Salvage Therapies for Autoimmune Hepatitis: A Critical Review

Stuart K. Roberts, MBBS, MD, FRACP, FAASLD1 William Kemp, MBBS, PhD, FRACP1

1 Department of Gastroenterology, The Alfred Hospital and Monash University, Melbourne, Australia


Address for correspondence Stuart K. Roberts, MBBS, MD, FRACP, FAASLD, Department of Gastroenterology, The Alfred Hospital and Monash University, 55 Commercial Road, Melbourne, Australia 3004 (e-mail: s.roberts@alfred.org.au).

Abstract

Several salvage therapies have been identified for autoimmune hepatitis refractory or recalcitrant to conventional therapy; however, the optimal salvage strategy remains unclear. High-dose prednisolone is currently recommended as the front-line salvage therapy, with alternative immunosuppressive therapies reserved for continuing treatment failure. Of the second-line therapies, the calcineurin inhibitors, cyclosporine and tacrolimus, and mycophenolate mofetil are preferred and have the most accrued clinical data. However, none of these have undergone rigorous clinical evaluation via randomized clinical trials. Tacrolimus is generally preferred over cyclosporine because of its higher potency and increased utility in organ transplantation. Mycophenolate is particularly useful for azathioprine intolerance but also for nonresponse to standard treatment. Subjects with progressive liver failure should undergo liver transplantation evaluation. The appropriate timing, dosing, and monitoring of salvage therapies require determination. Several promising immunosuppressive therapies have been developed for autoimmune diseases including molecular agents that may enhance regulatory T cell activity and function.

Autoimmune hepatitis (AIH) is an immune-mediated chronic inflammatory liver disease of unknown etiology characterized by high gamma-globulins, serum autoantibodies, and a predominantly periportal interface hepatitis with lymphoplasmacytic infiltrate on liver histology.1 Occurring worldwide and among all ethnic groups, AIH has a relatively high prevalence in European countries, particularly Scandinavia where prevalence rates are between 11 and 24 per 100,000 persons.2–4 Untreated, the prognosis of severe AIH is poor with an early mortality of 40 to 50%5 and progression to cirrhosis, liver failure, and portal hypertension common in survivors.6 In contrast, according to existing literature, the 5-year survival of patients with treated AIH is ≥ 90% and may be similar to that of the general population.7–9

First-line treatment regimens for AIH include prednisolone therapy, either alone or in combination with azathioprine.10–12 Three randomized controlled trials (RCTs) conducted in the 1970s demonstrated that both therapies were effective in achieving remission and reducing liver-related morbidity and mortality.13–15 Subsequently, a systematic review of all RCTs of AIH therapies concluded that both prednisolone monotherapy and prednisolone in combination with azathioprine were equally effective in inducing clinical, biochemical, and histological remission.16 While efficacy is similar, two-thirds of patients receiving prednisolone therapy experience steroid-related side effects, including cosmetic changes, diabetes, hypertension, cataracts, neuropsychiatric changes, peptic ulceration, and osteoporosis. The incidence of these steroid-related effects is reduced via dose minimization/titration and/or the use of combination prednisolone plus azathioprine.17 For these reasons, the combination regimen is preferable as the first-line treatment. More recently, budesonide in combination with azathioprine has been shown in a multicenter RCT to be as effective as prednisolone plus azathioprine in achieving remission and to have less steroid-specific side effects in patients with AIH without cirrhosis.18 Corticosteroid therapy is successful in improving survival and achieving a reduction in alanine aminotransferase (ALT) levels.
to < 2 × ULN (upper limit of normal) in 65% of patients within 18 months and 80% of patients within 3 years.\textsuperscript{14,18,19} However, around 20% of subjects have a suboptimal treatment outcome either with worsening liver disease despite treatment adherence in 9% of cases or an inability to meet remission criteria in 13%.\textsuperscript{20,21} Moreover, relapse rates are quite common being around 80 to 90% following complete drug withdrawal.\textsuperscript{22,23} while around 10% discontinue azathioprine and 13% cease corticosteroids due to drug toxicity and/or intolerance with both physical and psychological side effects impacting the ability of subjects to complete prescribed therapy.\textsuperscript{24–26} For these patients, alternative immunosuppressive therapies are required to induce and/or maintain remission.

Over the past decade, several noncorticosteroid-based salvage therapies have been evaluated and incorporated into clinical practice guidelines to facilitate treatment of patients who fail or are intolerant of standard treatment with corticosteroids with/without thiopurine(s). Such immunosuppressive agents include calcineurin inhibitors, mTOR inhibitors, mycophenolate mofetil (MMF), methotrexate, cyclophosphamide, and biological therapies, such as recombinant monoclonal phenolate mofetil (MMF), methotrexate, cyclophosphamide, and biological therapies, such as recombinant monoclonal antibodies that target key components of the immune system responsible for liver damage (e.g., rituximab and anti-TNF, Table 1). In this report, we critically review the outcomes of salvage treatment strategies that have been tried, to date, for difficult to treat AIH and discuss future therapeutic options, including site-specific targeted therapies in the context of recent advances in the understanding of the immunopathogenesis of the condition.

Immunopathogenesis of AIH

While the immunopathogenesis of AIH is incompletely understood, current opinion is that AIH could develop from an interaction between an environmental trigger(s) and genetic factors in a genetically susceptible host.\textsuperscript{27–29} At a genetic level, susceptibility to AIH is strongly linked to the human leukocyte antigen (HLA) region and in particular DRB1*0301 and DRB1*0401 alleles among Europeans and North Americans.\textsuperscript{30,31} Potential environmental triggers for AIH include viral infections and medications. There are several classes of medications that have been implicated in inducing an autoimmune-like drug-induced–liver injury (DI-AIH). Examples include minocycline, nitrofurantoin, \(\alpha\)-methylidopa, fibrates, hydralazine, 5-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins), and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) antagonists.\textsuperscript{32,33} While a detailed discussion of DI-AIH and drug induced liver injury is beyond the scope of this review, it is important to realize that AIH and DI-AIH may share many clinical, histological, and even serological patterns, which can complicate their differentiation, although histological features of chronicity/cirrhosis or relapse after immunosuppressive dose reduction or withdrawal favors a diagnosis of classical AIH rather than DI-AIH.\textsuperscript{34}

Regardless of the trigger, initiation of the autoimmune attack is mediated via the presentation of an autoantigenic peptide within a major histocompatibility complex (MHC) molecule by antigen presenting cells (APCs) to undifferentiated CD4 effector cells.\textsuperscript{27,32} When this occurs in the presence of costimulatory cytokines, including interferon-\(\gamma\) (IFN-\(\gamma\)) and interleukin (IL)-12, naive CD4 helper T cell differentiation is encouraged, which in turn results in IFN-\(\gamma\) secretion. IFN-\(\gamma\) production triggers several effector immune mechanisms, including activation of monocytes and cytotoxic CD8 T-cells and promotion of killing of natural killer (NK) cells. In addition, IFN-\(\gamma\) results in upregulation of MHC class I and induction of aberrant class II expression by hepatocytes that in turn further exacerbate liver injury via further activation of CD4 T cells and antigen presentation to CD8 T cells.\textsuperscript{27,32}

At the humoral level, exposure to IL-4 stimulates Th2-cell differentiation, which promotes humoral immunity via secretion of several cytokines (IL-4, IL-10, IL-13) that stimulate plasma cell production and the subsequent development of autoantibodies.\textsuperscript{27} Current evidence indicates that autoantibody production may contribute to hepatocyte injury via antibody-mediated cellular cytotoxicity and complement activation.\textsuperscript{28}

Regulatory immune mechanisms also appear to be impaired in AIH resulting in an imbalance between regulatory and effector mechanisms and ultimately a breakdown in immune tolerance.\textsuperscript{27} In particular, functional deficiencies in the CD4(+)CD25(+)CD127(low)FOXP3(+) regulatory T cells (Tregs) have been described in patients with juvenile-onset AIH, with the frequency of these cells inversely correlating with the markers of disease activity.\textsuperscript{34} Such deficient Tregs produce less IL-10 and have reduced ability to suppress CD4 target cells. In addition, when exposed to proinflammatory stimuli, Tregs in AIH appear to be more susceptible to exhibit effector cell-like behavior via enhanced production of IFN-\(\gamma\) and IL-17\textsuperscript{35}; this in turn may exacerbate liver injury.

