

# Anti-neutrophil cytoplasmic antibody-associated vasculitis: biological insights and biomarker-guided disease management

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## Abstract

Earlier recognition of anti-neutrophil cytoplasmic antibody-associated vasculitis has led to more timely treatment initiation and improvements in patient survival, despite limited available therapeutic options. Current drugs can induce remission in most patients, but therapies are not individualized, with consequent over-treatment of some patients and under-treatment of others, leading to considerable morbidity from both disease and therapy. Diverse research strategies, from large clinical datasets to single-cell molecular analyses, have improved understanding of antibody-associated vasculitis biology substantially, with implications for patient management. Longitudinal data from registries and trial databases have highlighted variations in the patterns and progression of kidney disease. Efforts to improve current biological readouts of disease activity have tried to identify more dynamic and clinically applicable biomarkers to inform, in real time, the level of therapy required. Examples of such readouts include urinary levels of CD163 and CC-chemokine ligand 2, urinary T cell numbers and serum levels of various immune biomarkers, although further validation studies are required. Several important questions – how to prevent kidney disease progression, predict relapse and determine immunological quiescence to inform duration of therapy – remain incompletely answered. Here, we highlight some of the latest research advances that can help to inform clinicians about the biology of disease and might ultimately enable customization of treatment.

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## Key points

- The role of ongoing kidney inflammation in anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV) as a driver of progression to kidney failure remains unknown.
- Kidney remission in AAV lacks a precise definition as clinical scores miss subtle activity and persistent urinary abnormalities might reflect ongoing disease but can also result from chronic damage. This imprecision complicates treatment management, risking over- or under-treatment.
- Few validated biomarkers reflect kidney disease activity in AAV, with even fewer predicting kidney relapse.
- The role of pro-inflammatory IL-6 as a driver, biomarker and therapeutic target in proteinase 3-anti-neutrophil cytoplasmic antibody and granulomatous polyangiitis requires further investigation. Urinary biomarkers (soluble CD163, CD4<sup>+</sup> T cells and complement components) and blood markers (IL-18 binding protein, calprotectin and complement components) might enable treatment personalization, particularly in AAV with kidney manifestations, but require validation.
- Avacopan and other complement-targeting therapies might improve remission rates in AAV, but their use is not yet guided by complement biomarker profiles. Future research to align complement inhibition with individualized disease monitoring would be helpful.
- Emerging treatments that can effectively eliminate B cells and plasma cells, including agents such as obinutuzumab, daratumumab and chimaeric antigen receptor T or natural killer cells, might be beneficial in cases of refractory AAV. The use of these treatments might require new biomarkers to track therapeutic responses and guide safe, effective immune reconstitution.

## Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAVs) – autoimmune syndromes characterized by endothelial injury of small blood vessels and, in some cases, granulomatous inflammation – are common causes of kidney injury, resulting in both acute and chronic kidney damage<sup>1</sup>. AAVs have been transformed from almost universally lethal diseases prior to the routine use of immunosuppression, to chronic relapsing–remitting conditions that can be managed with a limited number of immune-modulating therapies (reviewed in Chevet et al. and Kronbichler et al.<sup>2,3</sup>), which have now themselves become contributors to morbidity and mortality. Genetic susceptibility is thought to contribute to the loss of immune tolerance that promotes the activation of autoreactive T and B cells leading to ANCA formation, with clinical disease flares triggered by environmental stimuli, particularly infections, that promote leukocyte activation<sup>4</sup>. Similar to most other autoimmune diseases, repeated disease flares are common and contribute to increasing treatment burden and both disease- and therapy-associated organ damage. Certain fixed factors have long been recognized to be associated with greater risk of relapsing disease (reviewed in King et al.<sup>5</sup>), such as autoantibody specificity (proteinase 3 (PR3)-ANCA, more commonly associated with relapsing disease than

myeloperoxidase (MPO)-ANCA), and clinical phenotype (granulomatous polyangiitis (GPA), more commonly associated with relapsing disease than microscopic polyangiitis). However, a better understanding of the disease pathophysiology and the changes induced by specific therapies has not yet enabled meaningful individual patient stratification for personalized therapy. The paucity of granular markers of disease activity means that assessment of disease activity is mostly empirical and, critically, validation or replication of promising biomarkers identified from smaller cohorts is lacking.

Encouragingly, the latest research has started to define kidney inflammatory signatures associated with subclinical disease, as well as the changes within individuals in whom a particular disease course is predicted and how they might vary following certain treatments. In addition, large dataset analyses have enabled recognition of disease patterns beyond current clinical subsets and highlighted cases of variable kidney involvement. These have included cohorts with different rates of progression to kidney failure and have emphasized the importance of inflammation in those with the most rapid progression, for whom more aggressive remission induction management might be required to avert kidney failure, whereas individuals at a lower risk might not require such intense treatment. These findings could lead to a promising era of biomarker-led individualized therapeutic strategies that can maintain efficacy and reduce the adverse impacts of therapy, including infections, cardiovascular events and malignancies, which drive mortality in over half of the 11% of patients who die within the first year after presentation<sup>6</sup>.

In this Review, we examine how current biological and clinical insights might enable a more rational therapeutic approach whereby patient management is tailored to their specific condition and disease stage. With an emphasis on kidney outcomes, we discuss promising biomarkers of disease activity, relapse prediction and disease progression to kidney failure, highlighting the particular conditions under which they were tested and how they are affected by therapeutic interventions. Ultimately, we consider how these biomarkers might be used in real time to inform patient management.

## Kidney disease in associated vasculitis Acute kidney injury pathology and recovery

In kidney biopsy series, pauci-immune glomerulonephritis (GN), characteristic of AAV, is a common diagnosis in patients biopsied for acute kidney injury, and this finding is especially true in older individuals (almost one third of cases)<sup>1,7,8</sup>. In addition to pauci-immune GN, the presence of extra-glomerular kidney involvement with tubulo-interstitial nephritis (with or without granuloma) or extra-glomerular vasculitis is well recognized in some patients with AAV and contributes to kidney outcomes<sup>9</sup>. For example, extraglomerular arteritis found in 13.5% of patient biopsy samples is associated with greater inflammatory responses and poorer kidney outcomes<sup>10</sup>. Unsurprisingly, worse kidney damage at presentation, assessed by higher serum creatinine levels, is associated with poorer kidney and patient outcomes<sup>6,11</sup>. However, unlike in anti-glomerular basement membrane disease, ~50% of patients who require kidney replacement therapy (KRT) at presentation might regain independent kidney function<sup>12,13</sup>. To understand how pathology might prognosticate kidney outcomes, a histological classification system was designed (now termed the Berden classification), which defined the extent of focally inflamed, crescentic or sclerosed glomeruli, and correlated these classes with medium- to long-term kidney outcomes in treated patients recruited from European Vasculitis Society (EUVAS) cohorts<sup>14</sup>. In another study, the Mayo Clinic Chronicity Index Score, which incorporates four histopathological features of

chronic damage – global glomerulosclerosis, sclerosed crescents, interstitial fibrosis and tubular atrophy – correlated inversely with dialysis independence at 4 months<sup>15</sup>. Although these histological scoring systems are helpful in standardizing the pathology descriptions and their associations with outcomes, they cannot be used for deciding on the futility of therapeutic interventions or withdrawal of treatment.

The combination of both histological and biochemical parameters, such as serum creatinine, can improve prediction of medium-term (3-year) kidney outcomes, especially regarding the development of kidney failure<sup>12,16</sup>. This kidney risk score (KRS) was validated in a large cohort of patients and a modified score with four subgroups was proposed to improve kidney-failure prediction<sup>12</sup>. Multivariable analysis in a limited Italian cohort showed that only the Mayo Clinic Chronicity Index Score and KRS were independent predictors of kidney failure<sup>17</sup>. Such information enables more open discussions with patients and other physicians regarding likely prognosis and the weighing up of benefits and risks from particular therapeutic approaches, as well as facilitating KRT planning. Of note, these prediction tools are based on datasets of patients treated with standard therapies, so they might need to be revised following the introduction of new therapies (such as the C5a receptor antagonist avacopan) or for patients treated with combination therapies such as rituximab and cyclophosphamide<sup>18</sup>. Others have proposed that scoring systems could also help to refine decisions of who should receive adjunctive therapies, such as plasmapheresis<sup>19</sup>, but further data are required to understand whether such tools can be used to guide standard therapy. Importantly, up to 50% of people with disease classified as Berden sclerotic class and 12–18.5% of people within the very high kidney risk score group or 49% in the high risk score group do not progress to kidney failure within the 3–5 years of reported follow-up<sup>12,14</sup>. Moreover, the modern strategies increasingly used for slowing rates of chronic kidney disease (CKD) progression, might prevent some individuals at a high risk (and especially older patients) from needing KRT in their lifetimes. The limitations of these histological scores underlie the need for more informative biomarkers that enable individualized therapeutic interventions.

Other histopathological features that can aid in prognostication include glomerular complement deposition. This feature can be found in a subset of patients and greater histological deposition or urinary excretion of complement components is reported to be associated with worse kidney inflammation and kidney outcomes<sup>20,21</sup>. However, no current data suggest that patients with the greatest complement activation benefit the most from complement-directed therapies such as avacopan.

Spatial transcriptomic and single-cell transcriptome analysis of kidney-biopsy tissue from people with AAV revealed the central role of pro-inflammatory T helper 1 (T<sub>H</sub>1) and T<sub>H</sub>17 cells in a limited number of patients in whom ustekinumab adjunctive therapy targeting the IL-17 pathway was clinically beneficial<sup>22</sup>. Moreover, in a similar type of analysis of another limited set of AAV kidney-biopsy samples, a cluster of four different gene transcripts and proteins was associated with improved kidney survival<sup>23</sup>. This expression signature is reported to be a better predictor of kidney outcomes than conventional histology tools such as the Berden classification or the KRS system<sup>23</sup>, although validation in other cohorts will be required. Of note, one of these gene transcripts encoded for clusterin (apolipoprotein J), which is a chaperone protein involved in the clearance of misfolded proteins and regulation of monocyte polarization (see discussion of monocyte biomarkers below). Such data suggest that defining unique transcriptomes in the biopsy sample of an individual might enable customization of

therapy, potentially even its withdrawal, and improve prognostication of longer-term outcomes.

