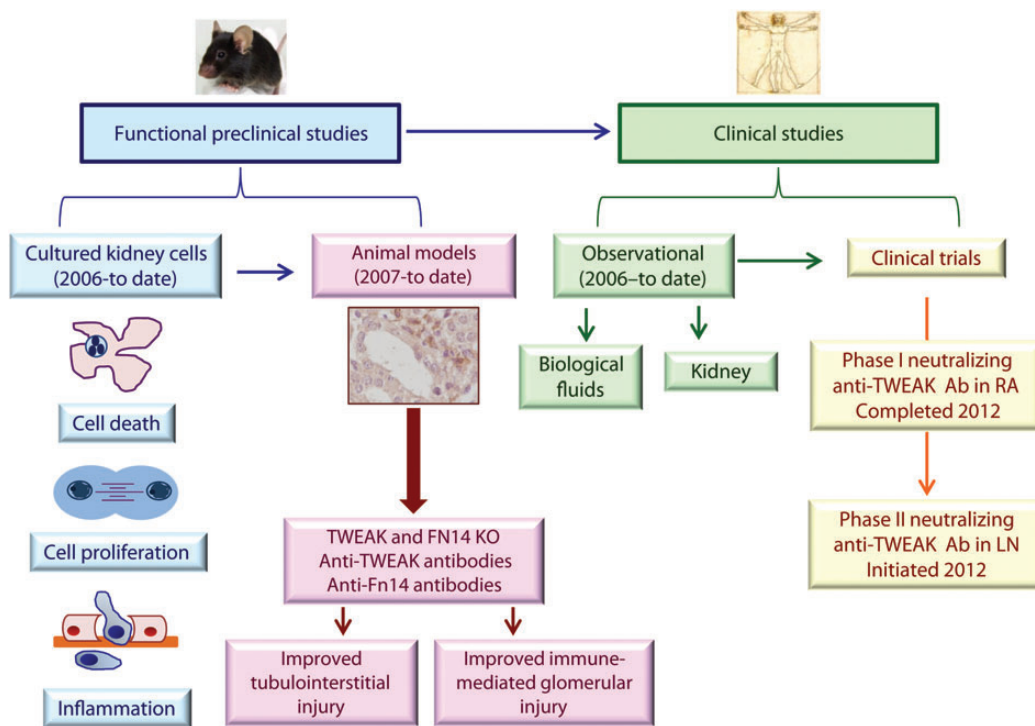
Fn14 is the only characterized signal-transducing TWEAK receptor. Both soluble and membrane TWEAK activate Fn14 [6, 12]. Fn14 was originally described as an immediate-early response gene regulated by growth factors in fibroblasts [13]. The human Fn14 gene encodes a type I transmembrane

protein (129 amino acids) that is processed into a mature protein (102 amino acids). The extracellular domain (53 amino acids) contains a cystein-rich domain necessary for TWEAK binding [14, 15]. In contrast to many TNFRSF members, the intracellular domain (29 amino acids) of Fn14 lacks a death domain. However, it contains TNFR-associated factor (TRAF)-binding sites [16]. TWEAK can also bind the monocyte/macrophage scavenger receptor CD163. TWEAK binding to CD163 results in TWEAK internalization, but not in signalling. This suggests that CD163 behaves as a TWEAK scavenger [17, 18].

In healthy tissues Fn14 expression is low [13]. However, cellular Fn14 levels are increased in response to stress or injury [19]. In the kidney tubular cells, mesangial cells and podocytes express Fn14 [20, 21]. Moreover, renal infiltrating cells, such as macrophages, may express Fn14 and CD163 [22, 23].

TWEAK regulates processes with potential physiopathological relevance for kidney injury such as cell death, proliferation, differentiation, migration, inflammation, neoangiogenesis and tissue regeneration. Fn14 binding to TWEAK leads to TRAF2 and TRAF5 recruitment and activation of intracellular signalling pathways [24–26]. The specific signalling pathways activated by TWEAK/Fn14 system and the outcome of TWEAK binding depend on cell type, cell state and the microenvironment [19].

