Second-line and Third-line therapy for Autoimmune Hepatitis

A position statement from the European Reference Network on Hepatological Diseases and the International Autoimmune Hepatitis Group

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Abstract

Autoimmune hepatitis in the majority of patients responds well to standard immunosuppressive therapy with steroids and azathioprine, and while untreated disease is usually fatal, patients responding well to therapy have an excellent prognosis. However, about 10-20% of patients present therapeutic challenges due to an insufficient response or intolerable side-effects to standard therapy, requiring dose-adaptation or switch of therapy. While there is a fairly good agreement on second-line treatment options, there is very wide variation in the indication and use of possible third-line therapies. The European Reference Network on Hepatological Diseases (ERN RARE-LIVER) and the International Autoimmune Hepatitis Group (IAIHG) here offer a treatment algorithm for both children and adults that should help to standardize treatment approaches, in order to improve patient care as well as to allow comparison of treatment results in scientific publications.

Lay summary

Autoimmune hepatitis is a chronic inflammatory disease of the liver. Most patients require lifelong immunosuppressive treatment, but because of insufficient control of liver inflammation or because of side effects some patients need alternatives to standard immunosuppressive medication. We here present an overview of second-line and third-line immunosuppression for patients with autoimmune hepatitis.

Graphical abstract

Treatment of Autoimmune Hepatitis
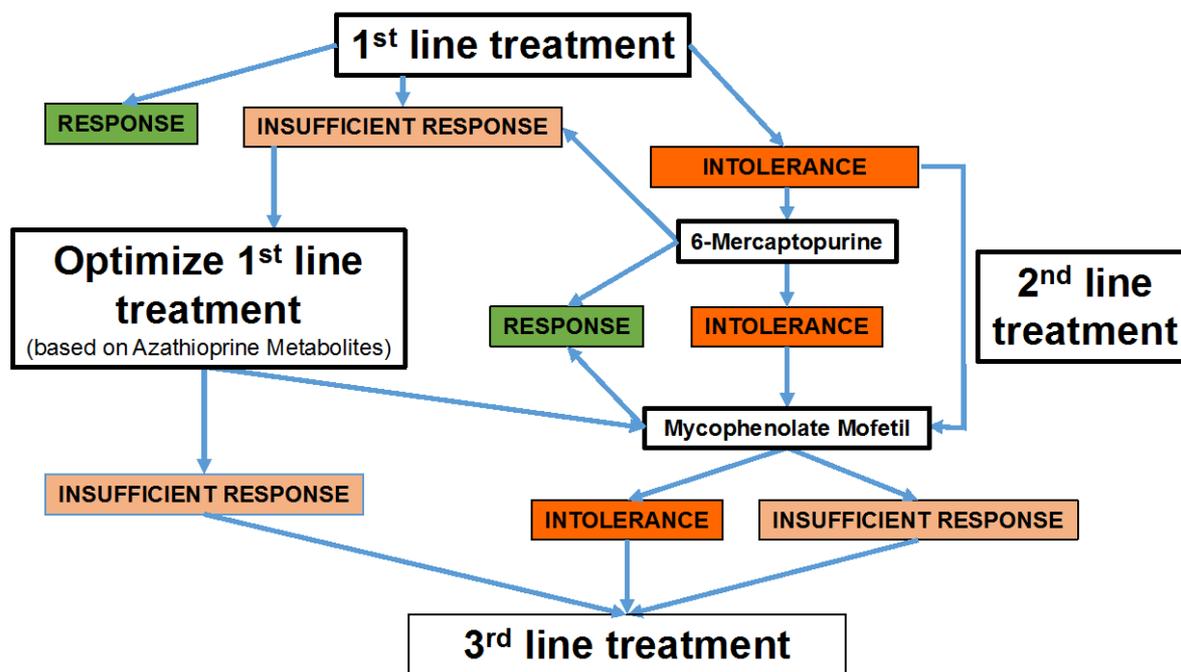
Key points

- Patients with autoimmune hepatitis (AIH) require alternatives for standard immunosuppressive treatment in two clinical scenarios: insufficient response, i.e not achieving remission, or intolerance due to side effects