Treatment Endpoints

The main objective of treatment of AIH is to achieve remission of disease that is defined as the complete biochemical and histological resolution of activity based, respectively, on normalization of serum aminotransferase, serum bilirubin, and gamma-globulin levels and the absence of interface hepatitis on liver biopsy.\textsuperscript{10–12} Those who show some or no improvement in clinical, laboratory, and histological features over 2 to 3 years of continuous therapy despite treatment compliance are deemed to have an incomplete response,\textsuperscript{10} although the majority have marked improvements in indices of activity occurring within the first 3 to 6 months.\textsuperscript{11,12} This is distinct from treatment failure, which is defined as the worsening of clinical, laboratory, and histological features in subjects on standard treatment including development of liver decompensation.\textsuperscript{10–12}

Prognosis

The prognosis of patients with AIH depends on several pre-treatment and treatment-related factors.\textsuperscript{12,36} Important pre-treatment factors associated with reduced survival or need for liver transplantation include female gender, African–American race (men), diagnosis in < 18 years old, Type 2 AIH, and soluble liver antigen (SLA)-antibody–positive disease.\textsuperscript{12,36} In addition, histologic cirrhosis, which is present in approximately 25% at
Table 1 Summary of salvage therapies for autoimmune hepatitis

<table>
<thead>
<tr>
<th>Drug regimen</th>
<th>Dose</th>
<th>Mode of action</th>
<th>Main indications</th>
<th>Treatment outcomes</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-dose prednisolone</td>
<td>60 mg daily for 4 wks, 100 mg/d IV in severe cases, dose reduced to 10 mg/mo, stabilized at 20 mg/mo&lt;sup&gt;10-12,53,211&lt;/sup&gt;</td>
<td>Binds with cytosolic glucocorticoid receptor resulting in reduced cytokine production and immunocyte proliferation</td>
<td>First line for recalcitrant AIH if cytopenias or liver failure present&lt;sup&gt;10-12,53,211&lt;/sup&gt;</td>
<td>Clinical and biochemical improvement in 75%, improves liver histology in 20% of patients&lt;sup&gt;10-12,53,211&lt;/sup&gt;</td>
<td>Frequent side effects: Cushing’s syndrome, osteoporosis, neuropsychiatric symptoms</td>
</tr>
<tr>
<td>High-dose prednisolone and azathioprine</td>
<td>Prednisolone 30 mg daily plus azathioprine 150 mg/d or 2 mg/kg/d for 4 wks, then reduce prednisolone by 10 mg/mo and azathioprine by 50 mg/mo&lt;sup&gt;10-12,53,211&lt;/sup&gt;</td>
<td>Azathioprine is a purine analogue that interferes with DNA synthesis in proliferating immunocytes</td>
<td>First line for refractory AIH in chronic disease with no cytopenias or liver failure&lt;sup&gt;10-12,53,211&lt;/sup&gt;</td>
<td>Clinical and biochemical improvement in 75%, improves liver histology in 20% of patients&lt;sup&gt;10-12,53,211,212&lt;/sup&gt;</td>
<td>Azathioprine side effects: Fever, nausea, vomiting, cytopenias, pancreatitis, rash</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>2–5 mg/kg daily, aim for trough levels of 100–300 mg/mL&lt;sup&gt;75,81,82&lt;/sup&gt;</td>
<td>Calcineurin inhibitor that binds to cytosolic cyclophilin in T cells, inhibits transcription of IL-2 and related cytokines, reduces effector T cell function and proliferation</td>
<td>Second line for refractory AIH, steroid sparing where drug toxicity</td>
<td>Clinical and laboratory improvement in 93% of patients&lt;sup&gt;10&lt;/sup&gt;</td>
<td>Side effects: Common hypertension, dyslipidemia, GI symptoms, renal impairment, electrolyte disturbance</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>Initial, 0.5–1.0 mg daily; maintenance, 1.0–3.0 mg bid; aim for trough levels 3 ng/mL&lt;sup&gt;85,90&lt;/sup&gt;</td>
<td>Calcineurin inhibitor that inhibits NFAT-dependent T cell cytokine release, including IL-2 via FK binding proteins, limits IL-2 receptor expression</td>
<td>Second line for refractory AIH, steroid sparing where drug toxicity</td>
<td>Clinical and laboratory improvement in 56% in refractory disease, &gt; 90% in treatment-intolerant patients&lt;sup&gt;37&lt;/sup&gt;</td>
<td>Frequent side effects: Hypertension, tremors, GI disturbance, renal impairment, electrolyte disturbance, cardiomyopathy</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>Initial, 0.5–1.0 g daily; induction dose, 1.5–2.0 g daily (range: 1–2 g daily); maintenance dose, 1 g daily</td>
<td>Prodrug of mycophenolic acid, potent inhibitor of type II isoform of inosine-5'-monophosphate dehydrogenase causing selective inhibition of B and T cell proliferation</td>
<td>Second line for refractory AIH, first line for azathioprine intolerance</td>
<td>Biochemical improvement in 23–57% in refractory disease&lt;sup&gt;108,110&lt;/sup&gt; and ~ 60% in azathioprine intolerance&lt;sup&gt;111&lt;/sup&gt; steroid sparing in 40% of patients</td>
<td>GI: nausea, vomiting, diarrhea, Cytopenias, Dermatologic reactions, Headaches, Teratogenic effects</td>
</tr>
<tr>
<td>Sirolimus (rapamycin)</td>
<td>No established dosing guidelines in AIH&lt;sup&gt;117–119&lt;/sup&gt;</td>
<td>mTOR inhibitor that binds to cytosolic FK-binding protein blocking response to IL-2, prevents activation and reduces proliferation of effector T cells</td>
<td>Potential third-line salvage therapy (investigational)</td>
<td>Limited experience, may improve liver chemistries, reduces steroid dose</td>
<td>Pulmonary: cough, pneumonitis, dyslipidemia, cytopenias, rash</td>
</tr>
<tr>
<td>Rituximab</td>
<td>No established dosing guidelines in AIH 2 × 1,000 mg infusions on D1 and D15 suggested&lt;sup&gt;140&lt;/sup&gt;</td>
<td>Monoclonal antibody to CD20 expressed on B lymphocytes, depletes B cell population and reduces antibody production&lt;sup&gt;198&lt;/sup&gt;</td>
<td>Potential third-line salvage therapy (investigational)</td>
<td>Limited experience in AIH, improves liver chemistries, widely used in RA, SLE, and vasculitides</td>
<td>Infections reactivation (e.g., TB, hepatitis B), anaphylaxis, Cardiac: heart failure, angina, arrhythmia</td>
</tr>
</tbody>
</table>

Abbreviations: AIH, autoimmune hepatitis; GI, gastrointestinal; IL-2, interleukin 2; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T-cells; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TB, tuberculosis; TNF, tumor necrosis factor.
initial presentation, is associated with reduced long-term survival in some,\(^8,18,36\) but not all,\(^7,37,38\) studies, with good outcomes reported from academic centers\(^7\) and/or where prompt treatment is initiated.\(^7,37\) Treatment-related factors linked to a poor long-term outcome include a poor,\(^9\) inadequate,\(^36,40\) or slow,\(^41\) biochemical response to therapy and multiple relapses after treatment withdrawal.\(^36,42\) Compliance with therapy also impacts patient outcomes with nonadherence to treatment during follow-up associated with a high relapse rate and liver failure.\(^43\) Pediatric patients entering puberty and adolescents are particularly at risk of poor compliance,\(^43,44\) and hence this population should receive additional support, motivation, and encouragement to achieve an optimal treatment response.

**Early Identification of Poor Responders**

It is important to identify early those patients who respond poorly to standard therapy, as they will require an alternative treatment strategy to induce remission. An estimated 13% of patients fail to enter remission after 36 months of treatment, and hence are classified as incomplete responders.\(^10\) In addition, subjects with treatment failure (i.e., recalcitrant AIH) who deteriorate despite compliance with therapy and subjects with treatment intolerance are candidates for consideration of alternate treatment strategies. The Model of End-Stage Liver Disease (MELD) score can facilitate the early identification of subjects likely to fail standard therapy with a MELD score of ≥ 12 at presentation having a sensitivity of > 90% and specificity of 68% for development of treatment failure.\(^20\) Other notable pretreatment risk factors for a poor treatment outcome include younger age at onset,\(^45\) severe acute/fulminant presentation,\(^14,46\) jaundice or significant hyperbilirubinemia,\(^47,48\) presence of HLA DRB1*03,\(^49\) and failure of laboratory indices to improve during the first 2 weeks of treatment.\(^47,48,50\) Several alternative salvage regimens are available for such patients as discussed below and outlined in *Table 1*.

