



Review article: experimental therapies in autoimmune hepatitis

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Summary

Background: Current therapeutic options for autoimmune hepatitis (AIH) are limited by adverse events associated with corticosteroids and thiopurines and the limited evidence base for second- and third-line treatment options. Furthermore, current treatment approaches require long-term exposure of patients to pharmacological agents. There have been significant advances in the understanding of the mechanisms underpinning autoimmunity and an expansion in the available therapeutic agents for suppressing autoimmune responses or potentially restoring self-tolerance.

Aim: To review the mechanisms and evidence for experimental therapies that are being actively explored in the management of AIH.

Methods: We have reviewed the literature relating to a range of novel therapeutic immunomodulatory treatment strategies and drugs.

Results: Drugs which block B cell-activating factor of the tumour necrosis factor family (BAFF) and tumour necrosis factor α are currently in clinical trials for the treatment of AIH. Experimental therapies and technologies to increase immune tolerance, such as pre-implantation factor and regulatory T cell therapies, are undergoing development for application in autoimmune disorders. There is also evidence for targeting inflammatory pathways to control other autoimmune conditions, such as blockade of IL1 and IL6 and Janus-associated kinase (JAK) inhibitors.

Conclusions: With the range of tools available to clinicians and patients increasing, it is likely that the therapeutic landscape of AIH will change over the coming years and treatment approaches offering lower corticosteroid use and aiming to restore immune self-tolerance should be sought.

The Handling Editor for this article was Dr Stephen Ryder, and this uncommissioned review was accepted for publication after full peer-review.

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1 | INTRODUCTION

Autoimmune hepatitis (AIH) manifests as an inflammatory disorder of the liver parenchyma and is associated with hypergammaglobulinaemia, circulating autoantibodies, specific HLA-DR alleles and characteristic histological changes.¹⁻⁵ AIH may result in a chronic, fluctuating hepatitis, potentially leading to progressive fibrosis, cirrhosis and chronic liver failure. It may also present as an acute severe hepatitis with the potential for acute liver failure.⁶ Defined as a rare disease, with a prevalence of 11.6-34.5 per 100 000 population,⁷⁻⁹ the condition accounts for 5% of elective liver transplants in Europe¹⁰ and is an indication for liver transplantation in the setting of acute liver failure.^{6,11}

Despite a long established evidence base for first-line therapy consisting of azathioprine and corticosteroids, and broadly agreed second-line therapy with mycophenolate mofetil (Table 1), 15%-20% of patients are intolerant of, nonresponsive to or have a contraindication to taking these therapies.¹² Both active disease and side effects from the currently available treatments are now recognised to have a significant negative impact on quality of life for patients, with corticosteroids being associated with the greatest reduction in

health utility.¹³⁻¹⁵ There is active interest in developing novel therapies that may reduce disease activity, enhance immune tolerance and avoid the adverse consequences associated with corticosteroid use in AIH.

Advances in our understanding of the mechanisms underpinning autoimmune responses and the recognition that similar genetic influences and effector mechanisms contribute to a wide range of single organ and systemic autoimmune diseases¹⁶⁻¹⁸ have resulted in a range of novel agents for controlling autoimmune responses. Due to the overlap in mechanism between different autoimmune disorders, it is plausible that treatments effective in one autoimmune disorder may be efficacious in AIH and hence should be considered for clinical trials in AIH.

In this review, we summarise the rationale and background to experimental therapies that have entered clinical trials in AIH, such as B cell-activating factor of the tumour necrosis factor family (BAFF) targeting therapies, anti-tumour necrosis factor (anti-TNF) therapy, pre-implantation factor and strategies that improve regulatory T cell function. We also discuss the potential for targeting proinflammatory cytokines IL1 and IL6 and Janus Kinase (JAK) inhibitors, which have received attention recently in a range of autoimmune disorders.

TABLE 1 Current treatment options for autoimmune hepatitis

	Drug class	Typical medications	Role in AIH
First line	Corticosteroids	Prednisolone	Strong evidence base for induction and maintenance of remission. Significant side effect burden.
		Hydrocortisone	
		Budesonide	
	Thiopurines	Azathioprine	Strong evidence base for induction and maintenance of remission.
		Mercaptopurine	No formal evidence of efficacy, small case series only. Possibly equivalent to azathioprine
		Tioguanine	
Second line		Mycophenolate mofetil	Used for maintenance of remission. Evidence of efficacy based on case series. RCT vs azathioprine ongoing
Third line	Calcineurin inhibitors	Tacrolimus Ciclosporin A	Probably effective in setting of intolerance and nonresponse to first- and second-line agents. Evidence from multiple case series. Ciclosporin used in paediatrics
	Anti-CD20	Rituximab or anti-CD20 biosimilars	Probably effective in maintenance of remission, evidence from small case series
Experimental therapies			
Current trials	Anti-BAFF	Ianalumab	Multicentre, randomised placebo-controlled phase II/III trial ongoing
	Anti-TNF	Infliximab	Small case series suggesting efficacy in rescue therapy in difficult-to-treat patients
Possible future trials	Novel peptides	Pre-implantation factor	Phase I safety study in patients with AIH complete. Experimental only
	Treg therapies	Adoptive Treg transfer	Early phase trials completed in other autoimmune conditions and transplantation. Feasible in AIH
		Low-dose IL2	Case reports of short-term efficacy in a few patients
		Treg stimulators	Not tested in AIH, in early phase trials in other autoimmune conditions
No trials currently planned	IL1 blockade	Canakinumab	No reports of use in AIH, ongoing trials in acute alcoholic hepatitis
	IL6 blockade	Tocilizumab	No reports of use in AIH
	JAK inhibitors	Filgotinib Ruxolitinib	No reports of use in AIH, increasing use in other autoimmune conditions

Abbreviations: BAFF, B cell-activating factor of the tumour necrosis factor family; IL, interleukin; JAK, Janus kinase; RCT, randomised controlled trial; TNF, tumour necrosis factor.

Finally, we discuss the potential approach for investigative studies that may bring novel agents into practice in AIH. Any novel therapies should only be offered in specialist centres with an interest in AIH, experience in clinical trials and within formal prospective clinical trials wherever possible.

Firstly, we will briefly review the current understanding of the immunopathogenesis of AIH to illustrate the context for possible treatment approaches.