TWEAK has a potential role in injury of different organs, including the central nervous system, liver, gut, the vasculature, skeletal muscle, heart and kidneys [26–28].



**FIGURE 1:** Progress in the study of TWEAK as a therapeutic target in kidney injury. Understanding the role and regulation of TWEAK and Fn14 in kidney injury has progressed very rapidly since the early functional studies published in 2006 to clinical trials in kidney disease launched in 2012. RA, rheumatoid arthritis; LN, lupus nephritis; Ab, antibody.

## TWEAK HAS MITOGENIC, LETHAL AND PROINFLAMMATORY ACTIONS ON CULTURED RENAL CELLS

TWEAK has actions on intrinsic kidney cells and on inflammatory cells of potential pathophysiological relevance (Figure 2).

Several intrinsic renal cells respond to TWEAK through Fn14 receptor signalling. The effects of TWEAK in tubular cells have been explored in most detail. In contrast, there is very little information on TWEAK and podocytes or kidney fibroblasts.

In cultured murine tubular cells TWEAK activates several signalling pathways. Thus, TWEAK activates mitogen-activated protein kinases (MAPK) such as extracellular signal-regulated kinase1/2 (ERK1/2) and p38 MAPK, phosphoinositide 3-kinase/Akt, JAK2 kinase and canonical or non-canonical NFκB activation [29–32]. As a result of canonical NFκB pathway activation and p65/RelA nuclear translocation, TWEAK induces the expression of inflammatory cytokines, such as MCP-1, IL-6, RANTES and CXCL16 and downregulates the expression of Klotho [30, 33, 34]. TWEAK also activates the non-canonical NFκB pathway, inducing the expression of CCL21 and CCL19 [32]. The proinflammatory effect of TWEAK on cultured tubular cells is enhanced by the simultaneous presence in the cell environment of additional inflammatory mediators, such as CXCL16, TNFα and interferon-γ [29, 33].

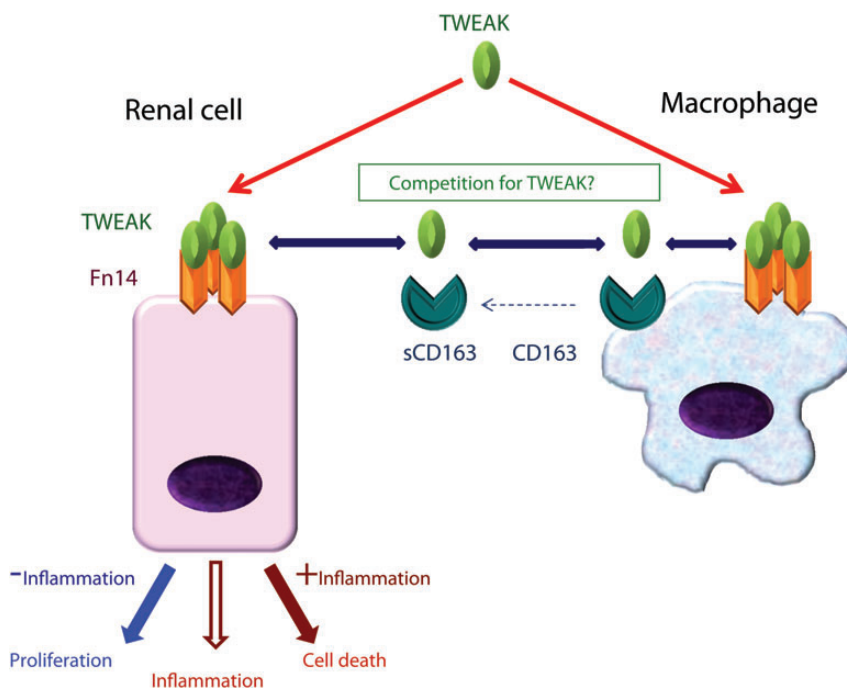
TWEAK has a proliferative effect in quiescent renal tubular cells [31]. This effect is modulated by the cell microenvironment and is increased by factors present in serum that enhance Fn14 expression [31]. TWEAK-induced tubular cell

proliferation requires ERK1/2, p38 MAPK, phosphoinositide 3-kinase/Akt and NFκB in a manner that disruption of one of them prevents proliferation [31].