However, all of these predictions are based on the initial presenting biopsy sample – more research is required to understand how changes in biopsy sample appearance following a therapeutic intervention might impact prognosis, as shown clearly in lupus nephritis<sup>24</sup> (see kidney remission discussion below). One study of protocolized repeat biopsies in a limited sample of mostly males with GPA treated for a year with cyclophosphamide and steroids, showed that although many acute lesions (cellular crescents and fibrinoid necrosis) improved after treatment, they were not absent in all individuals. Moreover, kidney CD20<sup>+</sup> B cells mostly disappeared, but CD3<sup>+</sup> T cells persisted, suggesting that inflammation might not completely resolve in all patients despite prolonged therapy<sup>25</sup>. No additional associations between repeat biopsy findings and relapse or progression were found in this limited cohort. Another study reported similar findings of persistent inflammatory lesions in tissue from repeat kidney biopsies as indicative of possible relapse 3–72 months following the initial biopsy<sup>26</sup>. In one study of repeat biopsies following urinary or creatinine changes, active GN was associated with progression to kidney failure in only 45% of cases<sup>27</sup>.

Alternative means of analysing biopsy tissue would also be helpful for more standardized predictions and artificial intelligence (AI) has considerable potential in facilitating the analysis of kidney biopsy tissue to predict kidney failure. Deep-learning algorithms can perform precise histopathological quantification of glomerulosclerosis, interstitial fibrosis and tubular atrophy that replicates the assessment performed by kidney pathologists<sup>28</sup>. A supervised machine-learning approach could predict and risk-stratify progression of CKD in IgA nephropathy, representing the first step in advanced prediction of GN through machine learning<sup>29,30</sup>. In people with AAV-GN, a deep-learning approach used podocyte depletion, a hallmark of glomerular injury, as a tool to risk-stratify progression to kidney failure and demonstrated that it was linked to poor outcomes<sup>31</sup>. Machine-learning-based assessment of glomerular lesions can reproduce pathologist classifications accurately, potentially removing some sources of variability in inter-pathologist biopsy interpretation<sup>32</sup>.

Whether future AI tools might identify better histological parameters currently overlooked by pathologists that correlate with treatment response or outcome, and can direct therapeutic management, remains unknown. Additional AI-related challenges involve data quality, model interpretability and the need for validation across diverse populations. However, the increasing and rapidly evolving work in this area is likely to be translated to standard clinical care in the near future.

## Chronic kidney disease progression

Large datasets from the FAIRVASC consortium recognizing diverse disease patterns have highlighted variations in kidney involvement in people with AAV and provide potential approaches of sub-stratifying clinical phenotypes. These phenotypes seem to be better predictors of kidney outcomes and relapse risk than traditional classification strategies based solely on clinical syndrome or ANCA subtypes<sup>33</sup>. Other nuanced clinical subsets have been previously suggested but were based on smaller cohorts with limited validation and perhaps overemphasized clustering into groups that included small numbers of patients with rarer manifestations (such as cardiac and gastrointestinal features), placing less emphasis on kidney phenotypes<sup>34,35</sup>. Similar to the FAIRVASC findings, these findings suggested 5–7 disease clusters, some demonstrating predominant kidney involvement.

**Table 1 | Factors associated with development of kidney failure**

Measurement	Association with kidney failure risk	Refs.
Presenting serum creatinine or eGFR	55% kidney failure with presenting creatinine > 500 µmol/l	6,11
Age and haemoglobin level	Age > 65 and low baseline haemoglobin	13
Inflammatory markers	Elevated serum levels of pro-inflammatory cytokines (TNF) and fibrinogen (in non-vasculitic CKD progression)	39,41
Berden histological class	Sclerotic class significantly associated with kidney failure within 5 years in EUVAS trial-treated patients	14
Kidney risk score	3-year kidney survival 96%, 79%, 54% and 19%, in low-, moderate-, high- and very-high-risk groups, respectively, based on serum creatinine levels, as well as % IFTA and % normal glomeruli in kidney biopsy tissue	12,16
Mayo Clinic Chronicity Score	Severe chronic damage associated with kidney failure	187
Glomerular transcriptomic signature	Transcriptomic <i>CLU</i> signature and serum <i>CLU</i> negatively associated with kidney survival	23

CKD, chronic kidney disease; *CLU*, clusterin; eGFR, estimated glomerular filtration rate; IFTA, interstitial fibrosis tubular atrophy; TNF, tumour necrosis factor.

The FAIRVASC consortium, which enrolled 3868 individuals with AAV, identified five clinical clusters, three of which had kidney involvement: one with severe kidney involvement characterized by significantly raised levels of inflammatory markers and variable ANCA specificity; another in which MPO-ANCA was associated with limited extra-renal disease; and one in which PR3-ANCA was associated with widespread extra-renal involvement<sup>33</sup>. This study also highlighted a large set of individuals with PR3-ANCA and limited kidney involvement, as well as a group with predominant ear–nose–throat manifestations (see granulomatous disease below). Kidney and patient survival varied significantly across these clusters, with the highest incidence of kidney failure in the severe kidney involvement cluster, emphasizing ongoing inflammation as a potential driver of kidney progression, followed by the MPO-renal and then the PR3-renal clusters. Interestingly, similar kidney and inflammatory clusters were found in a smaller Japanese cohort of individuals with predominant MPO-ANCA; the rate of kidney failure was highest in individuals with higher presenting creatinine and raised C-reactive protein (CRP), compared with those without signs of systemic inflammation<sup>36</sup>. The FAIRVASC data represent the largest of such analyses to date and, although validation, including in non-European cohorts, is required, these cluster analyses reinforce the existence of subtle subtypes of AAV beyond the clinical- or ANCA-based classifications, and highlight the variability in kidney involvement and outcomes. Application of these clusters to patient stratification, as well as studies exploring the impact of specific therapies or duration of treatment for each subset, might enable tailored management strategies and improved outcomes.

The mechanisms underlying the development of progressive CKD in patients with AAV and kidney involvement remains incompletely understood. In some patients, disease progresses following recurrent inflammatory disease relapses, but in the EUVAS cohort, of the patients with kidney involvement who attained remission and eventually reached kidney failure, 76% progressed without overt kidney relapses, whereas only 24% did so with a noted kidney relapse, which

occurred relatively infrequently (in 9.5% of the whole cohort) over 5 years. This finding might reflect limitations in detecting ongoing kidney inflammation or it might be due to other non-vasculitic drivers of CKD progression in people with AAV<sup>37</sup>. Unsupervised clustering of data from a predominantly non-vasculitic Chronic Renal Insufficiency Cohort (CRIC) showed that the greatest rate of CKD decline and need for KRT (as well as cardiovascular outcomes) occurred in patients with the greatest inflammatory response, who also had the worst overall health measures and greatest prevalence of diabetes and hypertension<sup>38</sup>. CKD progression was fastest in people with elevated plasma levels of fibrinogen, decreased serum albumin and elevated levels of circulating pro-inflammatory cytokines, such as tumour necrosis factor (TNF)<sup>39</sup>. The relationship between CKD progression and inflammation is complex<sup>40</sup>. Similar associations of inflammatory markers and CKD progression were found in other large non-vasculitis patient cohorts (Atherosclerosis Risk in Communities and the African American Study of Kidney Disease and Hypertension)<sup>41</sup>.

Interestingly, compared with other forms of GN, the rate of CKD progression to kidney failure in most people with AAV is relatively slow, perhaps reflecting the more efficacious therapies currently available to treat AAV compared with those used to treat other inflammatory glomerular conditions such as focal segmental glomerular sclerosis and IgA nephropathy<sup>42</sup>. However, kidney trajectories vary within these AAV cohorts. A single-centre study of 225 individuals with AAV highlighted four different trajectories – some patients presented with kidney failure or reached it very soon afterwards, whereas others maintained a degree of stable kidney function (preserved; based on estimated glomerular filtration rate (eGFR)) or stable CKD over a prolonged period of time (impaired); a small minority had significant improvement in kidney function<sup>43</sup>. The researchers suggested that decline to kidney failure was almost universally due to active vasculitis in the rapid progressors, but was a contributing factor in only 17–44% of patients in the other subgroups, although how this contribution was assessed and how many patients were re-biopsied is unclear. Some fixed demographics were associated with these subgroups (for example, the rapid progressor group had a lower eGFR at presentation and a greater number of comorbidities), but no individual biomarker could usefully predict these patterns of progression (Table 1).

### Remission, relapse and refractory disease

Defining kidney relapse in AAV is challenging because, for relapse to occur, remission must first be achieved. However, no firm definition of kidney remission currently exists and even scoring systems such as the Birmingham Vasculitis Activity Score (BVAS), BVAS for Wegener's granulomatosis (BVAS-WG) or AAV Patient Reported Outcomes do not adequately represent this state. Although overt disease activity (rising creatinine, worsening urinary abnormalities) is readily identified, subtle disease activity often goes unnoticed. Remission is typically defined based on clinical signs rather than a set of defined measures (as have been set out for lupus nephritis or membranous GN, with specific levels of proteinuria and GFR) or sensitive immunological or inflammatory markers, both in general and especially in kidney disease. Various studies have shown that during clinical remission levels of diverse inflammatory and immunological molecules are abnormally elevated<sup>44,45</sup>, suggesting that complete immunological remission might be overestimated using current conventional biomarkers<sup>46</sup>. Kidney-biopsy evidence remains the gold standard approach to detecting ongoing kidney inflammation, but its invasive nature precludes repeated

use, hence the need for non-invasive biomarkers that reflect subtle disease activity.

Remission is variably defined in clinical trials (Table 2), but generally requires a zero score on the BVAS or BVAS-WG, and therefore a zero score on kidney parameters. These scoring systems cannot adequately deal with changes in kidney parameters and, although they are based on less dynamic biomarkers such as creatinine, haematuria and proteinuria, they fail to take some of these into account after a fixed set period of time (BVAS after 3 months) or at all (proteinuria in BVAS-WG). Kidney Disease: Improving Global Outcomes guidelines, on the other hand, define kidney remission in AAV as stable or improving kidney function based on eGFR, whereas other groups do not attempt to define remission (including kidney remission) beyond a BVAS of zero. Moreover, limitations exist in using current parameters such as changes in creatinine levels, elevated levels of proteinuria or detectable haematuria, as these disease manifestations can persist for long periods, representing either ongoing inflammation or chronic damage, making them less reliable indicators of acute disease activity<sup>47</sup>. Following induction therapy in patients enlisted in selected EUVAS trials, almost 30% of patients had persistent haematuria and 34% had a urinary protein-to-creatinine ratio >0.05 g/mmol. After a median follow-up of 28 months, elevated levels of proteinuria (>0.05 g/mmol) and creatinine following induction therapy were the only factors associated with the end point of kidney failure or death, whereas haematuria and proteinuria were associated with subsequent kidney relapses<sup>48</sup>. This association was not found in the post hoc analysis of other clinical trials, including the RAVE trial, comparing rituximab with cyclophosphamide induction therapy, and the WGET trial, examining the adjunctive use of etanercept compared with standard induction therapy, in which 42% and 43% of patients had persistent dipstick haematuria and proteinuria (beyond 6 months), respectively,

and only haematuria was associated with subsequent kidney relapses but not progressive CKD or kidney failure<sup>49</sup>. Notably, most patients with persistent haematuria (almost 80%) did not have a kidney relapse, highlighting the poor predictive value of this biomarker. Of note, some studies report haematuria based on dipstick testing whereas others refer to erythrocyturia, which might have different sensitivities and specificities. However, persistent or worsening urinary abnormalities can inform the issue of who to re-biopsy. In 29 individuals with AAV who underwent ‘for cause’ interval biopsies (at a median of 34 months) indicated by persistent haematuria, new haematuria, persistent or new proteinuria, or worsening creatinine, active AAV was identified in 20%, 70%, 30% and 50% of cases, respectively<sup>27</sup>. In another study of repeat biopsies over a shorter interval (median 130 days) haematuria was present in 59% of those with inactive disease, whereas 60% of those with active disease had no haematuria<sup>50</sup>. Additionally, whereas clinical impression of inactive disease was correct in most patients (97% of cases), clinical suspicion of active AAV was only confirmed histologically in 42% of the cases. Overall, these data confirm that urinary abnormalities and clinical assessments are inconsistent means of predicting kidney disease activity defined by gold standard histological assessment.