1.1 | Current treatment approaches

The current treatment paradigm in AIH is induction of remission with corticosteroids (typically prednisolone 0.5-1 mg/kg) and then maintenance of remission with azathioprine started at 1 mg/kg while corticosteroids are weaned, with the aim of azathioprine monotherapy in the long term (Table 1).^{12,19} Remission is defined as normalisation of serum transaminases and IgG with minimal histological activity on biopsy (although this is rarely performed to confirm remission).¹⁹ Some patients require long-term low-dose corticosteroids in addition to azathioprine to maintain remission. Inadequate response to azathioprine should prompt consideration of dose optimisation to 2 mg/kg,^{19,20} therapeutic drug monitoring for toxicity²¹ and assessment of treatment concordance.

The majority of patients respond to treatment with corticosteroids and azathioprine, with remission rates of 44%-100% observed,^{13,20,22-25} although these studies often used high doses of corticosteroids for maintenance. Despite the potential efficacy of this treatment approach, failure to achieve remission is common; a large, real-world assessment of current treatment in >1200 patients in the UK demonstrated that biochemical remission was achieved in <60% of patients.²⁶ Furthermore, among patients in complete biochemical remission, relapse rates of >50%²⁷ and significant corticosteroid side effects in 30%-40% of patients¹³ are observed, demonstrating that despite current treatment approaches being potentially effective, a significant proportion of patients will continue to experience disease activity or treatment toxicity.

Intolerance, inadequate response or contraindication to corticosteroid and azathioprine treatment, including a requirement for high-dose corticosteroids (>10 mg prednisolone per day) to maintain remission, should prompt consideration of second-line therapies. Mycophenolate mofetil is typically offered as a second-line agent for those patients intolerant to azathioprine (Table 1)²⁸ and although there is no high-quality trial evidence to support this approach, there are extensive case series data suggesting efficacy.²⁹⁻⁴² The efficacy of mycophenolate mofetil as a second-line drug for those patients with insufficient response to standard therapy is questionable, and optimising first-line therapy by checking adherence and drug levels needs to be considered. Third-line therapies, including tacrolimus, infliximab and anti-CD20 monoclonal antibodies (rituximab), may be employed (Table 1) depending upon local availability, monitoring and clinician and patient choice.²⁸

Despite this range of treatment approaches, there remain up to 20% of patients who do not respond adequately to treatment or experience significant side effects.^{43,44} There is, therefore, a need for novel agents to expand the range of options for patients with AIH and their treating clinicians.

2 | IMMUNOPATHOGENESIS OF AIH

The immunopathogenesis of AIH is underpinned by the loss of self-tolerance and resultant immune-mediated damage of the liver. An increased risk of AIH is associated with a range of genetic variants; variants at human leucocyte antigen (HLA)-DRB1, -DRB3 and -DRB4 loci have most commonly been identified.^{2,45-47} Non-HLA associations, including immune regulation or signalling pathways such as cytotoxic T lymphocyte antigen 4,⁴⁸⁻⁵⁰ the transcription factor STAT4,⁵¹ SH2B3,² Fas⁵² and the vitamin D receptor,^{53,54} and several cytokine pathways, including TNF α ^{55,56} and transforming growth factor- β ,⁵⁷ among others, have been described. A range of environmental factors have been suggested to trigger autoreactive immune responses resulting in the loss of self-tolerance in susceptible individuals, including drugs and viral infections.⁵⁸

Loss of self-tolerance results from autoreactive CD8 and CD4 T cells and B cells recognising autoantigens presented on class I and class II HLA leading to their activation and proliferation. B cells mature into plasma cells and secrete antibodies capable of recognising self-antigens, cytotoxic CD8 T cells directly damage tissues and CD4 T cells secrete a range of proinflammatory cytokines resulting in the recruitment and activation of other immune effectors,⁵⁹ including mucosal-associated invariant T cells, natural killer cells and natural killer T cells, all of which may be important in AIH.⁵⁹⁻⁶¹

Adaptive immune responses are typically constrained by a range of mechanisms: restriction of antigen presentation, control of (co-) stimulatory signals, regulatory cytokines and specific cell populations, termed regulatory cells, which suppress the proliferation and activation of adaptive immune effectors. Regulatory CD4 T cells (Tregs) and regulatory B cells (Bregs) control T cell responses and are important in the maintenance of self-tolerance.^{62,63} Impairment of Treg function and frequency has been recognised as a potential factor in AIH⁶⁴⁻⁶⁷ and similarly Breg have been recognised to be important in a range of autoimmune diseases.⁶⁸

Immune cell activation in the liver results in changes in the cytokine profile and alteration of the recruitment and activation of other immune cell subsets. CD4 T cells typically differentiate into subtypes with specific cytokine profiles: Th1 CD4 T cells secrete interferon- γ (IFN γ) that induces upregulation of class I and class II HLA expression, resulting in increased antigen presentation and also stimulates CD8 T cells and macrophages resulting in IL1 and TNF α release. Th2 T cells produce IL4, IL10 and IL13 resulting in B cell maturation.^{59,60} The presence of IL6 prevents the differentiation of Treg and results in the generation of Th17 cells,⁶⁹ which have been implicated in AIH.^{70,71} Th17 cells secrete IL17 which in turn induces release of IL6 by hepatocytes⁷²; this may enable a feedback loop increasing pathogenic

Th17 cell differentiation.⁷³ Furthermore, the inflamed liver microenvironment appears to suppress Treg function directly.^{59,60,74} The inflammatory response in the liver results in an inflammatory infiltrate consisting of CD4 and CD8 T cells, B cells, plasma cells, natural killer cells, monocytes, macrophages and eosinophils.⁷⁵

The pathology observed in AIH is a result of the activation of a network of cells, signalling pathways, regulatory mechanisms and cytokines from both the innate and adaptive limbs of the immune system. This offers a range of therapeutic targets where the inflammatory process in AIH may be interrupted and potentially even restore immune tolerance. Therefore, therapies with efficacy in other autoimmune diseases that target these pathways may be of use in AIH.

3 | NOVEL THERAPIES UNDERGOING CLINICAL ASSESSMENT IN AIH

3.1 | B cell-activating factor of the tumour necrosis factor family (BAFF) targeting therapies

The association of hypergammaglobulinaemia and circulating autoantibodies with AIH and other autoimmune disorders suggests that B cell activity may be an important contributor to autoimmunity. Therefore, therapeutic targeting of B cells to reduce the production of potentially pathogenic antibodies and B cell antigen-presenting activity in autoimmunity⁷⁶ is an attractive proposition.