**Salvage Therapies for AIH**

**Corticosteroid-Based Immunosuppressive Regimens**

**Prednisolone with/without Azathioprine**

The initial management of subjects with treatment failure on standard therapy is greatly dependent on the severity of the underlying liver disease. In those without liver failure, current recommendations are to treat patients with high-dose prednisolone 30 to 60 mg/day orally with azathioprine 150 mg/day or 2 mg/kg/day (if no cytopenias) for up to 4 weeks (*Table 1, *Fig. 1).\(^10–12\) In addition, hospital admission should be considered initially for intravenous steroids (e.g., methylprednisolone 100 mg/day), particularly if treatment compliance is questionable.\(^12\) Following the initial 4-week induction period, the steroid dose should be reduced by 10 mg/month and then stabilized on a dose of 20 mg/day (*Fig. 1).\(^10–12\) The evidence, however, for such recommendations is relatively weak and of low quality being mainly derived from expert opinion and a single case series.\(^50,51\) In poor responders, with severe liver disease, including those with an acute fulminant presentation, patient management should be in conjunction with a liver transplant center. There is limited evidence that such patients benefit from a course of high-dose prednisolone (≥ 1 mg/kg) intravenously.\(^11,52\)

**Budesonide**

Budesonide, a potent second-generation corticosteroid, with high first-pass metabolism in the liver following oral ingestion, is an effective first-line therapy for AIH but is considered to be relatively ineffective in steroid nonresponders because of its similar mode of action.\(^53\) It may, however, be useful in noncirrhotic subjects who are intolerant to standard therapy because of steroid-specific side effects.\(^54\) In a small series of 13 patients with relapsed AIH, 11 of whom were intolerant to steroids, treatment with budesonide in a dose of 6 to 8 mg/day for the first 6 to 10 weeks improved serum ALT and immunoglobulin levels without causing significant steroid side effects.\(^54\) In comparison, in a pilot study of 10 patients with steroid-dependent disease, budesonide 9 mg/day was unable to control disease activity in the majority of patients with 7 developing worsening disease and/or treatment intolerance.\(^54\) Still, results are not uniform with another study that showed budesonide 3 to 9 mg/day to suppress disease activity in seven of nine patients with steroid-dependent or refractory disease without causing major side effects.\(^36\) Similarly, in another open-label pilot study involving 18 patients treated with budesonide 9 mg/day for up to 24 weeks, all 8 patients who received treatment for steroid refractory AIH achieved remission.\(^57\) Data are limited, however, on the ability of budesonide to achieve a durable response and/or histological remission when used in such settings.

**Deflazacort**

Deflazacort is an oxazoline derivative of prednisolone that has both immunosuppressive and anti-inflammatory properties.\(^55\) It is considered more attractive to use in subjects with or at risk of steroid intolerance, as it has fewer side effects than prednisolone at equivalent doses.\(^59\) Thus far, the limited number of studies conducted with this agent have mostly focused on its role as maintenance therapy for AIH.\(^60\) In one open-label study of 15 patients, who were in remission on maintenance prednisolone, conversion to deflazacort 7.5 mg/day did not lead to biochemical deterioration or delerious side effects.\(^60\) Nevertheless, like budesonide, it is unlikely to be effective as a salvage therapy for those with a poor or nonresponse to prednisolone, given that it acts via the same glucocorticoid receptor.

**Azathioprine-Based Regimens**

Azathioprine is a purine analogue that interferes with DNA synthesis and is particularly applicable to rapidly proliferating cells such as lymphocytes. Around 10% of subjects will have a suboptimal response to combination therapy due to a lack of therapeutic effect or intolerance to azathioprine.\(^10,12\) In this context, azathioprine is converted nonenzymatically to 6-mercaptopurine but achieves maximal effectiveness as an immunosuppressive agent via subsequent enzymatic conversion by
hypoxanthine phosphoribosyltransferase to its pharmacologically active metabolite, 6-thioguanine (thioguanine) nucleotides (6-TGN, \( \text{Fig. 2} \)). However, 6-mercaptopurine also undergoes enzymatic conversion by xanthine oxidase to the hepatotoxic thiopurine metabolite, 6-methylmercaptopurine (6-MMP), which in some patients may be preferentially generated (61,62). Thiopurine methyltransferase (TPMT) is the enzyme central to this metabolic process with the activity of TPMT determining the degree to which methylated breakdown products such as 6-MMP are produced (\( \text{Fig. 2} \)). As TPMT activity is known to vary among individuals likely because of differing TPMT phenotypes, (63) so the levels of 6-TGN and 6-MMP.

Experience from the literature on inflammatory bowel disease has shown that the generation of high levels of 6-MMP at the expense of 6-TGN is associated with azathioprine hepatotoxicity and an inadequate therapeutic response to azathioprine. (64) Conversely, achieving higher concentrations of 6-TGN is associated with the development of remission in AIH. (65) Table 2 outlines the potential utility of azathioprine metabolites in AIH in relation to monitoring for compliance, efficacy, and toxicity. However, further studies are needed to clarify the appropriate use of 6-TGN and 6-MMP metabolite testing in dose titration and treatment optimization. Still, TPMT activity and azathioprine metabolites testing could be considered in patients with a suboptimal response to standard therapy, as the results can assist in identifying patients with poor compliance (i.e., low 6-MMP, low 6-TGN). Furthermore, baseline TPMT testing may help identify patients at higher risk of developing azathioprine toxicity and therefore identify a cohort of patients more suitable for alternate treatment regimens (\( \text{Table 2} \)).
Table 2 Summary of the potential utility of thiopurine metabolite results in managing autoimmune hepatitis

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Thiopurine metabolite</th>
<th>Interpretation</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Low (&lt;260)</td>
<td>Nonadherence</td>
<td>Patient education</td>
</tr>
<tr>
<td>2</td>
<td>High (&gt;5,700)</td>
<td>Preferential 6MMP producer (“shunter”)</td>
<td>Consider adding allopurinol and reduce dose to 25% of original thiopurine dose</td>
</tr>
<tr>
<td>3</td>
<td>Low (&lt;260)</td>
<td>Preferential 6MMP producer (“shunter”)</td>
<td>Consider adding allopurinol and reduce dose to 25% of original thiopurine dose</td>
</tr>
<tr>
<td>4</td>
<td>Low or high</td>
<td>Refractory disease</td>
<td>Change therapy (e.g., MMF)</td>
</tr>
<tr>
<td>5</td>
<td>High (&gt;450)</td>
<td>Overdosed or refractory</td>
<td>Consider dose reduction or change therapy (e.g., MMF)</td>
</tr>
</tbody>
</table>

Abbreviations: 6TGN, 6-thioguanine nucleotide; MMF, mycophenolate mofetil; 6MMP, 6-methylmercaptopurine.


Note: All values in pmol/8 x 10⁸ red blood cells.

**Allopurinol**

The concomitant use of the xanthine oxidase inhibitor, allopurinol with low-dose thiopurine causes a preferential increase in 6-TGN production and reduction in 6-MMP formation (Fig. 2). Such an approach has been successfully adopted in patients with inflammatory bowel disease to optimize 6-TGN levels in thiopurine nonresponders. This treatment strategy was also reported to be successful in a small case series of patients with AIH with nonresponse or intolerance to thiopurines. In an open-label trial, allopurinol 100 mg/day was coadministered with thiopurine at 25 to 33% of the original dose in eight patients with AIH and unfavorable thiopurine metabolism and intolerance to or inadequate response to conventional thiopurines. All subjects showed improved liver chemistries, with sustained biochemical improvement seen in seven, associated with a significant increase in 6-TGN levels. While this approach has sound rationale and appears to be safe and simple, more objective data are needed before recommending it to be widely adopted.

**Noncorticosteroid-Based Immunosuppressive Regimens**

**Calcineurin Inhibitors**

Cyclosporine and tacrolimus are both calcineurin inhibitors (CIs) that interfere with the T lymphocyte-mediated response via interference with lymphocyte proliferation. Despite extensive experience with the use of these medications in the organ transplant setting, there are only small and predominantly nonrandomized trials to support their use in AIH. Coupled with the potential for serious adverse effects and the requirement for frequent drug monitoring, both cyclosporine and tacrolimus are employed less frequently than MMF as the second-line therapy, usually being reserved for a more selective group of patients (Fig. 1).