- Biochemical remission of AIH is defined as normal transaminases as well as normal IgG levels and histological remission is defined as a hepatitis activity index (HAI-score) of up to 3 out of 18

- The European Reference Network on Hepatological Diseases (ERN RARE-LIVER) and the International Autoimmune Hepatitis Group (IAIHG) here present a position statement on second-line and third-line treatment of AIH

- 6-Mercaptopurine and mycophenolate mofetil are the treatment of choice for AIH patients being intolerant to standard treatment

- For AIH patients not achieving remission under standard treatment, optimization of azathioprine dosage on the basis of 6-TGN levels should first be aimed at before starting alternative immunosuppressants

- In paediatrics and adolescents, growth development, problems with adherence and availability of liquid formulations of immunosuppressive drugs for small children can be additional reasons for adapting the treatment strategy

- No clear preference can be given for single immunosuppressants as third-line treatment since comparative studies are missing and none of the therapies used are approved, yet. However, standardized approaches are needed in order to make studies comparable and to enable comparative analysis of efficacy in the future

Introduction

Response to initial corticosteroid therapy is so universal in autoimmune hepatitis (AIH) that it is considered a diagnostic criterion.[1,2] Studying the effect of prednisone or prednisolone in AIH was the subject of a number of randomized trials in the 1960s and 1970s, some of the earliest randomized trials in medical history, with a clear result across all trials: steroid therapy improves survival impressively.[3,4] Subsequent trials have established that the addition of azathioprine spares steroids and has better success rates in the maintenance of remission than steroid monotherapy.[5,6] Thus steroids remain the drug of choice for remission induction, and azathioprine the drug of choice for maintenance of remission.[7] There is still some debate as to the optimal dosage of these drugs, and to what extent the azathioprine dose should be increased in order to be able to withdraw steroids versus lower dose combination therapy.[7-9] A recent study has suggested that the initial steroid dose is not decisive, and that 0.5 mg/kg body weight prednisolone is probably sufficient in the majority of patients.[10] There is consensus that azathioprine should be added within 2 weeks after starting steroids, given that total bilirubin levels are about < 5 mg/dl, and that azathioprine should be the backbone of any maintenance therapy.[7] The exact titration of doses during maintenance therapy, and the relative preference of higher doses of azathioprine as possible monotherapy versus low-dose prednisone (e.g. 5 mg/day) combined with low doses azathioprine (around 1 mg/kg body weight) depend on individual risk factors and preferences, and should be decided together with the patient. There is also consensus that relapse rates after withdrawal of therapy are extremely high, and withdrawal should only be attempted in patients who have been in stable remission on low-dose therapy for at least two years.[11] Liver biopsy before treatment withdrawal has been recommended by many guidelines on AIH, but are considered optional in the EASL Clinical Practise Guideline. Due to the limitations of

liver biopsy with to sampling errors, and due to the increasing evidence for biochemical remission as a reliable predictive marker, the role of liver biopsy in the assessment of remission has become less important. Patients with ALT levels below half the upper limit of normal in combination with IgG levels below 12 g/L have a very good chance for successful treatment withdrawal[12] and therefore probably do not need liver biopsy before weaning of immunosuppression. The main role of liver biopsy on follow-up is to distinguish remaining AIH activity from other causes of elevated liver enzymes (drug toxicity, associated NASH etc.), and to adapt management accordingly. Furthermore, whenever there is doubt that biochemical parameters are reliable in the assessment of remission, e.g. if IgG levels were not elevated in the beginning of treatment, or if levels of transaminases are close to the upper limit of normal, liver biopsy may also provide valuable information for patient management. Withdrawal of treatment should be avoided during puberty independent of the duration of remission and only with great alertness in patients with type 2 AIH, as well as in all patients in whom the disease first manifested in childhood.