Rituximab, an anti-CD20 monoclonal antibody, is an established therapy in autoimmune disorders and may have a role in

the treatment of AIH.⁷⁷⁻⁷⁹ A recent retrospective report collating outcomes for 22 patients in Northern Europe and Canada demonstrated an improvement in liver biochemistry and reduction in corticosteroid doses following two 1000 mg doses of rituximab given 2 weeks apart (five patients received repeat dosing),⁸⁰ suggesting that therapeutic targeting of B cells might be a useful approach in AIH.

Recently, a range of agents targeting BAFF have been developed. BAFF is a 285 amino acid protein produced by myeloid cells that may be membrane bound or cleaved by proteases producing a soluble cytokine. BAFF transmits a B cell survival signal important for B cell development (Figure 1) and interacts with three receptors on B cells, binding either as multimers or heteromers with a related protein called A proliferation-inducing ligand (APRIL).⁸¹

While the importance and pathogenic role of B cells and autoantibodies in AIH are debated, several pieces of evidence support the therapeutic targeting of B cells. Soluble BAFF levels are elevated in AIH and correlate with serum transaminase, immunoglobulin G and bilirubin levels, and BAFF levels fall with corticosteroid treatment.^{82,83} Similarly, BAFF levels are elevated in a range of autoimmune disorders, including primary biliary cholangitis,⁸⁴ systemic lupus erythematosus,⁸⁵⁻⁸⁷ rheumatoid arthritis,^{85,86,88} coeliac disease,⁸⁹ Sjögren's syndrome,⁹⁰⁻⁹² systemic sclerosis,⁹³ myasthenia gravis^{94,95} and correlate with autoantibody titres.^{85,86,88,90,91,95,96} BAFF levels are also elevated in chronic inflammation in the settings of chronic infection, malignancy and allergy.⁹⁷ Additionally, liver-infiltrating B cells are seen in acute AIH, more so than when compared to other conditions, for example drug-induced liver injury.⁹⁸

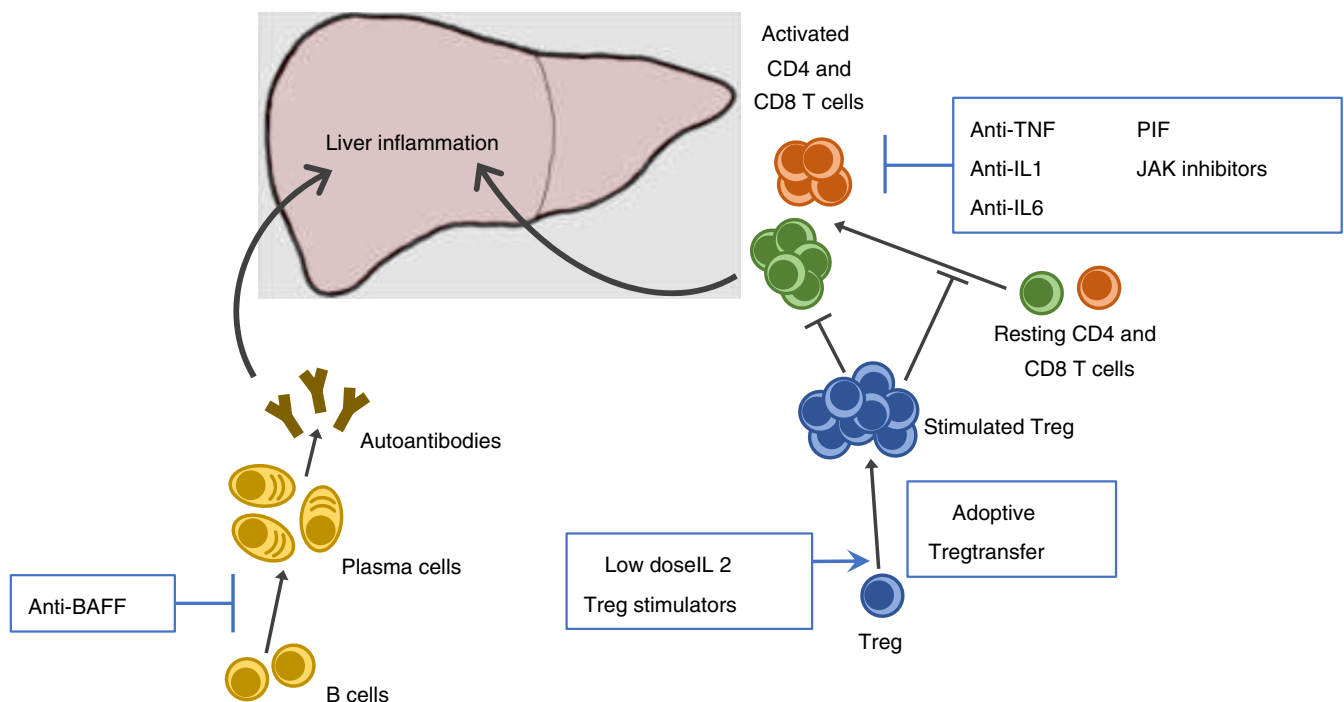


FIGURE 1 Proposed immunopathogenic mechanisms in AIH and sites of action of experimental therapies. BAFF, B cell-activating factor of the tumour necrosis factor family; JAK, Janus kinase; PIF, pre-implantation factor; Treg, regulatory T cells

Lack of efficacy or toxicity observed in trials for rheumatological disorders has led to the abandonment of several drugs targeting BAFF.^{99,100} Despite this, belimumab, a monoclonal anti-BAFF IgG1, has been FDA approved for the treatment of systemic lupus erythematosus based upon favourable trial outcomes.¹⁰¹⁻¹⁰³ Blisibimod, a peptibody made by the fusion of a BAFF-binding peptide to IgG1Fc,¹⁰⁴ has been demonstrated to improve fatigue in systemic lupus erythematosus, with an acceptable safety profile,¹⁰⁰ although other clinical benefits were limited.

While no formal testing of targeting the BAFF pathway in AIH has been reported to date, a phase II multicentre, randomised, double-blind, placebo-controlled trial of an anti-BAFF receptor monoclonal antibody (VAY736, inalumab) (Figure 2A) in patients with incomplete response or intolerance to current standard of care is underway (ClinicalTrials.gov: NCT03217422). Eighty adult patients with type 1 AIH and elevated alanine aminotransferase (ALT) (>1.5X upper limit of normal) and elevated IgG, despite (or intolerant of) adequate conventional first-line therapy will be randomised to one of four arms: placebo, inalumab 5 mg every 4 weeks, 50 mg every 4 weeks or 300 mg every 4 weeks for 24 weeks, after which the placebo arm will be switched to 150 mg active drug until week 52. A second phase of the study aims to enrol 280 patients randomised 5:2 inalumab:placebo for 52 weeks. The primary endpoint for both study phases is normalisation of ALT at 24 weeks with histological assessment at 24 (phase 1 only) and 52 weeks. The study is currently recruiting for phase 1.