Interestingly, the sclerotic subclass of ANCA-associated GN (with >50% of glomeruli showing sclerotic lesions and hence a less inflammatory phenotype) is also associated with increased risk of kidney relapses<sup>37</sup>. However, here the definition of kidney relapse was a rise in serum creatinine of >30% or a fall in eGFR >25% and/or new haematuria or proteinuria (all attributable to active vasculitis), as indicated by the BVAS. As discussed above, these parameters might not reflect recurrent active disease but rather progressive permanent kidney damage. Therefore, what persistence of proteinuria and/or haematuria following remission induction treatment truly represents,

**Table 2 | Definitions of kidney remission in ANCA vasculitis**

Scoring systems, guidelines and trials	Definition of kidney remission	Limitations
BVAS v3	Score of 0 including kidney components defined by: hypertension, proteinuria >1+, haematuria ≥10 RBCs/HPF, serum Cr <sup>a</sup> 125–249/250–499/≥500 μmol/l, rise in serum Cr >30% or fall in Cr clearance >25%	Persistence of kidney components >3 months considered ‘kidney damage’ (and not scored) Unless thresholds of serum Cr or Cr clearance are met, kidney function does not contribute to score after the first visit No consideration of Cr Clearance conversion to eGFR and formula used to calculate it
BVAS-WG	Kidney disease activity defined by: haematuria (>10 cells/HPF or >+1), RBC casts, rise in serum creatinine >30% or fall in Cr Cl >25% Remission defined by no new, worse or persistent kidney parameters	Proteinuria is not considered at all; change in creatinine not meeting the threshold would not be scored as active disease No consideration for Cr clearance conversion to eGFR and formula used to calculate it
KDIGO (2024)	Absence of manifestations of vasculitis and GN (BVAS=0), including stable or improved GFR; haematuria and proteinuria persistence does not necessarily imply active disease	Intentionally vague — threshold of stable or improved kidney function is not defined, equivocal stance on haematoproteinuria Use of BVAS with its associated limitations
AAV induction and maintenance trials <sup>b</sup>	Predominantly: BVAS=0 (or BVAS-WG) with or without cessation or reduced-dose steroids (prednisolone 5–7.5 mg) for a predefined time period	The primary outcome measures of these studies have not been kidney remission but almost exclusively remission, as defined by the BVAS with heterogeneity relating to whether steroid cessation or reduced dose for a predefined time period is mandated and how primary outcome is assessed (time to reach outcome or proportion of cohort reaching outcome) Limitations of the BVAS apply

BVAS, Birmingham Vasculitis Activity Score; BVAS-WG, BVAS-Wegener’s granulomatosis; Cr, creatinine; eGFR, estimated glomerular filtration rate; GN, glomerulonephritis; HPF, high-powered field; KDIGO, Kidney Disease: Improving Global Outcomes; RBCs, red blood cells. <sup>a</sup>Not measured after the first visit. <sup>b</sup>Including the CYCLOPS, RAVE, RITUXIVAS, MYCYC, ADVOCATE, RITAZAREM and MAINRITSAN trials.

**Table 3 | Fixed factors associated with an increased risk of disease relapse**

Fixed clinical factors	Increased overall risk of relapse	Increased risk of kidney relapse	Notes	Refs.
ANCA rises, persistence or reappearance	Yes	Unknown	Rise or persistence of ANCA more predictive of major relapse in patients with initial kidney involvement; relapse HR of 26.2 in seronegative patients with MPO-ANCA reappearance	78,82,83
PR3-ANCA	Yes	Unknown	Compared with MPO-ANCA or seronegative AAV	51,65,188
GPA diagnosis	Yes	Unknown	Compared with MPA	51,188
<i>Staphylococcus</i> carriage	Yes	Unknown	In GPA, 58% relapse rate compared with 10% in patients without chronic nasal carriage	54
Cardiovascular involvement	Yes	Unknown	Subhazard ratio 1.59 for overall relapse	51
Lung involvement	Yes	Unknown	HR 1.66–1.68 French and CGCN US cohorts	53
ENT involvement	Yes	Unknown	HR 1.58, CGCN cohort and 0.96 French cohort	53
Lower presenting serum creatinine	Yes	Unknown	Higher risk of creatinine <100 µmol/l compared with creatinine >200 µmol/l or GFR >30 ml/min/1.73 m <sup>2</sup>	51
Persistent haematuria	No	Yes	Increased risk in follow-up of both EUVAS (HR 2.16) and RAVE/WGET trial patients (sub HR 3.99)	48,49
Persistent proteinuria	No	Yes	Increased risk with uPCR ≥0.05 g/mmol in follow-up of EUVAS (HR 2.22) but not RAVE/WGET trial patients	48
Induction therapy type	Yes	Unknown	Higher risk in patients treated with MTX (NORAM) and pulse CYP (CYCLOPS) compared with oral CYP	65, 189,190
Maintenance therapy type	Yes	Unknown	MMF treatment associated with greater risk than AZA (IMPROVE), AZA associated with greater risk than RTX (MAITRISAN, RITAZERAM)	66,67
Sclerotic class on biopsy	No	Yes	Kidney relapse risk for sclerotic class (42.9%) > mixed class (22.2%) > focal class (21.7%) > crescentic class (15.5%)	37
Absence of interstitial infiltrates on biopsy	No	Yes	Kidney relapse risk increases by ~11-fold (HR = 0.09 for those with infiltrates)	37

ANCA, anti-neutrophil cytoplasmic antibodies; AZA, azathioprine; CGCN, Glomerular Disease Collaborative Network; CYP, cyclophosphamide; ENT, ear–nose–throat; GFR, glomerular filtration rate; GPA, granulomatous polyangiitis; MMF, mycophenolate mofetil; MPA, microscopic polyangiitis; MPO, myeloperoxidase; MTX, methotrexate; PR3, proteinase 3; RTX, rituximab; uPCR, urinary protein-to-creatinine ratio.

and whether prolonged immunosuppression in people with persistent urinary abnormalities would improve outcomes, remains unknown.

Treatment with conventional immunosuppression (predominantly cyclophosphamide) in combination with glucocorticoids is associated with an overall AAV relapse risk of 38% at 5 years, based on follow-up data from 535 patients enrolled in four European Vasculitis Study Group trials. Independent predictors of relapse included anti-PR3 antibody positivity, GPA phenotype and cardiovascular involvement<sup>51</sup>. Interestingly, higher serum creatinine levels were associated with a substantially lower risk of relapse. This paradoxical finding might reflect the relatively immunosuppressed state associated with kidney dysfunction, or perhaps a poor ability to detect ongoing kidney relapse in patients with established kidney impairment<sup>52</sup>. Other established susceptibility factors for overall disease relapse include lung involvement, ear–nose–throat involvement<sup>53</sup> and nasal carriage of *Staphylococcus aureus*<sup>54</sup> (Table 3). Although newer treatment protocols have reduced relapse rates, the pathogenicity of non-modifiable risk factors such as PR3-ANCA seems to persist. For example, in the PEXIVAS study, relapse occurred in 22.7% of patients at a median of 423 days (interquartile range (IQR) 141–846) following remission<sup>55,56</sup> and PR3-ANCA (versus MPO-ANCA) along with non-haemorrhagic pulmonary involvement were the most significant factors associated with risk of relapse. Kidney failure was again associated with a reduced relapse risk. In this post hoc analysis, kidney relapses were the most common manifestation (found in 46% of relapses), higher than previously reported in

EUVAS cohorts of patients with primary kidney involvement. Among 174 patients enrolled in the MEPEX (examining methylprednisolone versus plasmapheresis adjunctive therapy) and the CYCAZAREM (prolonged versus shortened cyclophosphamide dosing and conversion to azathioprine) trials with poor kidney function (median creatinine of 738 and 172 µmol/l, respectively) the 5-year cumulative incidence of kidney relapse was 9.5% (95% confidence interval (CI), 4.8–14.3%), considerably lower than overall relapse rates. In this cohort, increased kidney relapse risk was associated with the Berden sclerotic class (see above) and the absence of interstitial infiltrates at diagnosis<sup>37</sup>. Importantly, beyond these histopathological parameters, regression analyses from large trial cohort data failed to identify additional independent predictors of kidney-specific relapse<sup>57</sup>.

**Extra renal disease — granulomatous inflammation.** A key feature associated with relapsing disease is a clinical diagnosis of GPA, which is most often associated with PR3-ANCA positivity and low rates of severe kidney disease. GPA is defined by granulomatous inflammation and seems to be less responsive to conventional therapies, leading to prolonged treatment courses and a greater share of adverse treatment-related reactions.

Modern clinical trials have not uniquely enrolled GPA patients and specific therapies directed at granulomatous inflammation have thus not been adequately tested. The WGET trial is an exception and demonstrated that, overall, adjuvant anti-TNF therapy was not effective

for maintaining remission in GPA<sup>58</sup>. However, data from *in vitro* and *in vivo* models (using zebrafish rather than rodents) emphasized that the granulomatous inflammation in GPA seems to be dependent on the IL-6–STAT3 pathway, providing potential novel therapeutic avenues<sup>59</sup>. Accordingly, clinical cohort data demonstrate elevated levels of serum IL-6 levels in individuals with GPA compared with those with microscopic polyangiitis (MPA), and especially in those with PR3-ANCA compared with MPO-ANCA. Additionally, rises in IL-6 levels preceding relapses were observed in patients treated with rituximab<sup>60</sup>, as well as in the pre-rituximab treatment era<sup>61</sup>. Nasal IL-6 levels were elevated in samples from people with GPA compared with those from healthy individuals and were associated with CRP levels, suggesting a local IL-6-driven inflammatory response that might serve as an activity biomarker and novel therapeutic target<sup>62</sup>. Interestingly, post hoc analysis of the Protect-V COVID-19 Prophylaxis Study, which tested the ability of nasal nicosamide (which has IL-6–STAT3 inhibitor activity) to prevent COVID-19 infection, demonstrated reduced disease activity in a small number of PR3-ANCA-positive individuals with GPA who were enrolled in the study<sup>63</sup>. Given the multiple IL-6-targeting therapeutics available, a PR3-ANCA GPA trial using one or more of these agents might be warranted.