TWEAK alone does not promote renal tubular cell apoptosis. However, co-stimulation with sensitizing agents present in injured kidneys, such as TNFα and interferon-γ, leads to tubular cell apoptosis [20]. Apoptosis induced by TWEAK/TNFα/interferon-γ recruits the mitochondrial apoptosis pathway [20]. Both the time course and the response to caspase inhibitors differ from TNFα-induced apoptosis in the same cells [20, 35]. The combination of cytokines accelerates cell death. Caspase inhibition prevents features of TWEAK/TNFα/interferon-γ-induced apoptosis but increases overall cell death through a reactive oxygen species-dependent necrotic pathway [20]. Studies are underway to further assign this form of cell death to one of the known regulated necrosis forms of death [36].

TWEAK also induced the NFκB-dependent expression of inflammatory mediators, such as MCP-1, RANTES, CXCL10 and CXCL1 in human glomerular mesangial cells [21, 37]. In human glomerular mesangial cells, TWEAK-induced more prolonged NFκB p65/RelA phosphorylation than TNFα [21]. TWEAK promoted proliferation in humans but not in murine mesangial cells [37]. TWEAK alone did not induce human mesangial cell death, but the combination of TWEAK and interferon-γ increased apoptosis [21, 37].

Podocytes are also TWEAK responsive. TWEAK stimulation of human and murine podocytes induces a proinflammatory response via the activation of the NFκB signalling pathway [21] (Sanchez-Niño *et al.*, *JASN Abstr Suppl* 2011; 656).



**FIGURE 2:** TWEAK and TWEAK receptors. TWEAK binds Fn14 and transduces proinflammatory signals in kidney cells. In the absence of inflammation TWEAK may also induce cell proliferation, while in the presence of inflammation it promotes cell death. Chimeric mouse studies have established a key role of renal cell Fn14 in kidney injury. Macrophages also express Fn14 and may respond to TWEAK. In addition, they express CD163, which may be released in a soluble form and scavenges TWEAK. The precise role of CD163 in TWEAK dynamics is not fully understood. There is not enough information on the site of Fn14 expression in polarized epithelia.

## TWEAK AND INFLAMMATORY CELLS

TWEAK and Fn14 have been reported to be expressed by NK cells, macrophages, dendritic cells and Th17 cells [38, 39], but not by T and B cells [38, 40, 41]. Fn14 was not detected in peripheral blood mononuclear cells [42]. In addition, monocytes and macrophages express CD163 that can scavenge TWEAK [17]. A variety of actions of TWEAK on leucocytes have been reported. In human NK cells and macrophages, TWEAK induced prolonged p65 phosphorylation, inhibited STAT-1 and repressed interferon- $\gamma$  and IL-12 through HDAC-1 activation [38]. In human macrophage-like THP-1 cells, TWEAK induced the expression of inflammatory mediators, such as IL-6, MCP-1, IL-8 and MMP-9 [11]. In naïve murine T cells, TWEAK promoted Th17 differentiation [39]. In addition, TWEAK has been reported to contribute to autologous human monocyte death induced by lupus T cells [10] and to human H9 CD4 + T cell line death induced by monocyte-derived dendritic cells [43]. Fn14 KO mice had milder chronic colitis induced by a hapten. Reduced epithelial cell synthesis of thymic stromal lymphopoietin appeared to contribute to reduced inflammation, fibrosis and T helper 2-type immunity [44]. Adaptive immunity defects described in a TWEAK KO mouse strain [38] were not observed in TWEAK KO mice generated by other authors [19]. Thus, the overall contribution of leucocytes to TWEAK availability and the *in vivo* significance of TWEAK effects on leucocytes are unclear.