Inalumab has been trialled in primary Sjögren's syndrome in a phase II, placebo-controlled study of 27 patients, demonstrating a good safety profile, without observed hepatotoxicity or increased infection risk.¹⁰⁵ An injection reaction with fever, headache, nausea and chills was common and may have been associated with higher circulating B cell frequencies at the time of treatment. Nonsignificant, dose-dependent trends towards improved clinical measures of disease activity and fatigue were observed.¹⁰⁵ The phase 2b extension study of 190 patients has been reported in an abstract, which described significant improvements in disease activity and similar safety profile.¹⁰⁶ Ongoing trials in chronic lymphocytic leukaemia (ClinicalTrials.gov: NCT03400176), systemic lupus erythematosus (ClinicalTrials.gov: NCT03656562) and rheumatoid arthritis (ClinicalTrials.gov: NCT02675803) will give further information regarding toxicity and tolerability. Advantages of inalumab include monthly subcutaneous injection making concordance easier, with the potential for self-injection at home and modest delivery costs. A positive trial outcome would offer robust evidence for B cell targeting therapies in AIH and potentially a new third-line agent; the results of this trial are awaited with interest.

3.2 | Anti-tumour necrosis factor therapy

Tumour necrosis factors (TNFs) are cytokines produced by macrophages and effector T cells. TNF α activates the NF κ B and MAPK

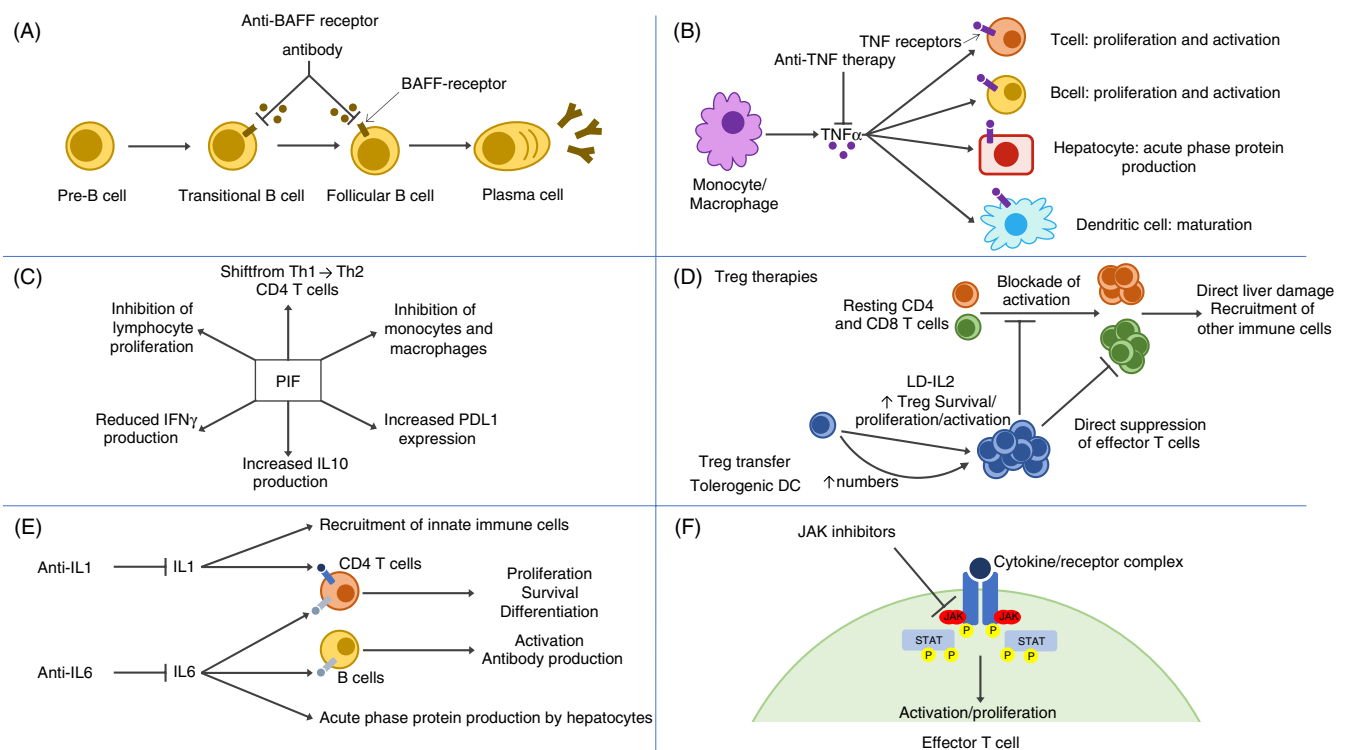


FIGURE 2 Mechanism of action of proposed experimental therapies in autoimmune hepatitis. BAFF, B cell-activating factor of the tumour necrosis factor family; DC, dendritic cells; IFN, interferon; JAK, Janus associated kinase; LD-IL2, low-dose IL2; PD-L1, programmed death ligand-1; PIF, Pre-implantation factor; Treg, Regulatory T cells

pathways via two receptors and complex signalling pathways, and can result in a range of proinflammatory responses (see Figure 2B), including cell apoptosis, proliferation or activation, that lead to necrosis and inflammation, depending upon which receptor is bound, and cross-talk with other signalling pathways.¹⁰⁷ The role of TNF α in autoimmune disorders is well established and several pharmacological agents that inhibit the actions of TNF- α are available, including monoclonal antibodies (infliximab and adalimumab) and soluble TNF receptor-IgG fusion molecules (etanercept). These drugs have established roles in the management of autoimmune and inflammatory disorders including rheumatoid arthritis, inflammatory bowel disease and psoriatic arthritis.¹⁰⁸

TNF α is important in inflammatory liver disease and there is growing evidence implicating it in the pathogenesis of AIH. TNF α -, IFN γ - and IL17-secreting T cells are believed to cause or co-ordinate hepatic damage; liver-infiltrating lymphocytes are enriched with populations of effector T cells that secrete these cytokines in AIH^{109,110} (Figure 1), and variants in the TNF α gene have been associated with AIH.^{55,56} Also, in a murine model of immuno-inflammatory hepatitis induced by the plant lectin concanavalin A, TNF α was pivotal in the generation of liver injury.¹¹¹ Therefore, with readily available drugs and evidence for a pathogenic role for TNF α , there has been growing interest in antagonism of TNF α to treat AIH.