There is wide consensus that remission can be defined both biochemically and histologically.[13-17] Biochemical remission is defined as normal transaminases as well as normal IgG levels.[7,8] In the paediatric literature decrease or even disappearance of autoantibodies is also considered an important marker of remission.[18] However, this may be very closely linked to IgG levels, and has thus so far not been confirmed as an independent indicator of remission. Histological remission is defined as a hepatitis activity index (HAI-score) of up to 3 out of 18, as this degree of disease activity is widely regarded as non-progressive.[7,19] A recent case series has demonstrated that patients reaching full biochemical remission do not only not progress, but overall even show a regression of liver fibrosis as measured by repeated evaluation of transient

elastography, e.g. by Fibroscan.[20] On the other hand, this and other studies have confirmed, that remaining disease activity is associated with progressive fibrosis and development of cirrhosis, thus underlining the need to achieve full biochemical remission if possible.[13-16,20]

While there is growing consensus on the standard management of autoimmune hepatitis, there is a considerable degree of uncertainty regarding how to deal with patients insufficiently responding to standard therapy, or not tolerating standard therapy. The EASL clinical practice guideline and the ESPGHAN position statement recommend mycophenolate mofetil (MMF) for those patients not tolerating azathioprine, and this also appears to be a widely held consensus beyond Europe.[7,8,21] While the data for MMF in patients intolerant to azathioprine are encouraging, MMF seems of little benefit in those patients not responding sufficiently to first line therapy, but there are only limited data on this issue.[22-26]

The largest controlled trial in autoimmune hepatitis addressed the role of budesonide as an alternative steroid to prednisone in induction therapy.[27] Besides its primary end-point result establishing budesonide as an alternative drug in remission induction with less steroid specific side effects, this trial demonstrated the very high rate of patients not achieving the endpoint biochemical remission within six months using normal ALT as endpoint. While some authors have criticised this trial for applying a rather low dose of prednisone and for tapering prednisone too strictly and too quickly in some patients, overall the treatment reflected widely used standards, and yet in both treatment arms more than 30 % of patients were not in biochemical remission at month six. Together with epidemiological studies showing increased liver related mortality in AIH despite therapy, this trial thus showed the unmet need for more effective and better tolerated

therapeutic options in AIH.[27,28] Therefore, in addition to the need for new drugs and new therapeutic approaches, we need recommendations, and in due course better data, on how to deal with patients not responding sufficiently to standard therapy in AIH, as well as with patients intolerant to standard therapy.

The scarcity of data, with only small studies and case series published so far (Supplementary Table 1), and the wide variation of inclusion criteria and drug usage, do not allow any recommendations at present as to which particular third-line therapy should be used in an individual patient. Multicentre studies are needed in order to accumulate data with sufficient power to make robust conclusions. In order to achieve this most efficiently, we need to structure, standardize and report our approach to patients with autoimmune hepatitis in need of third-line therapy. The following position statement is the result of repeated discussions within the scientific community coordinated by the European Reference Network on Hepatological Diseases (ERN RARE-LIVER) and the International Autoimmune Hepatitis Group (IAIHG) in order to help in the management of these patients, and in order to agree on a standard process for the use of in particular third-line therapies to allow the collection of comparable data from treatment centres. It is hoped that based on this treatment algorithm such data can be collected in the future, helping us to improve management of this important patient population, with the final goal of establishing third-line treatment options based on high quality data in adequately powered studies. This position statement will cover both adult and paediatric data and address the two key problems, sometimes intertwined: insufficient response and intolerance (Fig. 1 and 2).

Insufficient response

Treatment aim in every patient with autoimmune hepatitis is prevention of progressive liver disease. Lack of progression, and even regression of fibrosis, is observed in more or less all patients with full biochemical remission defined as normal transaminases and normal IgG levels.[13-16,20] It is uncertain whether histological proof of remission by demonstration of no or only minimal inflammatory activity (HAI<3/18) on liver biopsy is more reliable than biochemical remission. On the one hand histology is considered the gold standard, but on the other hand due to the risk of sampling error, biochemical remission parameters might provide a better overall picture than liver biopsy. The two approaches can be considered complementary. In particular in patients with borderline biochemical parameters, a liver biopsy may help to assess the remaining disease activity more reliably. Furthermore, liver biopsy may be able to detect co-morbidity such as non-alcoholic steatohepatitis (NASH) responsible for not achieving normal transaminase levels, or possible azathioprine hepatotoxicity.[29] When full biochemical remission is achieved, histological confirmation of remission is not required. Similarly, if both IgG and transaminases show a clearly insufficient response, histological confirmation of insufficient response is also not required. For all other cases, histological diagnosis is recommended, as the resulting treatment decisions may have important consequences, and are likely to determine the long-term therapy of this chronic disease.