**Treatment type and duration.** Based on the inter-patient variation in treatment responses, suppression of inflammation and tendency to relapse highlighted above, the duration of treatment required is not likely to be the same for all patients. Although this variability has been acknowledged by clinicians caring for patients for many years, scant evidence is available to guide any specific changes in management, so many patients remain on treatment for fear of disease relapse. Perhaps one exception is that patients achieving ANCA negativity following induction therapy clearly have lower relapse rates than those who remain ANCA positive<sup>64,65</sup>. Notably, many studies have not specified whether relapses are renal- or non-renal, rather classifying them as major or minor relapses. A small number of studies have examined the risk–benefit of prolonging therapy. In these studies, extended maintenance treatment was generally associated with fewer relapses but greater adverse effects. Surprisingly, in the REMAIN trial (comparing 2 years of maintenance treatment with 4 years) this pattern was consistent across all participants regardless of ANCA subtype, (despite PR3-ANCA generally being associated with a greater relapse tendency)<sup>66</sup>. By contrast, long-term follow-up of patients enrolled in several other EUVAS trials suggested no additional benefit in prolonging treatment beyond 18 months but highlighted increased rates of relapse in those treated with intravenous compared with oral cyclophosphamide, and in those with PR3-ANCA (compared with MPO-ANCA)<sup>67</sup>.

Importantly, not all maintenance therapies are associated with the same rates of relapse – the hierarchy of agents from the lowest to highest rates of relapse is rituximab, azathioprine equal to methotrexate, followed by mycophenolate<sup>68–71</sup>. However, even the improved rates of remission with rituximab are lost once maintenance treatment is discontinued. This variable efficacy in maintaining remission and the optimal duration of treatment is now reflected in the latest Kidney Disease: Improving Global Outcomes guidelines, emphasizing the need for remission maintenance therapy for 18–48 months<sup>72</sup>, although it is based on very limited datasets. Current biomarkers cannot inform treatment duration, but more granular testing of some urinary markers might provide a clearer view of when kidney inflammation is attenuated and, if confirmed by kidney histology, could reduce treatment duration in some patients.

In a small subset of patients, prolonged disease-free and treatment-free remission is maintained (termed tolerant or long-term remission off treatment (LTROT)) but the factors underlying this phenotype remain unclear. In the largest cohort analysed, 277 of 427 patients stopped treatment for some time (at a median of 20 months from presentation, and for a median of 45 months over the entire course of the follow-up). Of these 277 patients, 63 (23%) stopped therapy and were in remission >5 years, and of these, 50 remained off therapy with no further relapses (13 patients had a subsequent flare). Some participants subsequently died (in remission) and only 2 of 50 progressed to kidney failure (without obvious kidney relapses)<sup>73</sup>. Apart from fixed demographic features (being female, having MPO-ANCA, clinical MPA and pulmonary involvement), no other biomarkers at presentation or later predicted who could discontinue treatment and remain in long-term remission. In a similar preliminary analysis of a small number of European patients, no particular ANCA subtype or demographic seemed to discriminate patients who achieved long-term clinical and immunological remission off-treatment compared with those who remained ANCA positive and on treatment<sup>74</sup>. However, patients in the tolerant cohort had greater numbers of regulatory B (B<sub>reg</sub>) cells. Clearly, defining this group using more granular markers might clarify the factors leading to long-term remission, which in some cases might reflect the re-establishment of immune tolerance.

## Conventional biomarkers of disease activity, remission and relapse

The NIH and FDA have produced the Biomarkers, Endpoints, and other Tools resource to support the consistent use and interpretation of biomarkers<sup>75</sup>, including guidance on what a biomarker is intended for and how it should be used. These considerations are critical in the context of AAV to ensure optimal translation of research data into clinical application.

### Kidney function tests: creatinine, proteinuria and haematuria

Presenting levels of creatinine and proteinuria can be associated with long-term kidney outcomes, as mentioned earlier, but changes in these parameters might not mirror the dynamic inflammatory changes of vasculitis. It can take over 3 months to reach a nadir creatinine and over 9 months for proteinuria<sup>47</sup>. Given that one of the greatest problems in AAV therapy remains treatment-related adverse effects, using more granular biomarkers might enable treatment duration reduction for some patients, especially older individuals, or prolongation of therapy for those with ongoing kidney inflammation, despite completion of a standard course of induction treatment.

### The role of anti-neutrophil cytoplasmic antibody

ANCA detection has an important role in the diagnosis and pathogenesis of AAV and in stratifying the risk of relapse, but its utility in assessing disease activity or predicting future relapses based on change in titres is still controversial and escalating treatment based on ANCA titre changes alone is generally not recommended. An increase in ANCA titre correlates with relapse risk in people with kidney involvement but was less predictive in those with non-kidney disease<sup>37</sup>. In the RAVE trial, doubling of ANCA titres was associated with an increased risk of relapse but only in people with PR3-ANCA treated with rituximab<sup>76</sup>. In other cohorts, persistence<sup>77</sup> and reappearance of MPO-ANCA<sup>77,78</sup> were associated with a higher risk of relapse. These observations support the finding that relapses are associated with high plasmablast frequencies<sup>79</sup> and expanded memory B cell subset repopulation post-rituximab<sup>80</sup>.

Several meta-analyses have highlighted the heterogeneity of the studies included regarding study design, methods, frequency and duration of ANCA testing, in addition to the definition of what constitutes a rise in ANCA or a relapse. One meta-analysis included 15 studies and found that a rise in ANCA titres was weakly predictive of a future relapse (pooled likelihood ratio of 2.84, 95% CI 1.65–4.90) with sensitivity and specificity of 0.56 (95% CI 0.33–0.79) and 0.82 (95% CI 0.75–0.90), respectively, and that persistent ANCA titres had even poorer predictive value (likelihood ratio of 1.97, 95% CI 1.43–2.70) with sensitivity and specificity of 0.38 (95% CI 0.23–0.52) and 0.78 (95% CI 0.71–0.85), respectively<sup>81</sup>. In another analysis including 20 studies, ANCA titre rise was not associated with concurrent relapse but rather with relapse in the subsequent 6 months (and less so within 1 year)<sup>82</sup>. Finally, ANCA rises seem to be associated with a greater risk of major versus minor relapse in patients with kidney involvement (hazard ratio (HR) 11.09 (95% CI 5.01–24.55) versus 2.79 (95% CI 1.30–5.98), respectively; overall risk was higher in PR3-ANCA than in MPO-ANCA<sup>83</sup>. The impact on kidney-specific relapses was not reported. Some studies did not separate ANCA subsets but the potential differences between MPO-ANCA and PR3-ANCA as biomarkers might affect their predictive performances.

In addition to titre, subtle changes in post-translational modifications of ANCA-IgG were also associated with an increased risk of relapse in a single-centre study of both MPO-ANCA and PR3-ANCA subtypes. Specifically, total IgG glycosylation differed between patients who relapsed and those who did not – IgG Fc-sialylation decreased in the 6 months prior to clinical relapse in people with PR3-ANCA positivity, whereas other glycan patterns remained constant<sup>84</sup>. Finally, changes in MPO-ANCA epitope specificity between acute disease and remission might explain ANCA persistence in the absence of clinically active disease, as some antibody epitopes are immunodominant and might promote more inflammation<sup>85</sup>. Such an analysis has not yet been successfully performed for PR3-ANCA epitopes.

Other autoantibodies, either co-expressed or independent of PR3 and MPO ANCA, have been reported in AAV. These include anti-lysosomal-associated membrane protein-2 (anti-LAMP2), anti-tissue plasminogen (anti-PLG), anti-tissue plasminogen activator (tPA), anti-moesin and anti-pentaxin 3 (PTX3)<sup>86–88</sup>. The presence of some of these antibodies correlated with worse overall and kidney outcomes, although they are only found in a subset of patients. Anti-plasminogen and anti-tPA antibodies were detected in ~25% and 20% of individuals with AAV, respectively, in UK and Dutch cohorts<sup>86</sup>, and anti-plasminogen antibodies were found in 18% of individuals in a Chinese cohort<sup>87</sup>. These studies demonstrated the correlation of at least one of these autoantibodies with higher BVAS, poorer kidney function and a higher proportion of fibrinoid necrosis and cellular crescents. Similarly, anti-moesin antibodies seem to correlate with BVAS and serum creatinine levels<sup>88</sup>. By contrast, anti-PTX3 antibodies found in as many as 30% of people with AAV and in up to ~25% of ANCA-negative patients are reportedly higher in disease remission and are associated negatively with systemic and kidney manifestations<sup>89</sup>. PTX3 levels were lower in individuals with higher anti-PTX3 antibody titres, suggesting that PTX3 might be a disease activity biomarker<sup>90</sup>.

## The role of B cells

Policies for monitoring peripheral B cell numbers and how to use this information to assess relapse risk and decide on timing of maintenance treatment are considerably heterogeneous. Both induction with rituximab (or other B cell-directed monoclonal antibodies) and cyclophosphamide deplete circulating B cell numbers, although at

different tempos<sup>76</sup>; B cell recovery after treatment suggests a potential for immunological reactivation. In the MAINRITSAN 2 trial, patients were randomly assigned to receive fixed rituximab dosing every 6 months, or tailored redosing based on the reappearance of B cells (>0/mm<sup>3</sup>) or change in ANCA status (negative to positive, or doubling of the titre), tested every 3 months. Overall, relapse rates did not differ, although the fixed-dose arm received more rituximab infusions (median 5; interquartile range (IQR) 5–5) than the tailored treatment cohort (median 3; IQR 2–4), where re-infusion was mostly indicated because of B cell re-appearance (51%) or combined B cell and ANCA re-appearance (37%). Notably, relapses occurred in 45% of patients with no circulating B cells and in 18% who had neither ANCA positivity nor B cell return<sup>91</sup>. In the RAVE study, although most relapses in the rituximab group were preceded by detectable B cell levels, 12.5% occurred without B cell reconstitution (in contrast to 30% in the cyclophosphamide group). Importantly, approximately two-thirds of patients had detectable B cells or rising ANCA titres and did not experience a flare over 18 months of follow-up<sup>92</sup>.