Cyclosporine

Cyclosporine is a lipophilic cyclic peptide that binds with high affinity to a family of cytoplasmic proteins called cyclophilins.
Table 3  Summary of clinical reports of the efficacy and safety of calcineurin inhibitors as salvage therapy for autoimmune hepatitis

<table>
<thead>
<tr>
<th>Study (y)</th>
<th>No.</th>
<th>Study design</th>
<th>Indication</th>
<th>Dose</th>
<th>Response</th>
<th>Safety comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mistilis et al</td>
<td>72</td>
<td>Case report</td>
<td>Steroid refractory</td>
<td>2.25 mg/kg/d</td>
<td>AST: 98 U/L ALT: 113 U/L</td>
<td>Well tolerated</td>
</tr>
<tr>
<td>Hyams et al</td>
<td>77</td>
<td>Case report</td>
<td>Steroid refractory</td>
<td>5 mg/kg/d</td>
<td>Complete biochemical response</td>
<td>Well tolerated</td>
</tr>
<tr>
<td>Person et al</td>
<td>78</td>
<td>Case report</td>
<td>Steroid refractory</td>
<td>3 mg/kg/d + Pred + Aza</td>
<td>Complete response after 3 mo</td>
<td>Well tolerated</td>
</tr>
<tr>
<td>Sherman et al</td>
<td>79</td>
<td>Case series</td>
<td>Steroid refractory or intolerant</td>
<td>Varied</td>
<td>Complete biochemical response in 4/6 and improvement in 2/6</td>
<td>Gingival hypertrophy, opportunistic infection</td>
</tr>
<tr>
<td>Senturk</td>
<td>80</td>
<td>Case report</td>
<td>Steroid refractory</td>
<td>3 mg/kg/d</td>
<td>Complete response</td>
<td>Increased creatinine</td>
</tr>
<tr>
<td>Jackson and Song</td>
<td>81</td>
<td>Case report</td>
<td>Steroid refractory or intolerant</td>
<td>5 mg/kg/d</td>
<td>Complete response</td>
<td>Well tolerated</td>
</tr>
<tr>
<td>Fernandes et al</td>
<td>82</td>
<td>Case series</td>
<td>Steroid refractory</td>
<td>2–4 mg/kg/d, titrated to response</td>
<td>Complete response in 4/5, treatment failure (n = 1)</td>
<td>Varicella zoster, gingival hypertrophy, hirsutism</td>
</tr>
<tr>
<td>Debray et al</td>
<td>83</td>
<td>Retrospective case series Pediatric</td>
<td>Steroid refractory or intolerant (n = 5), naive (n = 10), cirrhosis (n = 8)</td>
<td>3.3–7.5 mg/kg/d (n = 13) 1 mg/kg/d IV (n = 2) Titrated for trough of 200–250 ng/mL (3 mo), then 100–150 ng/mL (12 mo)</td>
<td>Complete response in all with CyA with/without Pred ± Aza</td>
<td>Decreased GFR (n = 3) Hypertension Headaches Gingival hypertrophy</td>
</tr>
<tr>
<td>Malekzadeh et al</td>
<td>75</td>
<td>Open label</td>
<td>Steroid refractory or intolerant (n = 10) and naive (n = 9)</td>
<td>2–5 mg/kg/d Titrated for trough of 100–300 ng/mL Pred 10 mg added if no response by wk 4</td>
<td>Biochemical response in 95% Mean ALT reduced from 453 to 79 IU/L</td>
<td>16% discontinued due to side effects (hypertension, diarrhoea, paraesthesia, tremors, gingival hypertrophy, hirsutism)</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqel et al</td>
<td>89</td>
<td>Case series</td>
<td>Steroid refractory</td>
<td>1 mg/d Titrated for trough of 3.0 ng/mL (range: 1.7–10.7 ng/mL)</td>
<td>Biochemical remission in 10 of 11 patients (91%) Median ALT reduced from 77 to 21 U/L</td>
<td>9% (n = 1) discontinued due to side effects (tremors, hypertension, and generalized edema) Headache (n = 4)</td>
</tr>
<tr>
<td>Larsen et al</td>
<td>90</td>
<td>Case series</td>
<td>Steroid refractory</td>
<td>3 mg/d (range: 2–4 mg/d) Titrated for trough below 6 ng/mL</td>
<td>Mean ALT reduced from 154 to 47 U/L (p = 0.006) Histologic improvement</td>
<td>Well tolerated</td>
</tr>
<tr>
<td>Tannous et al</td>
<td>88</td>
<td>Retrospective case series Second-line therapy</td>
<td>Steroid refractory</td>
<td>2–6 mg/d</td>
<td>Biochemical remission in 12 of 13 patients (92%)</td>
<td>Nausea/vomiting, hair loss, and hemolytic uremic syndrome</td>
</tr>
<tr>
<td>Than et al</td>
<td>86</td>
<td>Retrospective case series)</td>
<td>Salvage therapy (n = 15) and Aza intolerance (n = 1)</td>
<td>0.5–5.0 mg/d Titrated for trough below 6 ng/mL</td>
<td>29% normal ALT at 12 months</td>
<td>47% (n = 8) discontinued due to side effects abdominal pain, headache, and vomiting (n = 3), noncompliance (n = 2), development of PSC overlap (n = 1), and liver transplant (n = 1)</td>
</tr>
<tr>
<td>Efe et al</td>
<td>87</td>
<td>Retrospective case series Pred/Aza refractory or intolerant</td>
<td>Varied</td>
<td>Biochemical response in 94.1% of Pred/Aza intolerant and 56.3% of refractory group</td>
<td>12.5% discontinued due to side effects liver-related deaths or liver transplantation in 10.3%</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; Aza, azathioprine; CyA, cyclosporine; Pred, prednisolone; PSC, primary sclerosing cholangitis.
opportunistic infections, and the potential for de novo malignancies.

Tacrolimus
Tacrolimus is a macrolide antibiotic that has more potent immunosuppressive effects than cyclosporine. Tacrolimus suppresses the proliferation of T lymphocytes via its binding to the cytoplasmic protein FK-binding proteins (FK506 [tacrolimus]-binding protein).\textsuperscript{83} The consequence of this binding is the inhibition of the translocation of NF-AT transcription factors via inhibition of calcineurin phosphatase activity that results in a similar downstream effect to that of cyclosporine. The depletion of activated nuclear factors leads to reduced transcriptional activation of cytokine genes for IL-2, IL-3, IL-4, TNF-α, CD40L, granulocyte–macrophage colony-stimulating factor, and IFN-γ.\textsuperscript{70}

Although tacrolimus has an established indication in the transplant setting, its use in AIH remains off label and therefore the decision to use tacrolimus needs to incorporate risk-benefit and cost-effectiveness analyses in the decision-making process. The published experience of tacrolimus in AIH is limited (\textsuperscript{84} – Table 3) with no prospective randomized trials comparing tacrolimus to conventional therapy having been performed. The efficacy of tacrolimus as first-line therapy in AIH was initially demonstrated by Van Thiel et al\textsuperscript{85} who published an early experience of tacrolimus in 21 patients. Dosing was commenced at 0.075 mg/kg in divided doses and incremented to achieve a trough level of 0.6 to 1.0 ng/mL. Significant improvement in hepatic necroinflammation was noted, although renal dysfunction was observed. Subsequently, several, mostly small, retrospective case series have established a role of tacrolimus as salvage therapy in AIH, particularly in patients who are nonresponders/partial responders or intolerant of steroid-based therapy.\textsuperscript{86–90} In this setting, tacrolimus is generally well tolerated and effective with some evidence suggesting it to be at least as effective as MMF and potentially more efficacious in those with an incomplete response to prednisolone and azathioprine.\textsuperscript{87} Monitoring drug levels is required with the schedule of dosing and monitoring of tacrolimus essentially derived from experience in the posttransplant setting rather than via clinical studies on AIH. In recent times, tacrolimus has generally been preferred to cyclosporine by transplant centers, and as a consequence, it has become the preferred calcineurin inhibitor as salvage therapy, although no comparative data are available to support this practice.

In a review of the literature that included a total of 113 subjects,\textsuperscript{84} two-thirds achieved a biochemical response to tacrolimus at a dose between 1 and 6 mg/day. A recent report\textsuperscript{87} of 80 patients collected from 19 centers indicated response rates of over 90% when used as a salvage therapy for steroid/azathioprine intolerance but only 56% if tacrolimus was used due to a failure of standard therapy. Overall, 12.5% required treatment withdrawal due to side effects. Further studies are needed to define the efficacy of tacrolimus in comparison to other regimens, although based on current literature and experience, it remains a viable salvage treatment option in steroid-refractory or intolerant patients.

As with cyclosporine, tacrolimus has the potential to cause significant drug toxicity. The most frequent side effects reported are gastrointestinal, such as anorexia, nausea, and diarrhea. However, nephrotoxicity, hypertension, neurotoxicity (tremors, headaches), and diabetes mellitus can also occur and should be monitored for.