While insufficient response in the initial treatment period is not well defined, and needs to be assessed individually depending on the severity of disease and co-morbidities, we have agreed that insufficient response is failure to achieve full biochemical remission within the first six months of treatment.[30] While in many patients full biochemical remission can be achieved even faster than that, six months appears a reasonable time to wait, as some patients respond slower than others.[31] In some

patients with initially severe disease and a clear tendency of improvement, achieving full biochemical remission may even take a few months longer than six months, but for the vast majority of patients the six months' time point is appropriate for assessing response and should therefore be standard. Histological remission does take longer than biochemical remission, and thus histological evaluation for degree of response, may need to be delayed up to one year, if there is uncertainty in the interpretation of biochemical response[5] – this can be the case in borderline values for transaminases or IgG, and discrepancy between transaminase response and IgG response.

We recommend that in patients with insufficient response, drug levels of azathioprine metabolites should be measured (Fig. 1).[7,32,33] There are two main reasons for this recommendation:

1. Azathioprine drug metabolism varies widely between individuals. It is the group of 6-thioguanine nucleotides (6-TGN) that contain the main active drug 6-tioguanine-triphosphate (6-TGTP), while the 6-methyl-mercaptopurine metabolites (6-MMP) are not immunosuppressive, but can reflect drug toxicity.

2. Non-adherence to recommended treatment is another important reason for insufficient treatment response, and can be detected by absent or very low levels of all drug metabolites.

Unfortunately, 6-TGN measurements are not trivial and can only be undertaken in a few specialized laboratories. Future studies will need to show the reliability and reproducibility of measurements across different laboratories, but data from transplant immunology as well as from inflammatory bowel disease have shown the value of these measurements in patient management.[34-36] A larger, albeit retrospective study of AIH patients from Sheffield, UK, showed that patients with 6-TGN levels below 220 pmol/8×10^8 red blood cells were more likely to experience relapse or have insufficiently

suppressed disease activity.[37] Therefore, it appears reasonable to try to achieve such a level in all patients with insufficient response and lower levels on drug measurement. This can normally be achieved by addressing adherence to therapy and adapting the dose up to 2 mg or even 2.5 mg/kg body weight, as is customary in treatment of patients with inflammatory bowel disease.

For those patients with inadequately low levels of 6-TGN and high levels of 6-MMP, the alternative drug metabolite, the underlying problem is altered drug metabolism in the presence of good patient adherence. In these patients, again a lesson learned from the inflammatory bowel disease colleagues, the combination of azathioprine with allopurinol can be very effective, as allopurinol blocks the 6-MMP pathway.[38,39] For this approach the dose of azathioprine needs to be reduced to about one fourth of the previous dose and 100 mg allopurinol per day added to the therapy, in order to achieve similar 6-TGN levels.[40] Allopurinol dosing for this indication is not standardized, as it is normally only used for chemotherapy-induced hyperuricaemia in paediatrics with very high doses up to 10 mg/kg body weight.[41] The dose required for the desired drug interaction is closer to 2–3 mg/kg body weight, thus allowing a very good safety margin. In order to achieve therapeutic levels, azathioprine then needs to be carefully increased keeping the allopurinol dosage constant, and drug levels need to be monitored along the way.

In countries in which 6-TGN measurements are unavailable, some colleagues have used mean corpuscular erythrocyte volume (MCV) as a surrogate marker for azathioprine dosage, as MCV should increase during sufficiently dosed azathioprine.[42,43] However, this approach has not been validated systematically and can only be regarded as a simplistic alternative when testing is not available. Normal MCV levels in azathioprine-treated patients should, however, always raise suspicion,

and once other causes for microcytosis such as iron deficiency or thalassaemia have been excluded, should raise the suspicion of below target drug levels, be they due to non-adherence or to altered drug metabolism.