The use of ANCA return compared with B cell reappearance to guide maintenance retreatment was formally compared in a single-centre cohort and showed that fewer relapses occurred if B cells were used to decide on treatment (4% relapse in the B cell-monitored group versus 20.5% in the ANCA-monitored cohort), with no difference in side effects but greater rituximab delivery in the B cell arm<sup>93</sup>.

Detailed analysis of re-emerging B cell subtypes following rituximab revealed that frequencies of transitional and naive B cell subsets increased, whereas those of memory B cells decreased compared with baseline, but none were associated with risk of relapse. However, PR3-antigen-specific plasmablast frequencies and ANCA titres were higher in relapsing patients<sup>94</sup>. By contrast, others have found that naive B cell repopulation at 6 months was associated with a significantly lower risk of relapse (HR 0.43 (0.22–0.84)) whereas total plasmablast repopulation was not<sup>95</sup>. The reasons underlying these discrepant findings are unclear.

## Novel biomarkers

Biomarkers that discriminate active disease from remission must be distinguished from those that can be used to predict future relapses (both overall and kidney-specific relapses), with careful consideration of the conditions under which the biomarkers have been tested. As a general principle, the latest research on biomarkers for diagnosis or prediction of active disease or kidney flares suggests that a multivariable approach that integrates various biomarkers (Tables 4 and 5) including the current standard of care (ANCA titre, proteinuria or albuminuria, and haematuria) and/or clinical data, is likely to be more predictive than univariable models. The most clinically useful biomarkers can be measured in biosamples that require the least fastidious methods of collection. Biomarkers can be considered according to the immune pathways in which they are involved, the cells from which they are derived or the biological samples to be tested (urine, blood or tissue).

## Innate immunity

**Complement.** The role of the complement system in AAV has been highlighted in animal and human studies<sup>96,97</sup>. Hypocomplementaemia has been reported as a poor kidney prognostic factor<sup>98,99</sup> and complement pathway components (Bb, P, C3d, C5b-9) have been detected in kidney-biopsy tissue from people with AAV. In these samples, greater Bb staining was associated with greater crescentic change and worse kidney function. Furthermore, Bb plasma levels correlated

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significantly with crescents, erythrocyte sedimentation rate (ESR) and BVAS, whereas urinary levels of Bb correlated with the number of crescents and serum creatinine, and inversely with the proportion of normal glomeruli in the biopsy but not with degree of glomerular complement deposition<sup>100</sup>. Urinary (but not plasma) Ba correlated similarly with disease activity, with modest sensitivity and specificity (76.2% and 68.4%, respectively)<sup>101</sup>. These findings were supported by a meta-analysis highlighting reports of increased plasma levels of C3a, C5a, C5-9 and factor B in active disease but not of Bb or properdin<sup>102</sup>, although they were not associated with clinical outcomes.

**Neutrophils.** Neutrophils have a central role in the pathogenesis of AAV through reactive oxygen species (ROS) production, degranulation and neutrophil extracellular trap (NET) release, all of which lead to endothelial cell injury<sup>103</sup>. NETs, which are structures composed of decondensed chromatin adorned with granule-derived peptides such as MPO and PR3, can additionally act as autoantigens in AAV<sup>104</sup>. NET levels are elevated in the serum of patients during active disease

and decrease upon remission; they are also detected in kidney-biopsy tissue of individuals with AAV<sup>105,106</sup>. Additionally, neutrophil microparticles containing cytosolic proteins and RNA are released following neutrophil activation by ANCA or apoptosis, are elevated in active vasculitis and can induce ROS release and endothelial cell damage<sup>107</sup>. Based on these findings, the analysis of neutrophil proteins, microparticles and NETs has potential as a biomarker for assessing AAV disease activity. Although a standardized assay for detecting these biomarkers has yet to be developed, a 2023 study that used a bioimpedance technique to measure serum NET-inducing ability showed that an increase in NET-inducing activity at 6 months could identify patients with relapsing disease<sup>108</sup>.

Neutrophil granule proteins also have biomarker potential. Serum MPO is elevated in active PR3-ANCA disease and significantly decreases in remission<sup>106</sup>, whereas an increase in serum calprotectin at month 2 or 6 compared with baseline identified a subgroup of patients with PR3-ANCA treated with rituximab who were at a higher risk of relapse by 18 months<sup>45</sup>. In addition, in a post hoc analysis of the MAINRITSAN

**Table 4 | Immune factors predicting disease activity or relapse in peripheral blood**

Immune factors	Test performance in active disease	Test performance as a predictor of relapse	Clinically translatable <sup>a</sup>	Refs.
CD8 T cell transcription signature	T cell memory and survival > T cell exhaustion signature	CD8 T cell transcription signature	Low	152,153
Serum calprotectin change	>1.5 higher in active disease than in remission (AUC 0.73)	Between presentation and 2 or 6 months following treatment; only in rituximab-treated patients with PR3-ANCA	High	45,109
B cells	Reduction in B <sub>reg</sub> cell frequency but not function Low sLag-3, high CD27	Repopulation or increase in memory B cell subset	Medium	127,129, 151,191
ANCA	Increase in titre by fourfold by IIF or 25% by ELISA or positive after being negative	Increased risk of kidney relapse (HR 11.09; 95% CI 5.01–24.55) more than non-kidney disease (HR 2.79; 95% CI 1.30–5.98)	High	78,83
Monocyte frequency and function	In MPA, increased activated monocytes and monocytes expressing interferon signature genes	In MPA, higher monocyte-to-PBMC ratio increased immature activated monocyte subsets	High	113
Neutrophil extracellular traps	Elevated in the serum of patients during active disease and decreased after remission	Increases at 6 months can predict relapse	Medium	104, 106,108
Granulocytes	Granulocyte gene signature of low-density granulocytes associated with decreased treatment response	Expression changes in granulocyte transcripts correlated with disease relapse	Low	112
Endothelial cells	EPC reduced numerically in active and relapsing disease; angiogenic T cells essential for EPC differentiation exhibit impaired proliferation	Circulating endothelial cells can remain elevated in remission, indicating low-grade inflammation	Low	155,158
Complement	Increased plasma levels of C3a, C5a, C5b-9 and factor B in active disease	Plasma levels of complement Bb correlate significantly with crescents, ESR and BVAS	Medium	100,102
IL-18BP	7.95-fold increase in active compared with inactive disease (sensitivity 77%, specificity 67%); improved predictor of active disease if combined with CRP, soluble CD25 and NGAL, but more so in patients treated with CYP	Significant upregulation in blood in active disease (AUC 0.71)	Medium	192
IL-6	Higher levels in patients with subsequent relapse	Higher in active disease, especially GPA; elevated prior to relapse; no assessment of sensitivity or specificity	Medium	60, 61,128
IL-10	Lower levels in those who subsequently relapse	Higher levels in acute disease correlating with BVAS and ANCA levels; decreased in remission and lowest in relapsed disease; no assessment of sensitivity or specificity	Medium	

AUC, area under the curve; B<sub>reg</sub>, regulatory B cells; BVAS, Birmingham Vasculitis Activity Score; CRP, C-reactive protein; CYP, cyclophosphamide; EPC, endothelial progenitor cells; ESR, erythrocyte sedimentation rate; GPA, granulomatous polyangiitis; IIF, indirect immunofluorescence; IL-18BP, interleukin 18 binding protein; MPA, microscopic polyangiitis; NGAL, neutrophil gelatinase-associated lipocalin; PBMC, peripheral blood mononuclear cells; PR3-ANCA, proteinase 3-anti-neutrophil cytoplasmic antibodies; sLag-3, soluble lymphocyte-activation gene. <sup>a</sup>Likelihood of translation into clinical practice in the next 3–5 years.

**Table 5 | Urinary biomarkers associated with disease activity or predicting relapse**

Biomarker	Correlation with kidney histology	Longitudinal monitoring	Large-scale validation	Access	Cut-off for detecting active kidney AAV versus remission	AUC	Sensitivity	Specificity	Positive LR	Negative LR	Clinically translatable in 3-5 years	Notes	Refs.
Bb, C3a, C5a, MAC	Bb correlates with percentage of crescents MAC correlates with kidney involvement and creatinine but not relapses	Yes	No	Easy	C5a: 0.05ng/ml	0.73	70	70	NA	NA	High	NA	100,102
Ba	Unknown	Yes	No	Easy	12.53ng/mg <sup>a</sup>	0.76	76.2	68.4	NA	NA	High	NA	101
MCP-1	Glomerular macrophage infiltration	Yes	Yes	Ex vivo instability	1.3-fold increase <sup>a</sup> 0.53ng/ml	0.93	94	89	8.5	0.07	Medium	Improved specificity and sensitivity in combination with CRP or uCD163	117,119, 120,193
CD163	Glomerular macrophage infiltration, percentage of crescents, percentage of fibrinoid necrosis	Yes	Yes	Easy	300ng/mmol <sup>a</sup>	0.93	83	96	20.75	0.17	High	Improved sensitivity for more subtle disease flares in combination with uCC2	115, 116,118, 120,169, 193-195
		Yes	Yes	Easy	72.9ng/mmol <sup>a</sup>	0.79	79.5	67.3	2.4	0.3	Medium	Can be combined with serum calprotectin measurements and assessment of haematuria for greater detection of disease activity	
		Yes	Yes	Easy	350ng/mmol <sup>a</sup>	0.94	75	94	13	0.27	Medium		
		Yes	Yes	Easy	250ng/mmol	0.95	96.8	86.8	NA	NA	Medium		
		Yes	Yes	Easy	20% increase or absolute increase of 20ng/mmol	0.96	100	89.3/87.5	NA	NA	Medium		
TWEAK	Weak correlation with percentage of crescents	Yes	No	Easy	302ng/mmol <sup>a</sup>	0.86	72	97	27.51	0.28	Medium		
		Yes	No	Easy	12ng/ml (not normalized to Cr)	0.88	88	80	4.57	NA	Medium		
Actin A	Significant correlation with percentage of crescents	Yes	No	Easy	NA	NA	NA	NA	NA	NA	Medium	NA	124
CD25	Unknown	Yes	No	Easy	10.7ng/g <sup>a</sup>	0.779	83%	70	2.76	0.244	Medium	NA	115,116, 118-120, 122,169
CD3 <sup>+</sup> , CD4 <sup>+</sup> , T <sub>H17</sub> , T <sub>reg</sub> , T <sub>H1</sub> cells	Unknown	Yes	No	Easy	125ng/mmol <sup>a</sup>	0.8	70.8	76.5	3	0.38	Medium	Better when combined with suCD163	116, 119,120
		Yes	No	Difficult (flow cytometry)	CD3: 3149 cells/100ml of urine (not normalized to Cr)	0.95	90	92	NA	NA	High	Improved prediction of relapse in combination with ANCA	116, 119,141, 142,195
		Yes	No	Easy	T <sub>reg</sub> : 60 cells (not normalized to Cr) <sup>b</sup> 490 CD4 <sup>+</sup> cells/100ml of urine (not normalized to Cr) <sup>b</sup>	0.92	74	96	NA	NA	Medium		

AAV, anti-neutrophil cytoplasmic antibody-associated vasculitis; AUC, area under the receiver-operating characteristic curve; Ba, complement factor Bb; C3a, complement component 3a; C5a, complement component 5a; CCL2, CC-chemokine ligand 2; CD25, soluble IL-2 receptor  $\alpha$ ; CD163, soluble CD163 (shed macrophage scavenger receptor); CD3, pan-T-cell marker; CD4, helper T cell marker; Cr, creatinine; HPF, high-power field; +LR, positive likelihood ratio; -LR, negative likelihood ratio; MAC, membrane attack complex (C5b-9); MCP-1, monocyte chemoattractant protein-1; NA, not available; RTX, rituximab; T<sub>H17</sub> cell, effector memory T cells; T<sub>reg</sub> cell, regulatory T cells; T<sub>H17</sub> cell, T helper-17 cells; TWEAK, TNF-like weak inducer of apoptosis. <sup>a</sup>Distinguishes active kidney AAV versus non-kidney AAV.

trial, a greater rise in calprotectin from the start of maintenance therapy over 6 months was a marker of kidney function decline (based on eGFR decline) in the subsequent 12 months and relapse in patients with PR3-ANCA<sup>109</sup>.