Mycophenolate Mofetil
Mycophenolate mofetil (MMF), the morpholinoethyl ester produg of mycophenolic acid, undergoes rapid conversion to mycophenolic acid following oral absorption.\textsuperscript{91} Like azathioprine, MMF is a purine antagonist that blocks de novo DNA synthesis; however, in contrast to azathioprine, its immunosuppressive properties are independent of the thiopurine methyltransferase pathway of catabolism.\textsuperscript{91} MMF has a selective inhibitory effect on both B and T lymphocyte proliferation via potent inhibition of the activity of the type II isofrom of inosine-5’-mono-phosphate dehydrogenase; this enzyme is involved in guanosine synthesis in lymphocytes. MMF is a more powerful and better-tolerated agent than azathioprine\textsuperscript{92} and is now a regular component of standard therapy for autoimmune diseases\textsuperscript{92} and one of the more commonly used salvage therapies for AIH.\textsuperscript{93}

In the absence of randomized controlled trials, data on the efficacy and safety of MMF as salvage therapy in AIH have been derived from several small retrospective, mostly single case series of patients with azathioprine intolerance and/or steroid-refractory disease (\textsuperscript{45,94–107} Table 4).\textsuperscript{45,94–107} Overall response rates in this setting have been between 30 and 84% with a subsequent analyses of four of these studies estimating the biochemical response achieved with MMF to be around 45%.\textsuperscript{108,109} The significant variability in response rates likely reflects several key differences between studies, including heterogeneity in study populations and lack of uniformity in the definitions used to codify response. Several small studies evaluating MMF as the salvage therapy suggest that biochemical improvement is significantly more common in patients treated for azathioprine intolerance compared with those treated for corticosteroid and/or azathioprine-refractory disease.\textsuperscript{104,105,110} A composite analysis of four studies\textsuperscript{100–102,106} demonstrating improvement rates of 58% in those with azathioprine intolerance versus 23% in those with refractory disease supports this view.\textsuperscript{108,109} More recently, Roberts et al reported the outcomes of 105 patients recruited from 17 centers who received MMF as the salvage therapy for AIH as part of a large multicenter study.\textsuperscript{111} Ninety-eight of these had received prior corticosteroids plus thiopurine(s) with the indication for MMF being treatment nonresponsive in 40% and treatment intolerant in 60%. Overall, 63 (60%) patients achieved biochemical remission on MMF with response rates being similar between those receiving MMF for inadequate response and those intolerant to standard therapy (57 vs. 62%). Four other studies assessing MMF as the second-line therapy also reported high rates of biochemical improvement of 70 to 100% in patients with a suboptimal response to standard therapy\textsuperscript{102,105,108,109}, two of these studies also reported response rates that did not differ according to treatment indication.\textsuperscript{108,109} However, the uncontrolled nature of studies to date and lack of uniformity in both
Table 4  Summary of clinical reports of the efficacy and safety of MMF as salvage therapy for autoimmune hepatitis

<table>
<thead>
<tr>
<th>Study (y)</th>
<th>Patient (n)a</th>
<th>Population</th>
<th>Cirrhosis (n)</th>
<th>Suboptimal response (n)</th>
<th>Treatment Intolerant (n)</th>
<th>Overall response n (%)</th>
<th>Response in suboptimal responders n (%)</th>
<th>Response in treatment intolerant n (%)</th>
<th>Side effects n (%)</th>
<th>Discontinuation due to side effects n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richardson et al95</td>
<td>7</td>
<td>Adult</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>5 (71)</td>
<td>–</td>
<td>–</td>
<td>1 (14)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Devlin et al96</td>
<td>5</td>
<td>Adult</td>
<td>–</td>
<td>3</td>
<td>2</td>
<td>5 (100)</td>
<td>–</td>
<td>–</td>
<td>1 (20)</td>
<td>–</td>
</tr>
<tr>
<td>Czaja et al97</td>
<td>8b</td>
<td>Adult</td>
<td>–</td>
<td>7</td>
<td>0</td>
<td>5 (63)</td>
<td>–</td>
<td>–</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Chatur et al104</td>
<td>13</td>
<td>Adult</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>8 (62)</td>
<td>–</td>
<td>–</td>
<td>4 (31)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Yu et al99</td>
<td>15</td>
<td>Adult</td>
<td>8</td>
<td>10</td>
<td>5</td>
<td>11 (73)</td>
<td>–</td>
<td>–</td>
<td>2 (13)</td>
<td>1 (6.5)</td>
</tr>
<tr>
<td>Hlivko et al100</td>
<td>29d</td>
<td>Adult</td>
<td>–</td>
<td>3</td>
<td>9</td>
<td>8 (67)</td>
<td>–</td>
<td>–</td>
<td>10 (48)</td>
<td>7 (24)</td>
</tr>
<tr>
<td>Aw et al103</td>
<td>18</td>
<td>Pediatric</td>
<td>–</td>
<td>12</td>
<td>6</td>
<td>16 (89)</td>
<td>–</td>
<td>–</td>
<td>13 (50)</td>
<td>3 (12)g</td>
</tr>
<tr>
<td>Wolf et al102</td>
<td>16n</td>
<td>Adult</td>
<td>–</td>
<td>6</td>
<td>7</td>
<td>12 (75)</td>
<td>–</td>
<td>–</td>
<td>1 (6.3)</td>
<td>1 (6.3)</td>
</tr>
<tr>
<td>Sharzehi et al105</td>
<td>21</td>
<td>Adult</td>
<td>–</td>
<td>15</td>
<td>6</td>
<td>8 (38)</td>
<td>0 of 12 (0)</td>
<td>8 of 9 (89)</td>
<td>1 (4.8)</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td>Baven-Pronk et al106</td>
<td>45</td>
<td>Adult</td>
<td>24</td>
<td>22</td>
<td>23</td>
<td>24 (53)</td>
<td>8 of 21 (38)</td>
<td>16 (70)</td>
<td>15 (33)</td>
<td>6 (13)</td>
</tr>
<tr>
<td>Park et al107</td>
<td>1</td>
<td>Adult</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>–</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Roberts et al111</td>
<td>105</td>
<td>Adult</td>
<td>38</td>
<td>42</td>
<td>63</td>
<td>63 (60)</td>
<td>24 of 42 (57)</td>
<td>39 of 63 (62)</td>
<td>27 (25)</td>
<td>10 (9.2)</td>
</tr>
</tbody>
</table>

Abbreviation: MMF, mycophenolate mofetil.

aRefers to number of patients with AIH or AIH overlap syndrome receiving MMF.
bOne patient received MMF first-line therapy.
cNone of the patients normalized pretreatment AST levels.
dSeventeen patients received MMF first-line therapy.
eCalculated from those receiving MMF second-line therapy.
fTotal study population was 26 patients including eight with autoimmune cholangitis.
gTotal study population was 26 patients including eight with autoimmune cholangitis.
hIncluded three patients receiving MMF for other indications.
the patient populations and definitions of “nonresponse” and treatment intolerance make it difficult to draw meaningful conclusions about the relative efficacy of MMF in those with refractory disease versus treatment intolerance. Prospective multicenter randomized controlled trials of MMF as salvage therapy in patients with AIH, are needed to address this uncertainty.

Response rates to MMF as salvage therapy may be lower in patients with cirrhosis compared with those without. In the study by Roberts et al, remission was achieved in 47% patients with cirrhosis compared with 66% in those without (\( p = 0.07 \)). Conversely, incomplete response rates were higher in those with cirrhosis compared with those without (47 vs. 25%, \( p = 0.02 \)). Other studies of MMF as rescue therapy have included patients with cirrhosis, but absolute numbers have been low and limited data have been provided on treatment outcomes in this population (– Table 4). Hence, there are insufficient data to determine whether patients with AIH and cirrhosis are a more difficult to manage group with MMF rescue therapy compared with those who are treatment naive and/or noncirrhotic.

The initial dose of MMF in studies has been 0.5 to 1.0 g/day, while doses of 1.0 to 2.0 g/day have been commonly used to induce remission. However, in two studies, a dose of up to 3.0 g/day was used during induction therapy. Thereafter, doses of 0.5 to 1.0 g/day were used to maintain remission usually in combination with steroids.

MMF treatment of AIH is generally safe and well tolerated including in those with cirrhosis and prior azathioprine intolerance. Significant side effects occur in 12 to 34% of patients with the majority of these involving the gastrointestinal tract, particularly nausea, vomiting, and diarrhea. Less common side effects include cytopenias, headaches, pancreatitis, neuropsychiatric symptoms, and dermatological reactions. Rates of treatment discontinuation due to drug-related side effects are generally low at around 9%, although in one study, 10 of 29 (34%) subjects discontinued treatment due to intolerance. Few data are available on the long-term safety of MMF therapy for AIH. MMF is potentially teratogenic (i.e., pregnancy category D) and is therefore contraindicated in pregnancy.

mTOR Inhibitors

Sirolimus

Sirolimus or rapamycin is an inhibitor of the mammalian target of rapamycin (mTOR), a regulatory protein that is intimately involved in the proliferation and survival of lymphocytes activated by antigen-stimulated binding of IL-2 to its T-cell receptor (CD25). Sirolimus exerts several immunomodulatory effects that include a potent reduction in the number of proliferating reactive CD4+ and CD8+ (cytotoxic) effector T cells via induction of cytotoxic T cell apoptosis, as well as impairment in the production of key proteins linked to T cell-mediated cytotoxicity including perforin and granzyme B. This results in a selective expansion in the pool of regulatory (CD4+ CD25 +) T cells that are innately resistant to the apoptotic effects of rapamycin, and play a key role in suppressing the proliferation and activity of cytotoxic T cells. The beneficial effect of sirolimus on the selective survival of Treg population is modulated by IL-2. This has prompted speculation about the potential benefit of combining calcineurin inhibitors that block IL-2 production with sirolimus, although this has not been formally evaluated in refractory AIH.