## IN VIVO TWEAK ACTIONS ON HEALTHY KIDNEYS MIMIC CELL CULTURE OBSERVATIONS

TWEAK actions *in vivo* in healthy kidneys have been explored in mice receiving a single intraperitoneal or intravenous recombinant TWEAK dose [21, 30–32, 37]. Systemic TWEAK-induced tubulointerstitial inflammation decreases kidney Klotho expression and promotes tubular cell proliferation within 4–24 h [31, 34]. TWEAK activated canonical (RelA) and non-canonical (RelB/p52) NF $\kappa$ B pathways in healthy kidney, resulting in early (4 h) RelA and delayed (24 h) RelB and p52 nuclear translocation in tubular cells [30, 32], and it increased total kidney MCP-1, IL-6, RANTES, CXCL16, CCL21, IP-10 and VCAM-1 expression [21, 30, 32, 33, 37]. Immunohistochemistry identified tubular cells as expressing inflammatory mediators and disclosed increased interstitial macrophages and CD3<sup>+</sup> cells [21, 30, 32, 33, 37].

Systemic injection of TWEAK also increased the number of proliferating kidney cells assessed as tubular PCNA<sup>+</sup> or Ki-67<sup>+</sup> cells or Ki-67<sup>+</sup> glomerular cells [21, 31]. However, at the times and doses tested TWEAK did not induce cell death in healthy mouse kidneys. This is an expected result since TWEAK-induced renal cell death requires a concomitant inflammatory microenvironment [20, 21, 37].

## TWEAK PROMOTES ACUTE AND CHRONIC KIDNEY DISEASE

The potential role of TWEAK and Fn14 in kidney injury has been explored in non-inflammatory compensatory renal growth, non-immune tubulointerstitial kidney injury and immune-mediated kidney injury (Table 1). However, the role of TWEAK in non-immune glomerular injury remains insufficiently studied.

### Non-inflammatory compensatory renal growth

Compensatory remnant kidney hypertrophy and hyperplasia follow unilateral nephrectomy. Clinical examples include nephrectomies for malignant tumour or living kidney donation and kidney transplant recipients [45]. Compensatory renal growth is thought to be a response to poorly characterized growth factors, including growth hormone [46]. TWEAK promotes remnant kidney growth following unilateral nephrectomy in mice [31]. Indeed, systemically administered TWEAK increased tubular cell proliferation in the remnant kidney, while TWEAK knockout mice had decreased remnant kidney tubular cell proliferation and kidney size. Tubular cell Fn14 expression was upregulated in remnant kidneys and may sensitize tubular cells to TWEAK-induced proliferation. In this regard, the remnant kidney expression of inflammatory cytokines, such as TNF $\alpha$  and interferon- $\gamma$ , was low [31]. Thus, compensatory kidney growth is a non-inflammatory model where the proliferative action of TWEAK on tubular cells can be fully appreciated *in vivo*, confirming the relevance of cell culture data. A role for TWEAK/Fn14 in tissue regeneration has been observed in other organs, including the liver [47] and skeletal muscle [48]. This effect of TWEAK/Fn14, observed in precursor cells in some organs [47, 48], may eventually be modulated to develop regenerative therapies in clinical situations characterized by an acute non-inflammatory loss of kidney mass or in tissue engineering.

### Non-immune kidney injury

Increased expression of TWEAK and Fn14 has been observed in human and experimental non-immune tubulointerstitial kidney injury [33, 49]. In addition, there is functional *in vivo* evidence for a role of TWEAK/Fn14 in acute kidney injury (AKI) and chronic kidney disease (CKD) of non-immune origin [30, 32–34, 50, 51].