In addition, neutrophil membrane PR3 levels are associated with increased relapse rates in people with GPA<sup>110</sup>. Similarly, a distinct neutrophil subset enriched for a type II interferon signature was identified in MPA and shown to expand during relapse<sup>111</sup>. Finally, expression of 2346 gene transcripts differed significantly between responders and non-responders in the RAVE trial. A granulocyte gene signature, likely reflecting the presence of low-density granulocytes (potentially pathogenic cells in AAV), was associated with disease activity and decreased response to treatment<sup>112</sup>. Further investigation of the impact of other therapies on these gene transcripts would be warranted, for example, in those treated with neutrophil-targeted therapies such as avacopan.

**Monocytes and macrophages.** Distinct patterns of monocyte markers associated with treatment responses and tendency to relapse were reported in a limited number of people with MPA. Activated immature monocytes and a higher ratio of monocytes in the peripheral blood mononuclear cell fraction were associated with subsequent relapses, whereas monocytes with greater IFN $\alpha$  signatures (and higher serum IFN $\alpha$ ) were associated with a good response to therapy (the details of which were not specified)<sup>113</sup>. Higher serum levels of apolipoprotein J following induction treatment at 6 months from diagnosis correlated with better kidney survival, although the underlying renoprotective mechanisms were unclear<sup>23</sup>.

Macrophages have increasingly been implicated in the initiation, perpetuation and eventual resolution of inflammation in AAV, with various macrophage markers having been investigated as biomarkers of disease activity. Soluble CD163 is a glycosylated membrane protein that functions as a scavenger receptor for haemoglobin–haptoglobin complexes and is released by anti-inflammatory macrophages following exposure to pro-inflammatory stimuli. CD163<sup>+</sup> macrophages are the most prevalent cells observed in the glomeruli of patients with AAV at sites of fibrinoid necrosis and cellular crescents<sup>114</sup>. Additionally, *CD163* mRNA expression was high in the glomeruli and tubulointerstitium of microdissected kidney tissue from individuals with AAV, and was also detected in glomeruli and urine in an experimental model of AAV<sup>115</sup>. Soluble urinary CD163 (suCD163) normalized to creatinine could reliably identify active kidney vasculitis in patients already diagnosed with small-vessel vasculitis<sup>115</sup>, and distinguish active vasculitis from flare-mimics with a sensitivity and specificity of 83% and 96%, respectively. Moreover, suCD163 levels correlated with histological changes, including the proportion of glomeruli with fibrinoid necrosis and cellular crescents<sup>116</sup>. However, 13–26% of patients with active kidney vasculitis have undetectable suCD163.

CC-chemokine ligand 2 (CCL2; also known as MCP-1) is produced by macrophages and recruits monocytes, macrophages and T cells towards inflammatory sites. Elevated levels of urinary (but not blood) CCL2 have been reported in patients with active kidney vasculitis compared with those in remission, those with active extra-renal disease and healthy individuals; importantly, CCL2 decreased following standard cyclophosphamide-based treatment<sup>117</sup>. Furthermore, urinary CCL2 levels correlate positively with glomerular macrophage infiltration and the kidney component of BVAS. Combining suCD163 with uCCL2 or with the addition of soluble serum or urine CD25, serum calprotectin, serum CRP or serum NGAL can improve its accuracy in defining active disease<sup>118–120</sup>.

Gremlin, a bone morphogenetic protein (BMP) antagonist (member of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily) co-localizes with CD163 and CD68 (a pan-macrophage marker) in glomerular crescents of patients with pauci-immune GN<sup>121</sup>. In addition, urinary gremlin levels correlate with the proportion of crescents, but with poor sensitivity for detecting renal vasculitis (sensitivity of 55%, specificity 100%). Activin A also belongs to the TGF- $\beta$  superfamily and is expressed following macrophage stimulation. Activin A was expressed within glomerular crescents and interstitial macrophages. Levels of urinary (but not serum) activin A correlate with the number of cellular crescents in kidney-biopsy tissue from patients with AAV, and with urinary proteinuria; activin A levels diminish following treatment and demonstrated better accuracy in identifying kidney vasculitis (sensitivity 83% and specificity 70%) than Gremlin, with undetectable levels in healthy individuals<sup>122,123</sup>.

TNF-like weak inducer of apoptosis (TWEAK) – a cleaved leukocyte-derived protein that binds to CD163 and fibroblast growth factor-inducible 14 (Fn14) – was also elevated in the urine of patients with active disease compared with remission and correlated weakly with BVAS, albuminuria and percentage of crescents on biopsy tissue. Moreover, TWEAK was detectable in both the glomerular and tubular compartments in patient biopsy samples. Of note, levels of urinary TWEAK were higher in individuals who had already received immunosuppression (including glucocorticoids or cyclophosphamide), potentially limiting its utility as a disease biomarker<sup>124</sup>. The interplay between TWEAK, CD163 and Fn14 is complex and might have an important role in modulating inflammation in the context of autoimmunity<sup>125</sup>.

## Adaptive immunity

**Humoral immunity.** Loss of B cell tolerance is central to the pathogenesis of AAV, as autoreactive B cells produce ANCA. The number of activated B cells correlates with disease activity<sup>76</sup> and circulating B cell subset repopulation can predict relapse<sup>126</sup>. B<sub>reg</sub> cells, which are crucial in maintaining tolerance via the production of the regulatory cytokine IL-10, are reduced in AAV compared with healthy individuals<sup>127</sup>. Additionally, low serum levels of IL-10 in patients under remission seem to be associated with a higher relapse risk<sup>61,128</sup>. The proportion of circulating CD19<sup>+</sup>CD5<sup>+</sup> B cells might be a surrogate marker for B<sub>reg</sub> cells in AAV and correlates inversely with disease activity<sup>129</sup>. Similarly, an increase in memory CD27<sup>+</sup> B cells has been associated with relapse and kidney disease severity<sup>130</sup>. Newer studies have described autoantigen-specific B cell populations, but they are challenging to identify, requiring fluorochrome-conjugated antigens, and are not yet suitable for use as routine disease biomarkers<sup>131</sup>.

B cell Activating Factor (BAFF) and A Proliferation-Inducing Ligand (APRIL) are both members of the TNF ligand superfamily; BAFF promotes survival and differentiation of immature B cells, whereas APRIL has a role in the late B cell maturation process and supports plasma-cell survival<sup>132</sup>. Increased BAFF levels have been reported in AAV<sup>133,134</sup> and might contribute to the positive selection and survival of autoreactive effector B cells after rituximab treatment<sup>135</sup>. Moreover, a pharmacogenetics study in patients with AAV treated with rituximab reported that an SNP in the regulatory area of *TNFSF13B* (encoding BAFF) was associated with a higher rate of rituximab failure within 12 months of administration<sup>136</sup>. Despite this link, the anti-BAFF monoclonal antibody belimumab did not reduce relapse rates in AAV when given as maintenance with azathioprine, following induction with rituximab or cyclophosphamide<sup>137</sup>. Results of a trial using a combination of belimumab and rituximab, compared with rituximab monotherapy are

awaited. Newer therapies targeting APRIL with clinical benefit in some glomerular diseases remain to be tested in AAV<sup>138</sup>.

**Cellular immunity.** In addition to humoral immunity, cellular immunity is important in AAV pathogenesis. Following activation, ANCA-activated neutrophils release autoantigens that can be recognized by CD4 and CD8 T cells within inflamed tissues. Experimental models of MPO-AAV demonstrate that depletion of CD4 or CD8 T cells reduces disease severity<sup>139</sup>. Furthermore, adoptive transfer experiments showed that T cells can induce glomerular injury independently of ANCA.

In people with AAV, the predominance of class-switched IgG1 and IgG4 circulating ANCA implies the participation of T follicular helper cells in the immune response<sup>140</sup>. Moreover, people with AAV have elevated levels of CD4<sup>+</sup> effector memory T cells in peripheral blood and urine<sup>141</sup>, with both CD4 and CD8 T cells also being detected within inflamed glomeruli<sup>105</sup>. Urinary levels of CD3<sup>+</sup> T cells (with either T<sub>H</sub>17 or regulatory T (T<sub>reg</sub>) cell profiles) are found in greater numbers in active disease and seem to perform better in detecting disease activity than either CCL2 or CD163 (sensitivity of 94% and specificity of 92%)<sup>142</sup>. Moreover, in a single-centre study, elevated urinary CD4 T cell numbers can predict future flares, with sensitivity and specificity of 60% and 97.8%, respectively. Cytokine profiling in people with AAV revealed a diverse array of effector cytokines, including IFN $\gamma$  and TNF<sup>143</sup>, which are prominent in granulomatous lesions. T<sub>reg</sub> cells in people with AAV have attenuated suppressive capacity and ongoing research has investigated T<sub>reg</sub> cell subset heterogeneity for potential biomarkers<sup>144</sup>.