Infliximab has been used as rescue therapy in patients with difficult-to-treat AIH; early experience demonstrated reduced inflammation; however, high rates of infectious complications were reported.¹¹²⁻¹¹⁴ However, a retrospective report of 11 paediatric cases with concomitant autoimmune liver disease (two with AIH and nine with autoimmune sclerosing cholangitis) treated with anti-TNF therapy for severe inflammatory bowel disease demonstrated stable or improved liver function tests and only a single infective complication of asymptomatic cytomegalovirus viraemia.¹¹⁵ There are concerns regarding idiosyncratic hepatotoxicity with anti-TNF therapy and the impact of this on patients with already impaired liver function needs to be carefully considered. In the largest series of 11 patients with AIH, some received infliximab following failure of first-line therapy with azathioprine or corticosteroids due to intolerance or inadequate response and a minority received it due to failure of second- and third-line therapies,¹¹² suggesting that infliximab may have a role at many points in the treatment algorithm for AIH (Table 1). Additionally, there is interest in the use of infliximab for induction therapy in AIH, and this is being actively explored in some centres, such as in Hamburg, Germany.

In the published experience with infliximab, 5 mg/kg intravenous infusion of infliximab at weeks 0, 2, 6 and 10 with ongoing treatment administered at monthly intervals has been used.¹¹² When used in inflammatory bowel disease, an immunomodulator is typically given concurrently to reduce the risk of the generation of anti-drug antibodies leading to reduced efficacy, and this should be considered in the setting of AIH. Due to the risk of infection with anti-TNF drugs, they should be avoided in the setting of active infection and used only in the setting of compensated liver disease. There are now a

range of biosimilar drugs available and these should be equivalent substitutes for infliximab.

3.3 | Pre-implantation factor

Pre-implantation factor is a 15 amino acid peptide secreted by mammalian embryos prior to implantation in the uterus.¹¹⁶ It functions to maintain pregnancy via a plethora of downstream actions mediated by binding to various proteins including insulin-degrading enzyme, Kv1.3b potassium channels, protein disulphide isomerases and heat shock proteins.^{117,118}

In addition to inducing alterations in the local endometrial tissues, direct trophic effects upon the foetus and neurodevelopmental and neuroprotective effects,¹¹⁶ pre-implantation factor also acts to modulate the maternal immune system, engendering maternal tolerance to the allogeneic embryo. This is achieved through pleiotropic mechanisms inducing changes in gene expression; including upregulation of IL1 receptor-associated kinase 1 binding protein and suppression of IL12-receptor subunit β -2, FK506 binding protein and immunoglobulin G gene and changes in adhesion proteins and regulators of apoptosis.¹¹⁷ Pre-implantation factor binds monocytes, activated CD4 and CD8 T lymphocytes and B lymphocytes and inhibits lymphocyte proliferation^{119,120} (Figure 1). Furthermore, pre-implantation factor influences lymphocyte cytokine production, increasing the production of immunoregulatory IL10, reducing proinflammatory cytokines, including interferon gamma, and induces a shift towards a Th2 profile of CD4 T cells.^{119,120} Pre-implantation factor also reduces nitric oxide production by macrophages, thereby reducing oxidative stress, and increases the expression of T cell inhibitory molecule PD-L1 by monocytes¹²¹ (see Figure 2C). Taken together, these data illustrate that pre-implantation factor is likely to modulate systemic immune responses and enhance immune tolerance.

Due to its potential effects on immune tolerance, the impact of pre-implantation factor on allo- and autoimmune responses has been considered. Pre-implantation factor reduces alloimmune-mediated damage in animal models of graft-versus-host disease.¹²¹ Administration of pre-implantation factor is protective against death and paralysis in experimental autoimmune encephalitis (a model of immune-mediated demyelination)^{122,123} and is protective in models of immune-mediated diabetes mellitus¹²⁴ and atherosclerosis.¹²⁵

Pre-implantation factor was awarded FAST-TRACK status by the FDA, and a phase I placebo-controlled study of pre-implantation factor administration in 18 patients with AIH demonstrated minimal toxicity.¹²⁶ Pre-implantation factor may now be taken forward to efficacy trials and represents a potential novel therapy for AIH, although a phase 2 trial was announced and then withdrawn as the manufacturer reported a change in the focus of drug development. The advantages of pre-implantation factor are that it is a naturally occurring peptide, with no evidence of anti-pre-implantation factor antibody formation in the phase I study. The required dosing interval

is currently unknown, although with a half-life of 91 minutes,¹²⁶ frequent subcutaneous dosing may be required.

3.4 | Strategies to enhance regulatory T cell function

CD4 regulatory T cells (Tregs) are defined by the presence of the transcription factor forkhead box P3 (FoxP3)¹²⁷ and constitutively express CD25 (IL2R α) and cytotoxic T lymphocyte antigen 4. They are capable of suppressing effector T cell responses by a variety of mechanisms including inhibition of CD28 co-stimulation,¹²⁸ sequestering IL2 via CD25 (thereby depriving effector T cells of this important stimulatory signal),¹²⁹ generation of the immunoregulatory nucleoside adenosine,¹³⁰ interleukin (IL) 10 and transforming growth factor- β secretion¹³¹ and direct cellular cytotoxicity.^{132,133}

Tregs are critical in the balance of self-tolerance and immune reactivity. Inability to produce functional Treg due to genetic deficiency of FoxP3 leads to immune dysregulation, polyendocrinopathy, enteropathy X-linked syndrome (IPEX) in humans and scurfy in mice, both of which are characterised by systemic autoimmunity and lymphoproliferation.¹³⁴⁻¹³⁷ Selective depletion of Treg in adult mice leads to a similar phenotype characterised by autoimmune hyper-reactivity,¹³⁸ suggesting that Tregs have a pivotal role in both the generation and maintenance of self-tolerance.

Tregs have been developed as a therapeutic option in autoimmune conditions, with focus recently in AIH. Ex vivo expansion, stimulation and transfer of Treg and the administration of low-dose IL2 are two strategies actively being explored in AIH (Figures 1 and 2D).