In addition to optimizing azathioprine dosing, patients with insufficient response should be re-evaluated diagnostically (Fig. 1). This entails questioning the initial diagnosis (Is this really AIH?) as well as excluding alternative and additional diagnoses. Primary sclerosing cholangitis (PSC) as well as primary biliary cholangitis (PBC) should be excluded in any patient with persisting cholestatic features. For possible PBC, tests should be done not only for antimitochondrial autoantibodies (AMA), but also for the PBC-associated antinuclear autoantibodies (ANA) anti-SP100 and anti-gp210, all of which can be tested by highly reliable immunoserological tests.[7,44] Drug-induced liver injury (DILI) is another important differential diagnosis to consider, including the possibility of azathioprine-induced liver toxicity. However, the diagnosis of azathioprine hepatotoxicity is often given to patients only based on raised liver enzymes during therapy, and may be a misinterpretation of insufficient response, leading to an unnecessary change to second-line therapy.

In view of the excellent treatment efficacy of azathioprine in most patients, drug toxicity should not be simply assumed, but needs to be proven, either by liver biopsy, or by drug withdrawal associated with improvement, and re-exposure associated with worsening of liver tests. Histological features of azathioprine drug toxicity are usually quite characteristic and, at least by an experienced liver pathologist, can be well differentiated from AIH activity.[45,46] If in doubt, liver biopsy should be sent to a reference centre for re-evaluation. Furthermore, viral infections, in particular Epstein-Barr virus

(EBV) and Cytomegalovirus (CMV) infection in previously seronegative patients, have also to be excluded as reasons for raised liver enzymes in treated AIH patients.

Non-alcoholic fatty liver disease (NAFLD) and NASH are frequent diseases in the general population, but may be even more frequent in AIH as a side-effect of initial steroid therapy. Thus, NAFLD may develop in a patient in whom initial liver biopsy did not show any suspicious features, and should be considered in insufficient responders, especially if the liver test pattern is compatible, with usually normal alkaline phosphatase (ALP), high gamma-glutamyl transferase (GGT) and alanine aminotransferase (ALT) higher than aspartate aminotransferase (AST).[29] Ultrasound screening showing increased echogenicity of the liver parenchyma, as well as high values of 'controlled attenuation parameter' (CAP) on Fibroscan can help in the diagnosis – and again liver biopsy may be required to make the final diagnosis and to try to differentiate the relative role of NAFLD/NASH and AIH activity in explaining the laboratory values.

Relapse during maintenance therapy may also be considered a version of insufficient response, but should be viewed slightly differently depending on the reasons for relapse. The most common form of relapse is due to lowering of maintenance therapy down to a level lower than required in the individual patient. Adaptation of the doses, and depending on the degree of relapse perhaps a transiently higher steroid dose, will solve the problem in the majority of patients, who can then be managed on acceptable levels of standard maintenance therapy. Relapse may also be due to non-adherence, and this problem is particularly common in adolescents and young adults, but can occur in any age group. Reconsideration of patient preferences and discussion of the risks and benefits of therapy should be undertaken in these patients, and psychological

help is sometimes necessary. Repeated relapses despite adequate maintenance therapy and adherence also represent insufficient response.

In patients with insufficient response, in whom alternative and additional liver disease has been excluded, standard drug therapy should be intensified taking into consideration the disease activity, the co-morbidities, and the drug side-effects (Fig. 1). Only in those patients who are adherent to therapy, and in whom despite intensified therapy the disease is active and presumably progressive, third-line therapy should be attempted according to one of the protocols given in Table 1. As an alternative for starting third-line therapy directly after unsuccessful intensification of first-line treatment, mycophenolate mofetil (MMF) as a second-line drug can be considered, although its effectiveness in poor responders to first-line therapy has been limited in present reports.[23] Fibroscan can be very helpful in assessing disease progression: Fibroscan measures the combination effect of inflammatory infiltration in the liver and fibrosis, and should thus improve upon remission induction.[47] Worsening of Fibroscan during follow-up especially beyond the first six months of therapy, indicates either re-activation of disease, or fibrosis progression, possibly even both.[20] The reasons for starting third-line therapy should be recorded, its pros and cons discussed with the patient, and the results of this discussion recorded. A liver biopsy before the start of third-line treatment is recommended in order to prove the necessity of third-line therapy, to exclude alternative diagnoses, and to have detailed information on disease activity (grading) and fibrosis (staging) prior to starting these experimental therapies. Patient (and in children also parents') preferences need to be taken into consideration when deciding on third-line therapy, both on initiation and on the drugs chosen. The recommendations for patients with insufficient response are summarized in Fig. 1.