The regulation of T cell activation is partly dependent on co-stimulatory signals, including activating and inhibitory pathways. Novel programmed cell death 1 (PD1) agonistic antibodies have been tested in some autoimmune diseases<sup>145</sup> and in kidneys with active vasculitis, PD1 staining was reduced in the interstitium, with raised levels of programmed cell death ligand 1 (PDL1)<sup>146</sup>; PD1 staining was also low in sclerotic biopsy tissue<sup>147</sup>. However, others found lower levels of PDL1 staining<sup>148</sup> in active AAV than in other forms of GN. In blood, results are also inconsistent, with greater PD1 cell surface expression on T cells<sup>149</sup>, and higher levels of soluble PD1 and PDL1 reported in individuals with AAV than in healthy controls; these increases were not reproducibly observed in acute disease compared with remission<sup>147,150</sup>. In the RAVE cohort samples, soluble PD1 (sPD1) and sPDL2 were lower in PR3-ANCA disease than in MPO-ANCA, and lower in relapsing patients than in those in remission<sup>151</sup>. Whether soluble PD1–PDL1 will be useful biomarkers of disease remains to be clarified. By contrast, PD1 and cytotoxic T lymphocyte antigen 4 (CTLA4) overexpression on CD8 T cells is characteristic of T cell exhaustion and this transcriptome phenotype is associated with lower relapse rates in AAV<sup>152,153</sup>. Soluble immune-checkpoint molecules beyond PDL1 and PDL2, including B and T lymphocyte attenuator (BTLA) were also investigated in the RAVE trial cohort. In patients treated with rituximab, lower baseline levels of sLag3 and higher soluble CD27 (sCD27) were associated with treatment failure, whereas higher sCD27, BTLA and TIM3 were associated with subsequent remission<sup>151</sup>.

Other markers of T cell activation demonstrate that T cells are persistently activated even during periods of clinical remission and with ongoing treatment, indicating the potential efficacy of anti-T cell therapies in relapse prevention<sup>154</sup>. Other co-stimulatory targets have been investigated in the context of AAV biomarkers (Table 6).

## Endothelial cells

Histological findings suggest that endothelial cells undergo necrosis, detach from the basement membrane and are released into peripheral blood in AAV. Of note, inhibition of neutrophil activation and NETosis reduces endothelial cell death in vitro<sup>106</sup>. Increased numbers of circulating endothelial cells are detected in patients with active AAV compared with those in remission, healthy individuals and disease controls (that is, people with infections or non-ANCA-associated glomerular diseases). Although these levels decrease within 6 months of immunosuppressive treatment, they remain higher than those observed in healthy individuals<sup>155</sup>. These findings suggest ongoing low-grade inflammation that can promote chronic vascular damage and thus contribute to the relapsing disease course and the increased cardiovascular morbidity and mortality of patients with AAV<sup>156</sup>. In addition, they might partly explain some of the associations of ongoing inflammation and CKD progression. Under homeostatic conditions, damaged vasculature is repaired by endothelial progenitor cells, which are mobilized from the bone marrow when vascular injury occurs<sup>157</sup>. The proliferative capacity of both endothelial progenitor cells and angiogenic T cells (CD3<sup>+</sup> cells with pro-angiogenic function that are pivotal for endothelial progenitor cell differentiation) is impaired during active and relapsing AAV<sup>158</sup> (Table 4).

## Biomarkers of over-immunosuppression and tissue damage

Although much emphasis has been placed on defining biomarkers of disease activity or impending relapse based on the pathophysiology of AAV<sup>159</sup>, treatment decisions could be facilitated by alternative strategies to guide immunosuppression, potentially based on markers of immune function, similar to the current approach used to manage solid organ transplant recipients (reviewed in Dendle et al.<sup>160</sup>). These markers might be related to immune effector cell function and mediators, or subclinical infection. Low levels of cellular or humoral immunity (such as total lymphocyte count, CD4 T cell numbers or IgG levels) are predictive of subsequent infection and associated mortality in both immunocompetent and immunosuppressed cohorts<sup>161–163</sup>. Detection of these alterations might prompt reduction in immunosuppression, when possible, or replacement therapy (such as intravenous immunoglobulin). Alternative functional immune assays have attempted to assess the responsiveness of CD4 T cells by measuring ATP levels after non-specific mitogen stimulation (ImmunoKnow assay). In liver-transplant recipients, infection incidence was reduced in those whose immunosuppression was adjusted based on the assay<sup>164</sup>. However, data on the use of such an assay in vasculitis cohorts are limited, especially in individuals with impaired kidney function.

Levels of viraemia associated with subclinical infections have been used historically as markers of immune competence (for example, EBV DNA levels), although these assays are neither standardized nor always easily interpretable. Measuring the load of Torque Teno virus, which is a ubiquitous and non-pathogenic virus, has been assessed in vasculitis and transplant cohorts, and was measured in a subset of patients from the RAVE cohort<sup>165</sup>. Preliminary analyses suggest that viral load increases after immunosuppression initiation and is lower in individuals who subsequently relapse. No associations between Torque Teno virus load and infection parameters were reported in the vasculitis cohort, but have been found among kidney-transplant recipients, implying over-immunosuppression<sup>166</sup>.

Several other urinary biomarkers that correlate with fibrosis rather than active vasculitis – including endotrophin, Dickkopf-3, Procollagen type VIC-terminal propeptide (PRO-C6), collagen type III degradation marker (C3M), epidermal growth factor, MMP-2 and

TIMP-1 – have been tested in AAV<sup>167,168</sup>. These fibrotic markers might be useful to confirm the presence of sclerosis and damage rather than inflammation, but only limited analyses on baseline samples have been carried out. Longitudinal changes could be informative and whether a composite measure of biomarkers of disease activity, fibrosis and immune competence might be useful to guide therapy in patients in clinical remission remains uncertain (Table 6). Whether these bioassays are sufficiently sensitive and reproducible to allow dynamic changes in immunosuppression for individuals is also unclear.

## Biomarker-based treatments and their impact on biomarker sensitivity

The type of treatment can also affect the reliability of biomarkers. For example, as mentioned above, the increase in serum IL-6 levels, as well as the changes in calprotectin between presentation, and 2 or 6 months post-therapy, only predict relapses in people treated with rituximab<sup>45</sup>, whereas urinary CD163 tends to decrease fastest in those treated with cyclophosphamide<sup>169</sup>. Whether these findings simply reflect the limited number of patients available to be studied or truly reflects a biological difference is not completely clear, but should be considered when contemplating biomarker panels for predicting disease activity, kidney progression or kidney relapse. This consideration becomes especially important as treatments become more targeted and selective. To date, no trials have used biomarkers beyond ANCA in patient selection or trial design, but with more robust data, this approach might change in the future.

## Complement antagonism

Avacopan was superior to a standard glucocorticoid-tapering regimen when given in combination with rituximab or cyclophosphamide, and has emerged as an effective adjuvant treatment for inducing remission in AAV<sup>170</sup>. Low serum C3 levels in people with AAV are often associated with thrombotic microangiopathy on kidney biopsy, poorer kidney outcomes and an increased risk of death<sup>20</sup>. Elevated Ba levels in urine but not plasma are also observed during a kidney flare<sup>101</sup>. However, to date, variations in complement levels and responsiveness to complement-targeting strategies have not been reported.

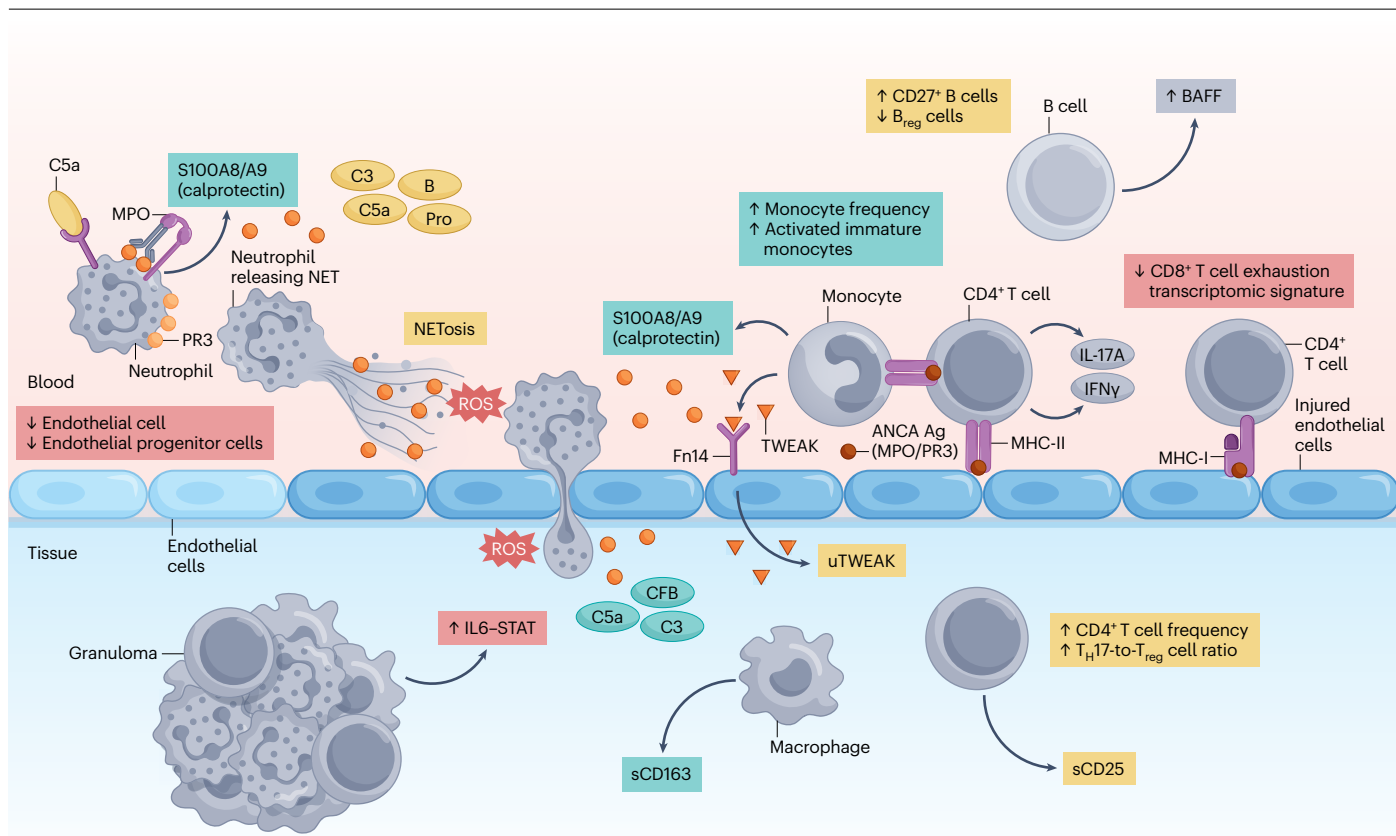
## CD4 T cell-targeted therapy

Given the role of costimulatory pathways in T cell activation, augmenting the action of regulatory costimulatory molecules could be an approach to attenuating autoimmunity. The efficacy of abatacept<sup>171</sup>, a fusion protein comprising the Fc region of IgG1 fused to CTLA-4, which acts as a CTLA4 agonist and prevents costimulatory signalling via CD80 and CD86, was tested in non-severe relapsing GPA but its addition to glucocorticoids had no disease control benefit<sup>172</sup>. PD1 agonistic monoclonals had some benefit in rheumatoid arthritis, but have not yet been tested in AAV<sup>145</sup>. Few other specific T cell-directed therapies have been tested, but might be beneficial as relapse rates in kidney transplant recipients with AAV seem to be much lower than in those with native kidneys, perhaps suggesting that calcineurin inhibitors have a disease-suppressing benefit.