Sirolimus and everolimus have nephroprotective effects and are therefore frequently used in the liver transplant setting, particularly in patients with renal insufficiency post-transplantation and in those with CI-induced nephrotoxicity.

Data on the efficacy and safety of sirolimus as salvage therapy in patients with steroid-refractory AIH are even more sparse than that for MMF being limited to just a few very small case series (Table 1). In the initial report, Kerkar et al reported their experience of the outcomes of sirolimus in six patients with either de novo or recurrent AIH post liver transplant of whom five were nonresponsive to conventional treatment with prednisolone and azathioprine. All patients responded to the addition of sirolimus with significant reduction in serum ALT levels, histology (when available), and immunoglobulin G (IgG) levels. The doses used were 1.0 to 3.0 mg/day and minimal side effects were noted, although the drug was withdrawn in one patient with severe colitis. In another small single center study, five adult subjects with disease, refractory to prednisolone and azathioprine or MMF, received sirolimus in an initial dose of 2 mg/day. Significant biochemical improvement with > 50% reduction in serum ALT levels was achieved in four (80%) patients with two (40%) achieving a sustained normalization of serum liver enzymes. All patients had a significant reduction in their steroid dose. Side effects were minimal apart from a rise in serum lipids in two patients. Less-positive results were reported in a small study of four pediatric patients with refractory AIH where sirolimus 1.0 to 2.0 mg/m2/day led to improvement in serum liver enzymes and/or steroid dose in only two patients.

Sirolimus has an acceptable safety profile and is generally well tolerated with the main side effects being respiratory in nature, involving cough, dyspnea, and fever. The overall risk of pulmonary toxicity with interstitial pneumonitis from the transplant literature is 2 to 7%, and this is an indication for immediate cessation of mTOR inhibitors. Other reported side effects include hyperlipidemia, fatigue, bone marrow suppression, skin rash, stomatitis, and edema.

Everolimus

Only one small study has evaluated everolimus as the second-line therapy for refractory AIH. Ytting et al reported on seven adults with nonresponse to standard therapy and empirical treatment with MMF, budesonide, and Cs. Treatment with everolimus in an initial dose of 0.75 to 1.5 mg bid led to rapid and significant biochemical improvement by 2 weeks in four subjects. Overall, four subjects achieved sustained biochemical remission and no worsening or improvement in liver fibrosis, while all patients achieved clinical remission within 5 months. Significant reductions in steroid dose were achieved in six patients. Side effects reported include arthralgias, asthma-like symptoms, and
rarely mTOR inhibitor-induced interstitial pneumonitis that, like sirolimus, is an indication for immediate cessation.

**Methotrexate**

Methotrexate is an antimetabolite that interferes with DNA synthesis via inhibition of folate metabolism. It has both anti-inflammatory and immunomodulatory properties and has been trialed as a disease-modifying agent in autoimmune diseases, including autoimmune cholestatic liver disease. Three small case series have assessed its use as an alternative agent in nonresponsive AIH. Following the initial two case reports of induction and maintenance of remission with once-weekly dosing for refractory AIH, interest in methotrexate waned following reports of methotrexate-induced AIH and hepatotoxicity in other diseases, as well as concerns surrounding its fibrogenic potential—hepatic and pulmonary—with long-term use. More recently, Hardiy et al reported their experience with methotrexate in 11 subjects of AIH including 4 with cirrhosis and 5 with an incomplete response to standard therapy. A total of five (46%) patients achieved or maintained remission on methotrexate associated with a reduction in corticosteroid requirements. However, 46% discontinued treatment within 12 months due to adverse events that were mostly hepatic related. Thus, given the safety concerns and limited evidence for efficacy, methotrexate cannot be recommended as salvage therapy for AIH.

**Cyclophosphamide**

Cyclophosphamide in lower doses is an immunosuppressant and has been used as such for the treatment of several immune-mediated diseases including vasculitis. Successful induction and long-term maintenance of remission have been reported in three subjects with AIH treated with the combination of cyclophosphamide 1.0 to 1.5 mg/kg and a tapering dose of corticosteroids commencing with 1 mg/kg. No significant side effects were observed over a cumulative observation period of over 12 years. However, the subsequent report that cyclophosphamide can activate the immune system by suppressing Treg function has dampened the enthusiasm for its use as an alternative therapy for AIH.

**Biological Therapies**

**Infliximab**

The use of anti-TNF-α therapies, such as infliximab, in AIH is based upon the recognition of the important role TNF-α plays in the cytotoxic T cell immune response including the proliferation and differentiation of lymphocytes. In addition, there are inconsistent reports of a relationship between genetic polymorphisms in the TNF-α promoter region and type 1 AIH. Furthermore, there is considerable experience in the use of biological agents in the treatment and management of other autoimmune conditions, such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease. Despite the biological plausibility of an effect, the use of agents such as infliximab in AIH is quite limited and hence it is not possible to draw any conclusions regarding the potential utility of these therapies in AIH. Infliximab was used in a small trial of 11 treatment-resistant patients using a dose schedule derived from the literature on inflammatory bowel disease (5 mg/kg body weight at time zero, 2 weeks, 6 weeks, and then every 4–8 weeks). Normalization of ALT was recorded in 8 of 11 participants, although 7 subjects developed treatment-related toxicities, predominantly involving opportunistic infections (see Table 1). To further complicate matters, anti-TNF-α antibodies have been associated with the induction of an immune-mediated liver disease resembling AIH. Considering the limited evidence available, the use of anti-TNF-α therapies outside of expert centers cannot be recommended until new efficacy and safety data emerge that support their use.

**Rituximab**

Rituximab is a chimeric mouse–human monoclonal antibody that promotes depletion of B lymphocytes via binding to the CD20 antigen expressed on the surface of B cells. This binding results in complement and antibody-dependent cellular cytotoxicity, and the resultant B cell depletion has implications for autoantibody formation and interference with both the B and T cell responses. The literature surrounding rituximab use in AIH has predominantly been case reports, with the largest published experience involving six cases that received two infusions of rituximab (1,000 mg) 2 weeks apart in patients refractory to prednisolone plus azathioprine. Biochemical response was observed in all subjects by week 12 and prednisolone was withdrawn in three subjects. The lack of robust clinical data, coupled with concerns regarding the reactivation of latent infections, such as hepatitis B and other potential drug toxicities, means that rituximab cannot be recommended for use in AIH outside highly specialized treatment centers with experience in its use.

**Nonimmunosuppressive Therapies**

**Ursodeoxycholic Acid**

Ursodeoxycholic acid (UDCA), an epimer of chenodeoxycholic acid, has several putative therapeutic properties relevant to treating liver diseases that include hepatocytopeno-protection and immunomodulation. The immunomodulatory actions of UDCA include alterations in class I and II HLA antigen expression on hepatocytes, reduction and/or inhibition of immunoglobulin and cytokine (IL-2, IL-4, and IFN-γ) production, stimulation of lymphocyte function, and inhibition of apoptosis. These actions could potentially alter the immune response and reduce hepatocellular injury in AIH. While UDCA is the standard of care for treating primary biliary cholangitis, only a few studies have explored its therapeutic role in AIH. Following the original pilot study that demonstrated UDCA to be efficacious in treatment-naive type 1 AIH subjects, a randomized placebo controlled trial of UDCA (13–15 mg/kg daily) was conducted as salvage therapy in 37 subjects with suboptimal responses to standard therapy. Adjunctive therapy with UDCA led to significant biochemical improvement compared with those receiving placebo, although no difference was observed in the need for corticosteroids or rates of clinical improvement and histological response.
Thus, in the absence of further evidence, UDCA cannot be recommended as a salvage therapy for AIH. However, it may still play a potentially important role in those with AIH overlap syndromes where significant cholestatic features exist.116

Plasmapheresis
Plasmapheresis removes semiselectively several large molecules pertinent to immune-mediated tissue damage, including immunoglobulins, autoantibodies, immune complexes, adhesion molecules, and cytokines.149 The technique has been employed in several different conditions in which humoral factors are implicated in the pathogenesis of disease.150 While considered potentially useful for the treatment of refractory autoimmune diseases, there have been only four case reports of its use in nonresponsive AIH.150–153 Three of these arose in the setting of overlap with other (auto)immune diseases,150–152 while the fourth involved a severe case of de novo AIH postliver transplant that was refractory to steroids, MMF, and CIs.153 Institution of plasmapheresis led to remission in the latter case, but maintenance treatment was required to prevent relapse.