Intolerance

Drug intolerance is precluding the use of azathioprine in a fair proportion of patients, estimated to be between 3 and 5%. Reliable prospective studies of the true incidence of azathioprine intolerance are missing. It is an idiosyncratic reaction usually manifesting within the first two weeks of therapy, and clinically characterized by general malaise and nausea, often associated with any of the following symptoms: fever, diarrhoea, muscle and body pain, vomiting. Symptoms may mimic acute gastroenteritis, which is a differential diagnosis. Symptoms typically subside within two to three days of stopping treatment, and resume rather more quickly upon re-exposure. Many colleagues will attempt a trial of re-exposure to be certain that it really is azathioprine intolerance, and the first recommendation is to undertake such an attempt by switching from azathioprine to 6-mercaptopurine (6-MP; Fig. 2), thus limiting possible intolerance to 6-MP and its metabolites and avoiding the pre-drug azathioprine. 6-MP is the first metabolite of azathioprine on the way to its active agent 6-TGN.[48] 6-MP is thus just as effective a drug as azathioprine, and in some countries the first drug of choice in this drug class of purine analogues. As in most countries only azathioprine, and not 6-MP, is licensed for use as immunosuppressive agent, and as in most countries there is a wider dose variation of azathioprine tablets on the market, most physicians prefer azathioprine, but from a pharmacological point of view, 6-MP is just as effective. The problem of inadequate dosing alternatives may also sometimes hamper optimal therapy in very young children, as azathioprine fluid, which allows exact dosing, is not available everywhere. As at the same time steroid maintenance therapy can be problematic in young children due to its negative growth effects, some specialists choose MMF as an alternative first-line therapy due to its wider availability in liquid form allowing individual dosing. Systematic data are lacking, and choice of preference should be individualised depending on the steroid dose required and

possible MMF side-effects. More data are here required to allow general recommendations. Furthermore, as children develop, a change to standard therapy should be then considered in these cases.

Up to 50-75% of patients intolerant to azathioprine are not intolerant to 6-MP, or their side-effects are markedly weaker.[49] It therefore appears prudent to attempt a challenge with 6-MP in a patient suspected to be intolerant to azathioprine (Fig. 2). Particularly patients with only milder, mainly gastrointestinal symptoms due to azathioprine are likely to tolerate 6-MP therapy. If symptoms of intolerance recur, this drug class should probably be avoided completely, even though there are some reports on the use of tioguanine as an alternative agent from this drug class with better tolerance.[50] If 6-MP is tolerated, it should be used, and dose adapted similarly to azathioprine based on optimal 6-TGN blood levels (starting usually at a dose of mercaptopurine of 0.5–1 mg/kg body weight).

In patients intolerant to both azathioprine and 6-MP, MMF should be used as a second line drug, starting with MMF 500 mg twice daily and dose increased to MMF 1 g twice daily, in children starting with 5 mg/kg body weight twice daily up to a maximum of 20mg/kg body weight twice daily (Fig. 2). MMF seems to be generally better tolerated than azathioprine, but does have gastrointestinal symptoms in a fair proportion of patients, as well as causing problems in wound healing, increasing also the risk of organ perforation for example in peptic ulcer disease. Furthermore, MMF is strictly contraindicated in pregnancy, and for men reliable contraception is recommended because the risk of genotoxicity cannot be completely ruled out.[51,52] Long-term family planning needs to be discussed with every AIH patients at reproductive age before switching to MMF. Thereby, re-changes of treatment can thus be avoided, in case that

family planning becomes first relevant soon after starting MMF. This is particularly relevant in adolescents and young adults, who may have been put on MMF at some stage, and in whom planned or unplanned pregnancies may lead to a complicated situation. Effectiveness for AIH appears to be good, and it has been reported that up to two thirds of patients intolerant to azathioprine achieve full biochemical remission using an MMF regimen.[53-55] This approach is summarized in Fig. 2.