Tubular cell Fn14 upregulation was observed within 24 h in experimental models of AKI induced by a folic acid overdose or by renal ischaemia–reperfusion injury (IRI). In folic acid-induced AKI, increased Fn14 expression persisted longer (up to 3 days) than in IRI (up to 1 day) [20, 49]. Kidney TWEAK was also increased in experimental renal injury, but to a lesser extent than Fn14 [20, 49]. Increased tubular Fn14 has also been observed in human ischaemic AKI and in acute or chronic human tubulointerstitial inflammation associated with glomerular injury or caused by acute tubulointerstitial nephritis [33, 49].

Functional studies in experimental models have characterized TWEAK/Fn14 as a therapeutic target in AKI. Targeting TWEAK with neutralizing anti-TWEAK antibodies, the use of



**Table 1. TWEAK targeting and kidney injury: animal models**

| Experimental model  | Target                        | Results   | References                           |
|---|-------------------------------|---|--------------------------------------|
| <b>Non-immune kidney injury</b>   |                               |   |                                      |
| Acute kidney injury (Folic acid)  | TWEAK                         | ↑Renal function<br>↓Interstitial inflammation<br>↓Tubular cell death<br>↓Tubular cell proliferation<br>↑Klotho                            | [30]<br>[31]<br>[32]<br>[33]<br>[34] |
| AKI-to-chronic injury (I/R)   | Fn14                          | ↑Renal function<br>↓Interstitial inflammation<br>↓Tubular cell death<br>↓Residual interstitial fibrosis                                   | [49]                                 |
| Chronic kidney injury (hyperlipidaemia-induced kidney injury in ApoE <sup>-/-</sup> mice) | TWEAK                         | ↓Inflammation   | [50]                                 |
| CKD: unilateral ureteral obstruction  | TWEAK                         | ↓Histological injury<br>decreased inflammation/fibrosis   | [51]                                 |
| <b>Immune injury</b>  |                               |   |                                      |
| SLE (cGVH model)  | TWEAK or Fn14                 | = Titres autoantibodies<br>↓Proteinuria<br>↓Inflammation<br>↓Glomerular IgG deposition  | [60]                                 |
| Nephrotoxic serum nephritis (NTN)   | Fn14 in haematopoietic cells  | = Initial nephritis   | [22]                                 |
|   | Fn14 in kidney resident cells | Delayed nephritis   | [22]                                 |
|   | Fn14                          | ↓Proteinuria<br>↓Histological renal injury  | [61]                                 |
|   | TWEAK                         | = Titres autoantibodies<br>↓IgG deposition<br>↓Histological renal injury<br>↓Proteinuria<br>↓Tubulointerstitial fibrosis<br>↓Inflammation | [61]                                 |
| <b>Non-inflammatory kidney regeneration</b>   |                               |   |                                      |
| Uninephrectomy-induced renal hyperplasia  | TWEAK                         | ↓Tubular cell proliferation   | [31]                                 |

SLE, systemic lupus erythematosus; cGVH model, chronic graft-versus-host model.

TWEAK knock-out mice or targeting Fn14 by blocking anti-Fn14 antibodies improved histological and functional features of AKI [1, 30–34, 49].

Better preserved renal function, histological tubular injury and kidney Klotho expression, as well as reduced chemokine expression, tubular cell apoptosis, compensatory tubular cell proliferation and interstitial leucocyte infiltration were observed in mice with folic acid-induced AKI treated with neutralizing anti-TWEAK antibodies or in TWEAK knock-out mice (TWEAK KO) [1, 30–34, 49].

Seven days after induction of AKI, renal function had recovered in control and TWEAK-targeted mice, suggesting that the decreased tubular cell proliferation was secondary to the lower initial injury and did not compromise recovery of kidney function [1].