Liver Transplantation
Liver transplantation is an important salvage therapy for AIH,49 particularly for severe acute or fulminant cases that are less responsive to corticosteroid therapy.46,49,148 Indeed, in one study of 16 cases of acute fulminant AIH, only 1 of 12 patients treated with corticosteroids improved with 13 needing transplantation.154 The principal indications for transplantation for AIH are fulminant liver failure from severe acute AIH (nonresponsive to steroids), decompensated cirrhosis, and hepatocellular carcinoma.155 Patient survival at 5 and 10 years following transplant is excellent being > 80 and > 75%, respectively, while 5-year graft survival is around 75%.156–158 Despite posttransplant immunosuppression and the absence of planned HLA donor–recipient matching, AIH recurs in the donor liver in a frequency that is quite variable but generally time dependent; around 12% of patients are affected at 1 year and up to 36% by 5 years.159–163 A systematic review of available literature estimated the weighted recurrence rate of AIH posttransplant at 22%.164 Factors predictive of recurrence include the severity of hepatic inflammation and level of serum IgG elevation at the time of transplant and coexistent inflammatory bowel disease.164,165 Furthermore, most transplant centers include prednisolone in the immunosuppressive regimen of patients transplanted for AIH. Liver histology, including a prominent plasma cell infiltrate with interface hepatitis, may be the only indicator of recurrent disease,166 although it may be difficult to make this distinction. However, progression to cirrhosis, graft dysfunction, and graft loss appears rare.159,162,164 Recurrent AIH usually responds well to increased immunosuppression with reintroduction or a higher dose of corticosteroids and/or optimization of CI dosing.155,167,168 However, in refractory cases, sirolimus117 or azathioprine may be required.

Emerging Salvage Therapies
There is a significant unmet need for novel salvage therapies for AIH that selectively target key components of the immune system involved in the immunopathogenesis of AIH rather than induce blanket immunosuppression. Several potential therapeutic interventions have been proposed for refractory AIH that have been detailed in recent reviews of this area (<Table 5>).158,169 Several molecular agents are in clinical development for other diseases and include those that (a) inhibit cytokotoxic effector T cell activity, (b) enhance Treg function and survival, and (c) induce cytokotoxic T cell apoptosis.108,169 Cellular interventions that manipulate immune cell populations are by comparison at a more rudimentary stage of development and include the adoptive transfer of freshly cultured Tregs to enhance Treg function,170 oral tolerization to improve T cell antigen specificity,171–174 glycolipid stimulation of NK cells,175 and mesenchymal stem cell transplantation108,176 and autologous bone marrow transplantation.108

Cytotoxic T Lymphocyte Antigen-4-Ig
Cytotoxic T lymphocyte antigen-4 (CTLA-4) is a protein expressed on the surface of CD4+ T lymphocytes and can interfere with the binding interaction with APC resulting in inhibition of lymphocyte activation.177 Abatacept is a dimeric recombinant human protein that represents the fusion of CTLA-4 and immunoglobulin (CTLA-4-Ig) and is capable of modulating the immune response by inhibiting the binding between CD4+ lymphocytes and APC.178–180 It has proven efficacy for the treatment of rheumatoid arthritis181 and is in clinical development in several other (auto)immune-mediated diseases.108,169,182,183 In autoimmune hepatitis, it has been the subject of a single case report,184 although a recent report suggested CTLA-4 was not involved in the pathogenesis of AIH.185

Interleukin-2
Interleukin-2 (IL-2) is an essential cytokine for Treg homeostasis, function, and survival.186,187 Recent proof-of-concept clinical trials have shown that the administration of IL-2 at low doses is safe and can improve several (auto)immune-mediated conditions, including systemic lupus erythematosus, type 1 diabetes, hepatitis C virus (HCV)- vasculitis, and graft-versus-host-disease.187 Preliminary work in patients with autoimmune liver disease has shown that very low-dose IL-2 can selectively enhance peripheral and hepatic Treg function and survival.188 These findings hold promise that this therapy could restore immune tolerance in AIH.

Recombinant Interleukin-10
Recombinant interleukin-10 (IL-10) can potentially enhance the anti-inflammatory effects of the type 2 cytokine response and blunt the type 1 cytokine response responsible for the proliferation and differentiation of cytokotoxic T cells targeting the liver.189,190 Animal and human studies of recombinant IL-10 in liver and nonliver diseases provide a sound rationale for its use in autoimmune liver disease.191–197 While administration of recombinant IL-10 reduces liver inflammation and fibrosis in mouse models197 and in patients with chronic hepatitis C,193,195 its side-effect profile, which is similar to interferon, is likely to limit patient acceptability.196–198

Preimplantation Factor
Secreted by embryos, preimplantation factor (PIF) is a novel biologic immune modulator that is responsible for maternal
<table>
<thead>
<tr>
<th>Drug regimen</th>
<th>Dose</th>
<th>Mode of action</th>
<th>Main indications</th>
<th>Clinical experience</th>
<th>Major side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monoclonal antibodies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-TNF-α (infliximab) or anti-TNF-α receptor (etanercept)</td>
<td>No established dosing guidelines in AIH, infliximab 5 mg/kg body weight as IV infusion q 2–8 wks suggested</td>
<td>Blocks proinflammatory cytokine TNF-α action that is implicated in disease pathogenesis, decreases cytotoxic effector T cells</td>
<td>Limited experience in AIH, improves liver chemistries and IgG levels,53,138,139 widely used in RA, IBD, and psoriasis</td>
<td>Infections: CMV, reactivation of infection (TB), anaphylaxis, immune-mediated injury, lymphoproliferative disease</td>
<td></td>
</tr>
<tr>
<td>Anti-CD3 (nonmitogenic)</td>
<td>Uncertain</td>
<td>Monoclonal antibody to CD3 expressed on T cells induces apoptosis of cytotoxic T cells and increases Treg number, survival, and function201-203</td>
<td>No experience in AIH, increases insulin production in type 1 diabetes</td>
<td>Few side effects: Fever, anemia; safety profile uncertain</td>
<td></td>
</tr>
<tr>
<td><strong>Molecular agents</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTLA-4-Ig (abatacept)</td>
<td>Uncertain</td>
<td>Recombinant human fusion protein that inhibits binding between CD4+ T cells and APC; impairs costimulatory signaling required for lymphocyte activation</td>
<td>Minimal experience in AIH,184 approved for RA, experience in MS, bone marrow transplantation</td>
<td>Safety profile uncertain</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>Low dose</td>
<td>Cytokine required for Treg cell homeostasis, function, and survival186,187</td>
<td>Minimal experience in AIH,188 experience in SLE, Type 1 diabetes, HCV-vasculitis</td>
<td>Few side effects at low doses</td>
<td></td>
</tr>
<tr>
<td>Recombinant IL-10</td>
<td>Uncertain</td>
<td>Recombinant protein that enhances anti-inflammatory type 2 cytokine response and blunts type 1 cytokine response,189,190 decreases liver-directed cytotoxic T cell numbers and effect192</td>
<td>No experience in AIH, improves liver fibrosis and inflammation in CHC,194 decreases inflammatory indices in IBD</td>
<td>Safety profile uncertain, flu-like symptoms, cytopenias</td>
<td></td>
</tr>
<tr>
<td>Preimplantation factor</td>
<td>Uncertain</td>
<td>Protein secreted by embryos that induces maternal tolerance of fetus, targets CD14+, CD4+, and CD8+ cells199</td>
<td>Limited phase I SAD/MAD experience in AIH100</td>
<td>Appears safe</td>
<td></td>
</tr>
<tr>
<td>B cell-activating factor</td>
<td>Uncertain</td>
<td>Involved in development, maturation, and survival of peripheral B cells, Ig production and class switch</td>
<td>Levels high in AIH and predictive of disease severity,209,210 reduced by corticosteroid therapy</td>
<td>Safety profile uncertain</td>
<td></td>
</tr>
<tr>
<td><strong>Cellular therapies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesenchymal stem cell transplantation/bone marrow transplantation</td>
<td>Uncertain</td>
<td>May improve function of several immune cell populations, including T and B lymphocytes, NK cells, macrophages, dendritic cells</td>
<td>Advanced refractory AIH</td>
<td>No experience in AIH</td>
<td>Uncertain safety profile</td>
</tr>
<tr>
<td>Adoptive transfer of autologous antigen-specific Tregs</td>
<td>Uncertain</td>
<td>May reduce activity of effector T cells to suppress autoimmune response</td>
<td>No experience in AIH</td>
<td>Uncertain safety profile</td>
<td></td>
</tr>
</tbody>
</table>
tolerance toward the fetus during pregnancy. PIF induces systemic immune homeostasis via the targeting of protective, immune regulatory, and cytoskeleton proteins in CD14+, CD4+, and CD8+ cells. This makes it a potential therapeutic agent in immune-mediated disorders including AIH. In a recent phase I randomized, double blind, placebo-controlled study, synthetic PIF administered in a single dose of either 0.1, 0.5, or 1.0 mg/kg subcutaneously to adult patients with AIH had an excellent safety profile and was well tolerated. The study, which was expanded to include multiple ascending dose arms of the above doses over 5 consecutive days, was recently completed with results expected soon (ClinicalTrials.gov Identifier: NCT02239562).