3.4.1 | Adoptive transfer of Treg

Due to the importance of Treg in maintenance of self-tolerance, the frequency and function of Treg in AIH have been investigated. Data on the frequency of Treg in AIH are conflicting,^{65,66,139-141} but the expression of proteins important for suppressive function, such as FoxP3, cytotoxic T lymphocyte antigen 4 and IL10, is reduced^{65,66,139} and their suppressive activity may be impaired.⁶⁵⁻⁶⁷ Intrahepatic Tregs are frequent at the time of diagnosis but decline with treatment¹⁴² and may become functionally impaired by an IL2 deficient, inflamed, liver microenvironment.⁷⁴ These data suggest that there may be a deficit in Treg function in AIH but further work is required to comprehensively define this. Augmentation of Treg function represents a potential treatment strategy for AIH irrespective of whether a Treg defect is required for the genesis of the disease as enhanced Treg function may improve the control of aberrant autoimmune responses.

Adoptive Treg transfer has shown early promise in the control of autoimmune and alloimmune responses. Two independent phase I studies of autologous Treg therapy in type 1 diabetes have demonstrated an acceptable safety profile, the sustained persistence of transferred Treg and possible clinical improvements.^{143,144} Adoptive

transfer of autologous, donor-specific Treg following living donor liver transplantation has been successful allowing 7/10 patients to be weaned off immunosuppression within 3 years of transplantation.¹⁴⁵ Additionally, several studies have demonstrated efficacy for adoptive Treg therapy in graft-versus-host disease.¹⁴⁶⁻¹⁴⁸ Taken together, this body of work illustrates an acceptable safety profile and potential efficacy of adoptive Treg transfer in preventing auto-immune and alloimmune responses.

No trial assessing the efficacy of Treg therapy in autoimmune liver diseases has been undertaken to date. However, a study tracking the distribution of autologous Treg after infusion in patients with AIH demonstrated both the feasibility of autologous Treg generation from patients with AIH and the homing of infused Treg to the liver of four patients, suggesting that this therapeutic approach is a realistic proposition.¹⁴⁹

The advantages of this approach include the autologous nature of the cell product, which reduces the likelihood of immune responses against the therapeutic product, and the potential that Treg therapies may restore self-tolerance. However, questions relating to the durability of Treg themselves, their clinical effect, the potential for de-differentiation to nonregulatory effector T cells that could exacerbate inflammatory processes and the risk of malignancy and infection remain.¹⁵⁰ Furthermore, this approach is costly, complex and difficult to administer compared to conventional pharmacological therapies.

3.4.2 | Low-dose IL2 therapy

Low-dose IL2 therapy is under active investigation as a treatment strategy for a variety of autoimmune disorders. IL2 is a critical survival factor for T cells and important for their proliferation. Treatment with low-dose IL2 exploits the greater IL2 sensitivity of Treg, compared to other effector T cells,¹⁵¹ to shift the balance of immune responses in favour of regulation.

Low-dose IL2 treatment increases Treg number and activity, without significant activation of other effector T cells or toxicity in a range of conditions including hepatitis C virus-induced vasculitis,¹⁵² graft-versus-host disease,¹⁵³ type 1 diabetes,¹⁵⁴ alopecia areata¹⁵⁵ and systemic lupus erythematosus.^{156,157}

Evidence for a specific role for IL2 in the pathogenesis of AIH is limited. Serum IL2 is higher in paediatric patients with AIH at disease onset compared to following treatment and similarly a reduction in intrahepatic Treg frequency is noted during treatment.¹⁵⁸ However, a study of adult AIH patients showed no differences in serum IL2 levels.¹⁵⁹ Treg from patients with AIH do appear to have defective responses to IL2, with a failure to increase production of the immunoregulatory cytokine IL10 after IL2 treatment.⁶⁵ Treg from patients with AIH may recover function following treatment with IL2. In vitro low-dose IL2 treatment of hepatic or peripheral blood lymphocytes of patients with autoimmune liver diseases leads to Treg-specific expression of effector proteins, without the loss of liver homing chemokine receptors.¹⁶⁰

Low-dose IL2 treatment has been reported in patients with AIH. Two patients with type 1 AIH who had inadequate response to conventional second-line immunosuppressive therapy received 1×10^6 IU IL2 subcutaneously for five consecutive days a month leading to short-term expansion of the circulating Treg compartment with no adverse events.¹⁶¹ A further two patients with AIH were included in an open-label trial of low-dose IL2 in a cohort of patients with a range of autoimmune disorders, again illustrating that the treatment was well tolerated and resulted in increased circulating Treg.¹⁶² These studies pave the way for the formal assessment of low-dose IL2 in AIH.

Modified versions of IL2 are being developed to improve Treg selectivity and longevity. Mutated forms of IL2 that selectively bind the trimeric, high-affinity receptor IL2 rather than the low-affinity dimeric receptor will hopefully enhance Treg selectivity and reduce conventional T cell activation, thereby further skewing immune responses towards regulation. Conjugation of IL2 to other moieties with the aim of prolonging its plasma half-life is also under investigation.¹⁶³

3.4.3 | Treg stimulators

In addition to the administration of ex vivo expanded Treg and low-dose IL2 to increase Treg numbers, enhancement of Treg differentiation and stimulation through the use of tolerogenic dendritic cells and mesenchymal stem cells¹⁶⁴ are being actively explored.

T cell stimulation requires engagement of the T cell receptor with its cognate antigen presented by an antigen-presenting cell alongside a co-stimulation signal. Dendritic cells act as antigen-presenting cells and, depending upon the cytokines and other signals such as damage and pathogen-associated molecular patterns present when they are exposed to antigen, can acquire a tolerogenic or proinflammatory phenotype. Tolerogenic dendritic cells maintain Treg populations and can divert naïve conventional T cells to become Treg¹⁶⁵ or induce anergy in reactive conventional T cells,¹⁶⁶ hence autologous tolerogenic dendritic cells have been tested in autoimmune conditions to change the balance between endogenous Treg and conventional T cell responses in favour of tolerance. In phase I studies, autologous, ex vivo expanded tolerogenic dendritic cells have been administered to patients with type 1 diabetes,¹⁶⁷ rheumatoid arthritis^{168,169} and inflammatory bowel disease.¹⁷⁰ As well as acceptable safety profiles and feasibility, increased frequencies of circulating Treg, impaired Th1-type T cell responses and reduced circulating effector T cell frequency have been observed,^{168,170} suggesting appropriate support for a regulatory T cell response can be achieved. Thus, it appears possible that endogenous Treg responses can be stimulated in vivo by the use of autologous cell therapies, although robust evidence of efficacy is awaited.