Fn14 blockade in renal IRI decreased peak serum creatinine and urea (at 24 h), tubular injury, inflammation, apoptosis (at 24 h) and residual fibrosis (at 30 days) [49]. This study addressed the transition from AKI to CKD and indicated that protection from a transient insult by TWEAK targeting resulted in longer term benefits over renal fibrosis. However, it did not explore potential specific anti-fibrotic functions of TWEAK/Fn14 targeting, or the consequences of TWEAK/Fn14 targeting when the kidney insult is persistent. A recent report revealed that TWEAK KO mice were protected from kidney fibrosis resulting from persistent unilateral ureteral obstruction [51].

In hyperlipidaemic ApoE<sup>-/-</sup> mice with non-immune chronic kidney injury repeated TWEAK administration activated kidney cell NFκB and increased the severity of tubulointerstitial and glomerular injury and inflammation [50]. In contrast, neutralizing anti-TWEAK antibodies improved these parameters [50].

#### Immune-mediated glomerular injury

Lu *et al.* [52] examined the expression of TWEAK and Fn14 in kidney biopsies obtained from LN patients. As compared with controls, lupus patients had significantly higher glomerular and tubulointerstitial mRNA expression of both TWEAK and Fn14. Moreover, TWEAK expression was significantly higher in class V than in class IV nephritis, and was inversely correlated with the activity index but not SLEDAI score, anti-DNA antibody titres, or complement levels. In a follow-up study, Lu *et al.* [53] looked at changes in TWEAK and Fn14 expression between an initial and a repeat kidney biopsy, and found that tubulointerstitial TWEAK, glomerular TWEAK and glomerular Fn14 increased when the histology evolved from membranous to proliferative nephritis. In contrast, glomerular Fn14 decreased when the pattern changed from proliferative to membranous histopathology. Glomerular Fn14 and tubulointerstitial TWEAK correlated with the renal histology index. Although the findings in these particular studies are not entirely concordant, the significant changes found in the expression of this pathway, and between

histological classes, do support a role for TWEAK and Fn14 in LN pathogenesis. Finally, Zhi-Chun *et al.* [54] reported that patients with LN also display significantly higher levels of TWEAK in PBMC, although this finding was not replicated [55].

We [56] and others [55] found that serum TWEAK levels cannot distinguish between lupus patients with and without nephritis. Since inflammatory mediators produced locally in the kidney are important in pathogenesis and urinary levels can closely reflect renal production, we investigated the use of urinary TWEAK (uTWEAK) as a possible LN biomarker. In a cross-sectional study, we found that uTWEAK levels were significantly higher in patients with renal involvement and correlated with the renal SLEDAI score (rSLEDAI). Moreover, renal flares were associated with significantly higher uTWEAK levels. Significant positive correlations were found between uTWEAK and serum anti-DNA antibody titres and urinary MCP-1, while a negative correlation was found with serum C3 and C4 [57]. We confirmed the potential of uTWEAK as an LN biomarker in a subsequent study as well [56]. uTWEAK levels distinguished between lupus patients with nephritis and lupus patients without nephritis, healthy individuals and RA controls, and correlated with the rSLEDAI. In lupus patients followed longitudinally, a significant association was found between uTWEAK levels and renal disease activity over time, with uTWEAK levels peaking at the time of flare. El-shehaby *et al.* [58] similarly found significantly higher uTWEAK in LN when compared with lupus patients without nephritis or healthy controls. While uTWEAK levels did not differentiate between the various histopathological classes in this study, they did show a good correlation with rSLEDAI. Finally, Xuejing *et al.* [59] found a similar association between uTWEAK and active LN. Furthermore, significant correlations were found between uTWEAK with glomerular and tubulointerstitial activity indices, but not with chronicity.