**Table 6 | Summary of available blood and urine biomarkers, and large datasets to support current and future patient assessment**

Disease status	Renal biopsy	Blood	Urine	Large datasets
<b>Current status assessment</b>				
Active disease	↑T <sub>H</sub> 1 and T <sub>H</sub> 17 cell infiltrates Complement deposition correlating with crescents High activity lesions (necrosis, crescents)	ANCA, anti-plasminogen, anti-tPA, anti-moesin ↑Calprotectin ↑Endothelial cells	↑ CCL2 and CD163 ↑ Urine complement ↑ sCD25 ↑ TWEAK, Gremlin, activin A	Kidney risk score FAIRVASC Trajectory groups (rapid progressors with inflammatory phenotype)
Remission	Glomerular transcriptomic CLU signature Mayo Clinic Chronicity Score <4	CD8 T cell exhaustion signatures (PD1, CTLA4) Anti-PTX3 antibodies (high) Naïve B cell repopulation post-RTX	No validated urine biomarker to predict complete kidney quiescence yet	
Over-treatment	Chronic scarring without active crescents	↑TTV and EBV viral load Lymphopenia Hypogammaglobulinaemia	↑ Fibrosis markers: endotrophin, Dickkopf-3, PRO-C6, C3M, EGF, MMP-2, TIMP-1	
<b>Future status prediction</b>				
Relapse	Berden sclerotic class initial biopsy Persistence of CD3 cells on repeat biopsies (limited data)	Rising ANCA (PR3 > MPO), return of ANCA after negativity ↓ Naïve B cell repopulation ↑ PR3-specific plasmablasts ↑ IL-6 prior to relapse in RTX-treated GPA patients ↓ Soluble PD1/PDL2 ↓ CD8 exhaustion signature (≠ tolerant group) ↑ NET-inducing activity	High urinary CD4 T cells; high T <sub>H</sub> 17 cells and low T <sub>reg</sub> cells Urinary CD4 T cells + ANCA combination Low PD1/PDL2 in PR3-ANCA	FAIRVASC clusters with higher relapse rates (PR3-ENT, renal-inflammatory cluster) Trajectory: fast-decline CKD group shows relapse and/or inflammation enrichment
LTROT/tolerant phenotype	CLU-rich 'protective' transcriptomic cluster	Higher B <sub>reg</sub> and T <sub>reg</sub> cells Female, MPO-ANCA, MPA phenotype (demographic predictors)	Low uCD163, low MCP-1 Low urinary CD4 T cells High urinary T <sub>reg</sub> cells	

ANCA, anti-neutrophil cytoplasmic antibodies; CKD, chronic kidney disease; CLU, clusterin; EBV, Epstein-Barr virus; ENT, ear-nose-throat; GPA, granulomatous polyangiitis; LTROT, long-term remission off treatment; MPA, microscopic polyangiitis; MPO, microscopic polyangiitis; NET, neutrophil extracellular trap; RTX, rituximab. These biomarkers and datasets have current or potential utility in assessing disease activity, remission, over-treatment, relapse and LTROT.



**Fig. 1 | Microvascular kidney injury and biomarker detection in anti-neutrophil cytoplasmic antibody-associated vasculitis.** Anti-neutrophil cytoplasmic antibody (ANCA)-activated, primed neutrophils are recruited by chemokines to endothelial cells in the kidney microvasculature, resulting in endothelial injury through multiple mechanisms. These neutrophils generate reactive oxygen species (ROS), degranulate to release proteases, ANCA antigens, serum calprotectin and neutrophil-derived microparticles, and form neutrophil extracellular traps (NETs) that induce endothelial cell death. The release of ANCA antigens facilitates their processing and presentation as antigenic peptides, thereby activating effector T cells. This activation promotes the differentiation of CD4<sup>+</sup> IL-17-producing T helper (T<sub>H</sub>17) cells, which support B cell responses, and CD8<sup>+</sup> T cells in which an exhausted

phenotype is associated with a lower risk of disease relapse. B cells differentiate into plasma cells and memory B cells at the expense of regulatory B cells, contributing to disease pathogenesis. Antigen-presenting cells in ANCA-associated vasculitis include endothelial cells, monocytes and dendritic cells. Notably, an increased monocyte frequency and the expansion of activated immature monocytes is associated with relapse. Extravascular tissue injury occurs through the infiltration of inflammatory leukocytes, which can be detected in urine, with soluble CD25 (sCD25) being shed from activated T cells. Tissue-resident and recruited macrophages contribute to inflammation and fibrosis, shedding soluble CD163 (sCD163) upon activation. Additionally, leukocyte aggregation within granulomas exacerbates tissue damage by activating the IL-6-STAT signalling pathway.

## B cell, plasma cell and natural killer cell depletion

The next-generation anti-CD20 antibody obinutuzumab, which is a fully humanized antibody, promotes higher antibody-dependent cellular cytotoxicity activity and enhanced direct B cell killing<sup>173</sup>. This treatment is currently being trialled as an alternative to rituximab to evaluate whether greater tissue B cell depletion can reduce relapse rates<sup>174</sup>.

Daratumumab, an anti-CD38 antibody used to target long-lived plasma cells in some autoantibody-mediated diseases, was used to treat two patients with severe refractory AAV and CD20<sup>-</sup> B cells in kidney biopsy tissue post-rituximab and cyclophosphamide<sup>175</sup>. Daratumumab led to clinical and immunological response, with the depletion of CD38<sup>+</sup> plasma and NK cells highlighting the need for plasma-cell-targeted therapies.

More profound B cell depletion might be achieved with bispecific CD3-CD19 or CD3-BCMA monoclonal antibodies that engage T cells with B cells or plasma cells and eliminate antibody-producing cells.

These antibodies have been used in haematological malignancies<sup>176</sup> and were recently used in isolated cases of autoimmune conditions such as systemic sclerosis<sup>177</sup>; no AAV data are available.

CAR T cell therapy is the most effective therapy for achieving profound tissue B cell depletion. To date, at least 18 trials are testing CAR T cells and two trials are using CAR NK cells for the treatment of AAV. CAR T cell trials are based on the hypothesis that CAR T cells in AAV will induce profound B cell depletion, eliminate autoreactive B cells, allow a 'B cell reset' and potentially cure the disease<sup>178</sup>. Preclinical data in an animal model of MPO-ANCA vasculitis suggest that this approach might be beneficial in AAV<sup>179</sup>, and three case reports of patients with refractory AAV (PR3- or MPO-ANCA) reported various degrees of success with CAR T cell therapy<sup>180-182</sup>. However, this treatment is costly, time-consuming and might not be suitable for all patients with AAV. T<sub>reg</sub> cell expansion and antigen-specific therapies in AAV have also been proposed (reviewed in Free<sup>183</sup>).

Data on the role of NK cells in AAV are largely lacking and somewhat inconclusive, with small studies reporting both increases and decreases in NK cell numbers and frequencies in disease, compared with healthy individuals, or between active disease and remission. These data come from heterogeneous AAV cohorts that include patients at different stages of disease activity, varying treatment modalities and differing disease phenotype<sup>184</sup>. In GPA, the number of circulating NK cells is reported to correlate negatively with disease activity<sup>185</sup>. Moreover, rituximab treatment in GPA enhanced activation and degranulation of NK cells, which contributed to B cell depletion; this effect was enhanced with obinutuzumab<sup>186</sup>. If validated, this finding suggests that NK cell count and function could potentially be used as a prognostic marker for the long-term effect of B cell-depleting treatments.

## Home monitoring and point of care testing with novel or conventional biomarkers

Many vasculitis centres care for patients who live beyond their immediate catchment area, which might require patients to undertake long journeys for appointments, with additional economic and work-related impacts. Overall, patients bear this burden for the reassurance of ongoing monitoring of disease activity, and the idea that early recognition of disease re-activation might prompt changes in management that could prevent a full-blown relapse and a return to the state of ill health they experienced at the initial presentation. However, this approach relies on the coincidental finding of changes in biochemical or immunological parameters during routine visits or requires additional return visits at times of perceived clinical change. Since the implementation of remote patient monitoring during the COVID-19 pandemic, home monitoring options have become increasingly available, including the use of local blood or urine analysis centres coupled with a tele-medicine approach, which saves travel time and reduces unnecessary journeys. In addition to these simple approaches, home monitoring with point-of-care testing or microsampling devices sent to central laboratories offer the hope of better and more timely analysis of disease states when patients notice that a change in clinical status might have occurred. Such devices are increasingly being trialled for home drug monitoring or kidney function testing and might have expanded applications with appropriate logistical support<sup>77</sup>. Which biomarker or set of biomarkers would be most appropriate for early flare recognition remains to be decided, but the methodology to enable such monitoring could be readily tested.

## Conclusions

Current methods for assessing ongoing kidney disease activity in AAV are somewhat limited and fail to adequately differentiate ongoing inflammation from damage caused by prior disease activity. In addition, the ability to predict imminent disease relapses is currently highly limited and does not enable treatment customization for individual patients. This limitation results in both over- and under-treatment for many patients, with resultant infection-related complications that contribute greatly to morbidity and mortality, especially in older patients with impaired kidney function or progressive disease. The search for better, ideally non-invasive biomarkers that can detect ongoing kidney inflammation and predict future disease activity has gathered pace, with many diverse potential candidates (Fig. 1), often based on urine testing, rather than blood. However, little or insufficient validation has been carried out for most of these candidates, further complicated by the observation that biomarker utility is affected not only by the disease phenotype but also by prior treatment exposure. These barriers have limited the clinical uptake of new biomarkers to date. Importantly,

many of these novel biomarkers should be integrated with clinical data into a usable platform that could inform clinicians in real time of immune status changes. However, whether such approaches will translate into improved kidney and overall patient survival remains unclear. Moreover, where biomarker data indicate the need for augmented immunosuppression, an unfavourable adverse effects profile, particularly in older patients with multi-morbidity, might ultimately become an important limiting factor. Models combining various biomarkers, clinical phenotypes and treatments will likely be required to translate the research findings of some of these biomarkers into daily clinical practice.

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## Author contributions

All authors researched data for the article, and reviewed or edited the manuscript before submission. M.A. and A.D.S. made substantial contributions to discussions of the content and wrote the manuscript.

## Competing interests

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## Additional information

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