The development of and increasing experience with Treg therapies, low-dose IL2 technology and dendritic cell therapies mean that these have become realistic experimental possibilities for the

treatment of AIH in the coming years. Targeting the Treg axis represents an attractive approach in AIH as it may offer immunoregulation without immunosuppression and correct a pathway known to be dysfunctional with minimal toxicity.

4 | POTENTIAL FUTURE THERAPEUTIC TARGETS IN AIH

The pivotal role of IL1, IL6 and that JAK signalling molecules in autoimmune responses has led to the development of therapies to specifically target these pathways. Here, we discuss below the evidence for these as targets in AIH and consider the potential for testing the efficacy of these in the future.

4.1 | Anti-interleukin 1 and anti-interleukin 6 therapy

IL1 and IL6 are proinflammatory cytokines and antagonism of their function may be of benefit in autoimmunity (Figure 2E). IL1 α is a cytoplasmic and cell surface molecule that is released following cell death, whereas IL1 β is actively secreted. Both molecules bind the IL1 receptor (IL1R1), which is expressed on all nucleated cells, but this interaction is competitively inhibited by the binding of IL1 receptor antagonist (IL1Ra) to IL1R1 or by binding of the cytokine to the non-signalling decoy receptor IL1R2.¹⁷¹ IL1 has pleiotropic effects upon the neurological, cardiovascular and hormonal systems and upon electrolytes and extracellular matrix turnover.¹⁷¹ Within the immune system, it induces acute phase protein production and recruits neutrophils and macrophages to sites of tissue damage or infection.¹⁷² IL1 also stimulates T cells resulting in the differentiation of Th17 cells¹⁷³ that are known to be important in many autoimmune disorders.

IL6 is produced by a broad range of cells, including most immune and stromal cells.¹⁷⁴ The regulation of IL6 expression is complex and influenced by many signals including cytokines, such as IL1 and TNFs, bacterial and viral products and several well-described signalling pathways.^{174,175} IL6 has a broad spectrum of actions within the immune system, regulating innate and adaptive responses. It is important in regulating the production of acute phase proteins¹⁷⁶ and the migration of neutrophils to the site of inflammation.¹⁷⁴ IL6 promotes the survival and expansion of T cells¹⁷⁷ and induces CD4 T follicular helper cell differentiation,¹⁷⁸ plasma cell formation and antibody production,¹⁷⁹ both directly and indirectly, via T follicular helper cells. IL6 also induces the survival and proliferation of Th1 and Th2 CD4 cells and its presence prevents transforming growth factor- β -mediated differentiation of CD4+ FoxP3+ inducible Treg, instead resulting in the differentiation of Th17 cells.⁶⁹

Due to the pivotal roles for IL1 and IL6 in inflammation, evidence for their association with autoimmune disorders and liver inflammation has been sought. IL1 production is elevated in

patients with fulminant hepatic failure¹⁸⁰ and alcoholic hepatitis,¹⁸¹ and elevated IL1 β :IL1RA ratio is observed in severe hepatitis C virus-associated inflammation.¹⁸² However, elevated IL1 levels have not been convincingly demonstrated in AIH, and AIH is not associated with gene variants in IL1 β or IL1Ra.^{55,183} Some evidence suggests that IL6 may be altered in AIH. However, while elevated serum levels of IL6 have been reported to be associated with AIH^{159,184,185} and fall during remission,¹⁸⁵ IL6 levels do not correlate with disease activity and the association is lost after adjustment for other cytokines and clinical parameters. Additionally, in another patient cohort, the vast majority of patients had undetectable serum IL6 levels despite active disease.¹⁸⁶ In vitro stimulation of peripheral blood mononuclear cells from patients with AIH demonstrated a greater capacity for the production of IL6 (among others) in patients with AIH compared to healthy controls or those with hepatitis B infection.¹⁸⁷

Ultimately, irrespective of whether variation in IL1 or IL6 are important in the genesis of AIH, they are key mediators of inflammation hence antagonism of these pathways may help in the resolution of hepatitis.

Therapeutic blockade of the IL1 axis has been successful in several conditions and a range of agents are available. These include anakinra (a recombinant, modified analogue of IL1Ra) that competitively inhibits IL1 binding to IL1R1, riloncept (a soluble form of IL1R) and canakinumab (a neutralising monoclonal antibody against IL1 β). Anakinra has moderate benefit in the treatment of rheumatoid arthritis¹⁸⁸ and psoriatic arthritis¹⁸⁹ and may be of use in systemic lupus erythematosus,¹⁹⁰ ankylosing spondylitis¹⁹¹ and type 1 diabetes,¹⁹² although randomised controlled clinical trial data have been disappointing.¹⁹³ Additionally, a range of inflammatory disorders can also be controlled with IL1 antagonism¹⁹⁴ and there is a multicentre, placebo-controlled phase 2 trial of canakinumab in moderate acute alcoholic hepatitis underway in the United Kingdom (ClinicalTrials.gov: NCT03775109).

The IL6 pathway has been targeted in several conditions. Tocilizumab is a humanised monoclonal against the IL6 receptor and has been approved for use in rheumatoid arthritis following a range of positive trial outcomes¹⁹⁵ including in patients refractory to anti-TNF therapies.¹⁹⁶ Tocilizumab is also efficacious for the treatment of systemic lupus erythematosus,¹⁹⁷ juvenile idiopathic arthritis¹⁹⁸ and giant-cell arteritis,¹⁹⁹ although other agents targeting the IL6 pathway in systemic lupus erythematosus have been less successful.²⁰⁰ Studies targeting the IL6 pathway in a range of other inflammatory conditions have also been completed.¹⁷⁵

The toxicity of agents antagonising the IL1 and IL6 pathways has been extensively assessed and hepatotoxicity, defined as elevated transaminases, has been reported with IL6 antagonism²⁰¹ but not IL1.²⁰² As IL6 is an important mitogen for hepatocytes and is important in liver regeneration,²⁰³ careful timing of IL6 antagonism would be needed to avoid disturbing native liver repair mechanisms. Both IL1 and IL6 antagonism are associated with increased risks of infection and suppression of leucocyte counts.^{194,201} However, these observations need to be considered in relation to the adverse events,

including hepatotoxicity and infection, associated with currently used therapies in AIH.