Based upon the proinflammatory effects of TWEAK on murine and resident kidney cells, the preliminary studies in humans, and an observation that kidney (but not splenic) expression of TWEAK and Fn14 increases with disease progression in a spontaneous murine lupus model (the MRL-lpr/lpr strain) [Xia *et al.*, *Arthritis Rheum* 63(10) (Suppl.): S212–S213 (abstract)], we hypothesized that TWEAK/Fn14 interactions are instrumental in LN pathogenesis. Furthermore, this pathway may be a novel target for therapeutic intervention. To examine this question, we used two models of antibody mediated nephritis that share many similarities with spontaneous disease. In the cGVH model [60], injection of MHC-mismatched bm12 splenocytes into non-autoimmune C57Bl6 mice induces an alloreactive reaction. This immune response

promotes the activation of recipient B cells, with production of autoantibodies and the development of nephritis. In the second animal model, nephrotoxic serum nephritis, passive transfer of preformed rabbit anti-mouse glomerular antibodies induces a necrotizing proliferative glomerulonephritis that is very similar histologically to LN [61]. Via a genetic approach, we found that both Fn14 deficient C57Bl/6 recipients of bm12 splenocytes, as well as Fn14 knockout 129/SvJ mice injected with nephrotoxic serum, were significantly less susceptible to the development of nephritis than wild-type, Fn14 sufficient littermates. Furthermore, an identical result was achieved pharmacologically, where nephritis was attenuated in those Fn14 wild-type mice treated with an anti-TWEAK mAb. The adaptive immune response was not affected by anti-TWEAK treatment; therefore, the most likely mechanism of protection was through the observed reduction in downstream targets of TWEAK/Fn14 signalling, particularly chemokines and other inflammatory mediators.

## TWEAK, INFLAMMATION AND AGEING

CKD is associated with accelerated ageing and premature death [62]. The reason for this association is probably multifactorial, but inflammation may play a prominent role as suggested by observational studies linking inflammatory biomarkers to adverse patient outcomes [63, 64]. In this regard, higher than expected circulating TWEAK levels were associated with accelerated mortality, especially in the context of systemic inflammation [65]. A recent hypothesis suggests that CKD is a state of Klotho deficiency and that Klotho deficiency might contribute to accelerated ageing in uraemia [62]. Klotho is an anti-ageing hormone with anti-inflammatory properties, which is highly expressed in tubular renal cells [34, 66, 67]. TWEAK reduces renal Klotho expression through canonical NFκB RelA activation and histone H3 and H4 deacetylation at the Klotho promoter [34]. Indeed, TWEAK was a key contributor to kidney Klotho downregulation in experimental AKI [34]. Thus, TWEAK/Fn14 system promotes both NFκB-mediated activation of inflammation pathways and suppression of anti-inflammatory/anti-ageing pathways and is a potential player in inflammation-associated ageing [68].

## CLINICAL TRIALS EXPLORING TWEAK/FN14 TARGETING

Clinical trials exploring TWEAK/Fn14 targeting are underway in inflammation and cancer (Table 2). A phase I dose ranging clinical trial was recently completed in rheumatoid arthritis

**Table 2. TWEAK/Fn14 targeting in clinical trials**

| ClinicalTrials.gov Identifier | Agent     | Target                  | Phase | Disease                | Status                  | Company           |
|-------------------------------|-----------|-------------------------|-------|------------------------|-------------------------|-------------------|
| NCT00771329                   | BIIB023   | Neutralizing anti-TWEAK | 1     | Rheumatoid arthritis   | Completed               | Biogen Idec       |
| NCT01499355                   | BIIB023   | Neutralizing anti-TWEAK | 2     | Lupus nephritis        | Recruiting              | Biogen Idec       |
| NCT01383733                   | RO5458640 | Anti-TWEAK              | 1     | Advanced solid tumours | Ongoing, not recruiting | Hoffmann-La Roche |
| NCT00738764                   | PDL192    | Anti-Fn14               | 1     | Advanced solid tumours | Completed               | Abbott            |

<http://www.clinicaltrials.gov/ct2/results?term=tweak&Search=Search> (5 May 2013, date last accessed).

patients [69]. A single intravenous dose of neutralizing anti-TWEAK antibodies resulted in undetectable serum TWEAK levels for up to one month as well in a trend towards decreased levels of several circulating biomarkers of systemic inflammation. No serious adverse effects were recorded.