**Anti-CD3 Antibody**

Immunosuppressive, nonmitogenic CD3 antibodies target the T cell antigen receptor, thereby inducing apoptosis of cytotoxic T cells. The phagocytosis of these apoptotic cells by macrophages and dendritic cells results in the release of transforming growth factor-β (TGF-β), which stimulates the expansion and activity of Tregs, in turn facilitating immune tolerance. Preliminary studies in patients with type 1 diabetes show that administration of nonmitogenic CD3 antibodies is generally well tolerated and can improve indices of glucose homeostasis and insulin requirements. Side effects associated with the use of anti-CD3 antibodies include fever, rash, and anemia. Still, it remains to be seen whether this treatment has sufficient safety, specificity, and durability to be a feasible salvage therapy for autoimmune hepatitis.

**B Cell-Activating Factor**

B cell-activating factor (BAFF) is a member of the TNF superfamily and is influential in the development, maturation, and survival of peripheral B cells that play an integral role in the humoral immune response. Other important biological functions of BAFF include immunoglobulin production and immunoglobulin class switch recombination in which activated B cells (plasma cells) change their antibody production from one isotype to another. Serum levels of BAFF are elevated in several autoimmune diseases, including AIH where it is predictive of the degree of hepatic necroinflammatory activity. Furthermore, BAFF appears to contribute to liver injury and disease development in patients with AIH, while corticosteroid therapy results in a marked reduction in serum BAFF levels. Thus, BAFF is a potential therapeutic target in the management of AIH.

**Management of Refractory/Recalcitrant Autoimmune Hepatitis**

While several potential salvage therapies have been identified for refractory (i.e., poorly responsive) or recalcitrant autoimmune hepatitis with treatment failure, as detailed above, uncertainty remains as to what the optimal strategy is of this difficult to treat population. Still, clinical practice guidelines developed by the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) have adopted broadly similar recommendations and treatment algorithms (Fig. 1) to manage refractory AIH populations that are supported by other key experts in this field. These recommendations are based predominantly on level III/IV evidence collected from single center observational studies and expert opinion with no significant randomized controlled trials of salvage therapies having been conducted in this setting. Included among these is the strong recommendation that poor compliance with treatment needs to be excluded, particularly in young patients before alternative treatment strategies are considered for nonresponders to conventional treatment. The accuracy of the diagnosis also needs to be reevaluated, including consideration to repeat liver biopsy, as the poor response to steroids may be due to an alternative diagnosis and/or the coexistence of other conditions, such as chronic hepatitis C, nonalcoholic fatty liver disease, autoimmune cholestatic liver disease, Wilson’s disease, α1-antitrypsin deficiency, and hemochromatosis.

The initial treatment for those with recalcitrant AIH, associated with significant acute deterioration, is high-dose prednisolone monotherapy 60 to 100 mg/day orally or IV. For subjects with a more subacute course of deteriorating liver chemistries and/or liver histology, prednisolone in a lower dose of 30 mg/day combined with high-dose azathioprine (150 mg/day or 2 mg/kg/day) for up to 4 weeks is preferred. Responders to this higher dose regimen should then have steroid and azathioprine doses reduced at monthly intervals until reaching maintenance dose levels.

Subjects who show evidence of treatment failure and/or progressive liver failure despite treatment should be managed in conjunction with a liver transplant center and undergo evaluation for liver transplantation. Those who fail to respond to first-line salvage therapy and have less severe disease, who are otherwise not suitable for transplantation, should be considered for alternative immunosuppressive therapy. Steroid-sparing calcineurin inhibitors are preferred in this setting with tacrolimus as the generally preferred agent over cyclosporine because of its higher potency and larger experience in organ transplantation. Commencement of mycophenolate should also be considered, particularly in those with azathioprine intolerance but also for suboptimal responders to standard therapy, as remission rates up to 60% have been reported when used as rescue therapy. Beyond these, there are limited data to recommend other immunosuppressive agents, including sirolimus, rituximab, and infliximab, as salvage therapies with use of these investigational agents best managed by specialized centers with expertise in managing this condition.

**Conclusion**

Several agents have been studied as front-line salvage therapy for patients with AIH, who respond suboptimally to conventional therapy, with the more promising of these being calcineurin inhibitors and MMF. However, none of these salvage therapies have undergone rigorous clinical evaluation via randomized clinical trials, with efficacy and safety data thus far being generated predominantly from single or multicenter, mostly retrospective observational cohort studies. There remains much...
to learn about the utility of these alternative therapies for AIH, including the appropriate timing, dosing, and therapeutic monitoring, following their introduction and the patient population to whom these should be directed. Liver transplantation remains an effective salvage therapy for those with severe fulminant AIH and those with end-stage disease, although recurrence of disease is not infrequent. Looking to the future, several promising specific immunosuppressive therapies have been developed for autoimmune diseases that may potentially facilitate immune tolerance via enhancement of Treg activity and function.

Main Concepts and Learning Points

<table>
<thead>
<tr>
<th>Major concept</th>
<th>Learning point</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Around 20% of patients with autoimmune hepatitis fail to respond to conventional therapy</td>
<td>• Patients with refractory or recalcitrant autoimmune hepatitis require salvage therapy</td>
</tr>
<tr>
<td>• The optimal salvage treatment strategy for autoimmune hepatitis is unclear</td>
<td>• Several potential salvage therapies have been identified, including calcineurin inhibitors, mycophenolate, and rituximab, but none have undergone rigorous clinical evaluation</td>
</tr>
<tr>
<td>• The first-line salvage strategy involves high-dose prednisolone monotherapy ± azathioprine</td>
<td>• Subjects with progressive liver failure should be discussed with a liver transplant center and evaluated for liver transplantation</td>
</tr>
<tr>
<td>• Second-line salvage therapy is typically with calcineurin inhibitors (i.e., cyclosporine, tacrolimus) and mycophenolate</td>
<td>• Tacrolimus is generally preferred over cyclosporine and is given in low dose with therapeutic monitoring required</td>
</tr>
<tr>
<td></td>
<td>• Mycophenolate is effective when used in patients with azathioprine intolerance and to a lesser extent in those with steroid-refractory disease</td>
</tr>
</tbody>
</table>

Funding
None.

Conflict of Interest
None.

Acknowledgments
None.

References
Seminars in Liver Disease Vol. 37 No. 4/2017

Salvage Therapies for Autoimmune Hepatitis


Sparrow MP, Hande SA, Friedman S, Cao D, Hanauer SB. Effect of allopurinol on clinical outcomes in inflammatory bowel disease


Fallatah HI, Akbar HO. Mycophenolate mofetil as a rescue therapy for autoimmune hepatitis patients who are not responsive to standard therapy. Expert Rev Gastroenterol Hepatol 2011;5(04):517–522


Salvage Therapies for Autoimmune Hepatitis

Kaplan MM, DeLellis RA, Wolfe HJ. Sustained biochemical and 124
123
Augustine JJ, Bodziak KA, Hricik DE. Use of sirolimus in solid 120
119
Kurowski J, Melin-Aldana H, Bass L, Alonso EM, Ekong UD. 118
117
Chatrath H, Allen L, Boyer TD. Use of sirolimus in the treatment of 116
115
Vierling JM. Autoimmune hepatitis and overlap syndromes: 113
112
Molhoek KR, McSkimming CC, Olson WC, Brautigan DL, Sling- 110
109
107
Park SW, Um SH, Lee HA, et al. Mycophenolate mofetil as an 104
103
Nakamura K, Yoneda M, Yokohama S, et al. Ef 100
99
Burak KW, Swain MG, Santodomingo-Garzon T, et al. Rituximab 96
95
Czaja AJ, Carpenter HA, Lindor KD. Ursodeoxycholic acid as 92
91
87
Kharidy J, Nicoll A, Sood S. Methotrexate therapy for autoim- 84
83
Haridy J, Nicoll A, Sood S. Methotrexate therapy for autoim- 80
79
Kaplan MM, DeLellis RA, Wolfe HJ. Sustained biochemical and 76
75
71
Czaja AJ. Current and prospective pharmacotherapy for autoimmune 68
67
Czaja AJ. Autoimmune hepatitis: focusing on treatments other than steroids. Can J Gastroenterol 2012;26(09):615–620 64
63
59
55
51
Kaplan MM, DeLellis RA, Wolfe HJ. Sustained biochemical and 48
47
43
39
35
31
28
25
22
19
16
13
10
7
4
1
-1
-2
-3
-4
-5
-6