The availability of agents that inhibit these key proinflammatory cytokines, with associated acceptable safety profiles, offers the option for clinical trials in AIH and should be actively considered. Initial review of existing trial data may illustrate whether any patients with AIH have been treated for other indications, and controlled clinical trialling in AIH seems appropriate.

4.2 | Janus Kinase inhibitors

Janus Kinases (JAKs), comprising of JAKs 1-3 and tyrosine kinase 2, are intracellular kinases that are integral to the signalling pathways for many cytokines. After a cytokine binds to its cognate receptor, receptor-specific JAKs are activated and phosphorylate themselves and the intracellular tail of the receptor. This enables receptor signalling, ultimately leading to the activation of specific members of the STAT family of transcription factors (Figure 2F). JAK expression is cell specific and the association of particular cytokine receptors and JAKs is specific,²⁰⁴ which may allow targeting of each JAK to modulate narrow ranges of cytokine signals.

JAKs are important in inflammatory responses and haematopoiesis and therefore attention has been focused on the inhibition of JAK signalling to limit inflammatory responses in autoimmune and inflammatory disorders. Nonselective first-generation JAK inhibitors are small molecule enzyme inhibitors that block the function of all JAKs (eg tofacitinib and peficitinib) or JAK1 and 2 alone (ruxolitinib and baricitinib).²⁰⁵ Efficacy has been demonstrated for tofacitinib in the treatment of rheumatoid arthritis,^{206,207} psoriatic arthritis²⁰⁸ and ulcerative colitis,²⁰⁹ although results in Crohn's disease have been disappointing.^{210,211} Ruxolitinib may reduce symptoms in rheumatoid arthritis (ClinicalTrials.gov NCT00550043) and is effective topically for psoriasis.²¹²

A range of second-generation JAK inhibitors, which selectively target JAK1 and/or JAK3, remain at early trial stages²⁰⁵ but efficacy of filgotinib in rheumatoid arthritis²¹³ and Crohn's disease²¹⁴ has been reported. Problems with leucopenia are encountered with these drugs and their relative sparing of JAK2 prevents blockade of the important proinflammatory Th1 cytokines IL12 and IL23.

JAK inhibitors represent a potential treatment for AIH as they have the potential to target cytokine producing and responsive lymphocytes that are important in liver autoimmunity.⁶⁰ Furthermore, the association of variants in Src Homology 2 adaptor protein 3 (SH2B3/Lnk) with AIH in genomic studies^{2,215} and evidence for mutations in SH2B3 driving JAK-STAT signalling^{216,217} make inhibition of JAKs an attractive target.

Other appealing features of these drugs are their rapid onset of action and oral route of administration. However, the lack of long-term safety data, the observations of transaminitis and myelosuppression and a concern about long-term cancer risks²¹⁸ represent challenges to their use at present. While there is growing evidence that JAK inhibitors may be useful in the management of a range of

autoimmune disorders, and they offer an intellectually attractive approach to managing inflammation in AIH, there has been no formal testing to date.

5 | EXPERIMENTAL APPROACH IN AIH

There are many novel treatment approaches that may be applicable to AIH in the coming years and with active trials open for anti-BAFF receptor and anti-TNF agents in AIH, new treatment options are a realistic proposition. Additionally, pharmacological agents targeting IL1, IL6, BAFF and JAKs are on the market and large trial databases exist that should be analysed to identify patients with AIH who were treated for other indications to determine signals of toxicity or clinical benefit in AIH. This approach could inform which drugs could next go forward into clinical trialling.

Experimental therapies, including pre-implantation factor, Treg therapy and low-dose IL2, are nearing the point where formal clinical trials are being established, and these treatments may be available in trial settings over the coming years.

There remains a need for new treatment approaches in AIH and as our understanding of the dosing, monitoring and toxicity of these new drugs become established, the barriers to establishing trials of these new agents are reduced. Novel and experimental therapies should be offered in specialist units with expertise in the management of AIH, experience in the delivery of therapeutic drug trials and wherever possible under the auspices of formal prospective clinical trials.

The positioning of novel treatments in the management of AIH is not straightforward. Currently, the approach of induction and maintenance of remission with corticosteroids and azathioprine with the aim of corticosteroid-free remission on azathioprine monotherapy has a strong evidence base. Despite this, a significant proportion of patients do not achieve corticosteroid-free remission,¹² experience a significant burden of side effects¹³⁻¹⁵ and have high rates of relapse both on treatment and following cessation of therapy.^{13,219} In patients with preserved hepatic synthetic function and without evidence of advanced liver disease, it may be acceptable to conduct non-inferiority studies comparing newer agents against corticosteroids for the induction of remission, especially once evidence of efficacy is observed in nonresponders, as delay in remission is unlikely to be associated with adverse outcomes. For patients with an intolerance of, or inadequate response to, conventional first-line therapy for the maintenance of remission, randomised controlled trials of novel agents should be offered. Ianalumab is currently available in a placebo-controlled randomised trial in this setting.

Assessment of new agents following the failure of second- or third-line treatments has the potential for poor outcomes; this group of patients have 'difficult-to-treat' disease by definition and may be more resistant to treatment meaning that the efficacy of novel agents may be underestimated. Furthermore, several trials have been limited by poor recruitment due to the difficulties

in identifying suitable candidates for enrolment. Thus, there is the potential to significantly delay the introduction of novel therapies for the treatment of AIH. Therefore, trial designs for novel therapeutics should consider their introduction at the point of failure of first-line treatments, as well as after second- or third-line failures, and consideration should be given to studies assessing new drugs for non-inferiority against first-line treatments in patients with low-risk disease.

6 | CONCLUDING REMARKS

There is an unmet clinical need in AIH for new therapeutic agents due to intolerance of, or inadequate response to, conventional agents, and the significant side effect burden experienced by patients.¹³⁻¹⁵ Robust evidence for currently used second- and third-line agents remains lacking and the pharmacological agents available to treat AIH have not been expanded for many years. Due to advances in our understanding of the pathophysiology of AIH and autoimmunity more broadly, several novel agents are in development or have an established role in other autoimmune disorders, which may be of use in the management of AIH. The challenge now facing the field is the funding and design of effective clinical trials that will allow the establishment of these novel agents as third-line, or potentially earlier, agents in AIH. There is an exciting wealth of experimental therapies in development and this, combined with the collaborative, international approach to the investigation and management of AIH, will hopefully encourage investment from industry to drive the development of novel therapeutics for this rare but important disease.

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