A phase II randomized placebo-controlled clinical trial (ATLAS, Anti-Tweak in Lupus Nephritis Patients) is exploring the efficacy, safety and tolerability of the BIIB023 anti-TWEAK antibody as add-on therapy in class III/IV LN patients that fail to undergo a complete remission within 3 months of initial therapy with steroids and mycophenolate mofetil [70]. The primary outcome measure is the proportion of subjects with complete and partial renal response from baseline to Week 52. ATLAS was launched in 2012 and is expected to recruit 300 patients. Results are expected no earlier than end of 2014.

In addition, several tumours express Fn14 and TWEAK may induce apoptosis, depending on cell type and the environment [71]. Both agonistic anti-Fn14 that recruit antibody-dependent cellular cytotoxicity and neutralizing anti-TWEAK antibodies are being studied in human clinical trials to treat cancer [72–75]. A potential side effect of Fn14 activation would be nephrotoxicity in those patients with prior kidney injury and upregulated kidney cell Fn14.

## THE FUTURE OF TWEAK TARGETING IN KIDNEY DISEASE

An increasing body of evidence suggests that TWEAK has a deleterious role in kidney injury associated with inflammation through actions on intrinsic renal cells that include further promotion of inflammation and cell death. Based on evidence of beneficial effects of TWEAK targeting in preclinical models of kidney injury, a clinical trial is exploring the contribution of TWEAK to LN. The eventual success of this clinical trial may expand the range of kidney disease in which TWEAK should be explored as a therapeutic target. In addition, a potential role of TWEAK in kidney regeneration should be explored. Finally, a better understanding of TWEAK signalling and its role in cytokine networks may identify therapeutic strategies beyond direct TWEAK/Fn14 targeting. Thus, a nanoconjugate molecule prevented TWEAK-induced cell death and inflammatory signalling in diverse cell types, including tubular cells [29, 76].

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## CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been published previously in whole or part.

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## Aldosterone synthase inhibitors in cardiovascular and renal diseases

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### ABSTRACT

Aldosterone is involved in various cardiovascular pathologies, including hypertension, heart failure, atherosclerosis and fibrosis. Mineralocorticoid receptor (MR)-dependent and -independent, genomic and non-genomic processes mediate its complex effects. Spironolactone and eplerenone, both MR antagonists, are the only commercially available compounds targeting directly the actions of aldosterone. However, due to the poor selectivity (spironolactone), low potency (eplerenone) and the fact that only MR-dependent effects of aldosterone can be inhibited, these drugs have limited clinical use. An attractive approach to abolish potentially all of aldosterone-mediated pathologies is the inhibition of aldosterone synthase. This review summarizes current knowledge on the complex effects mediated by aldosterone, potential advantages and disadvantages of aldosterone inhibition and novel directions in the development of aldosterone synthase inhibitors.

**Keywords:** aldosterone synthase inhibitors, fibrosis, inflammation, mineralocorticoid receptor, oxidative stress

### INTRODUCTION

The renin–angiotensin–aldosterone system (RAAS) is actively involved in various cardiovascular pathologies. Aldosterone, together with angiotensin II (Ang II), mediates oxidative stress, inflammation and tissue fibrosis. The underlying mechanisms are complex in their nature and involve mineralocorticoid receptor (MR)-dependent and -independent, genomic and non-genomic processes, and the interplay with Ang II, sodium and potassium. Moreover, the effects of aldosterone may vary in different tissues depending on aldosterone receptor distribution. Therefore, the pharmacological intervention targeting aldosterone pathway requires a broad knowledge about the mechanisms involved in aldosterone synthesis, molecular pathways involved in MR-dependent and -independent processes, discovery and description of putative aldosterone receptors and appropriate animal models of the human diseases. Here, the novel group of drugs, aldosterone synthase inhibitors (ASI), will be presented and their spectrum of action and side effects will be compared with MR antagonists.