Side-effects of corticosteroids may also be severe and be considered to cause intolerance in some patients. In particular, during adolescence, non-adherence to steroid-based therapy may be a major problem, both due to real as well as to perceived or feared potential steroid side-effects. As high-dose steroids are usually only required for short periods, dose adaptation should first be tried in this situation, preferably by optimizing azathioprine treatment as rapidly as possible in order to spare steroids. Budesonide can be considered as an alternative in patients with severe steroid side-effects, but is contraindicated in all patients with cirrhosis, in whom first-pass effect is impaired with increased risk of steroid-related side effects as well as a high risk of portal vein thrombosis.[56] While being an option for remission induction, budesonide is more difficult to taper due to its short half-life and limited dose availability on the market. Both prednisolone and budesonide can cause considerable long-term steroid side-effects, and in view of the better long-term effectiveness of systemic immunosuppressants, steroids should in any case not be the mainstay of therapy in AIH.[27,57,58] Nonetheless, in patients intolerant to azathioprine, and also intolerant to MMF, steroid monotherapy may be a valid treatment alternative, if bone mineral density is good and remains good, and if the prednisolone dose required can be kept at a maximum of 10 mg/day (in children probably at a maximum of 2.5-5 mg/day). This approach is summarized in Fig. 3, and should be discussed as a possible option with

the patient before entering the alternative of third-line therapy. In this discussion, it needs to be mentioned that depression is found quite frequently in patients with AIH, and this seems to be closely associated with steroid use, thus hinting towards a pathogenetic role of steroids in AIH-associated depression.[59] Therefore, existing depression and depression risk should be assessed carefully, and psychological support may be required in these patients. An algorithm summarizing all recommendations is given in Fig. 3.

Third-line therapy

A number of drugs have been reported to help in the treatment of patients with AIH not responding sufficiently to first- and second-line therapy (Supplementary Table 1). Various schedules of third line drugs have been used, often depending on local expertise as well as reimbursement rules. In order to learn the degree of effectiveness of each third-line protocol, a collaborative effort of all expert centres managing such patients is required. We suggest that patients should only receive third-line therapy on the basis of the algorithm presented in Fig. 3. Patients should be stratified to the reason they are receiving third-line therapy: Because of insufficient response to standard therapy (expected to be difficult-to-treat), or because of intolerance to standard therapy (expected to be easier-to-treat). Furthermore, third-line protocols should be as standardized as possible, and therefore we have listed the possible regimens including dosing and dosing intervals in Table 1. Results of case series of patients treated according to these protocols should be shared and the results published. Collection of data can be undertaken via the ERN RARE-LIVER.

This paper does not give firm guidance on the choice of third-line therapy, nor on the question, if third line drugs should be combined with standard drugs, or given as an

alternative, as no reliable data for such a recommendation are available. However, experience suggests that for patients being intolerant to first- and second-line treatment, a well-tolerated single third-line drug will probably be sufficient to control liver inflammation. However, for cases with insufficient response under first- and second-line therapy, double or even triple immunosuppression may frequently be needed for remission induction, at least during the initiation of the third-line drug. But the aim of starting third-line treatment is always that the third-line drug will maintain remission on its own. However, for future evaluation of the effectiveness of third- line drugs, it is important to stratify results both according to intolerance or insufficient response as reasons for giving third-line drugs, and to record, if combination therapy, and in what form, has been applied. When initiating third-line therapy, in addition to checking and proving the need according to the above recommendations, re-evaluation of the overall health status of the patient is required, not only in order to evaluate the indication for third-line therapy, but to also determine and limit the risks. First of all, in older patients and patients with comorbidities the overall life expectancy and quality of life needs to be assessed: Is AIH really the most important disease of this patient, and would progressive disease limit life-expectancy and quality of life more than the side-effects of higher dose standard therapy? Secondly, particularly in children, growth and developmental assessment needs to be performed regularly in determining the risks of disease, side-effects of previous therapy and potential risks of alternative third-line therapies. Overweight should be tested in all patient populations, and additional measures for weight control may be required, particularly in patients still requiring steroids. Furthermore, cardiovascular and renal risk factors should be checked, as many of the third-line drugs have relevant cardiovascular and renal side-effects. Chronic infections need to be excluded; in case of anti-TNF therapy in particular tuberculosis. Finally, assessment of vaccination status and performing (re-)

vaccinations as required should be undertaken, preferably prior to institution of third-line therapy.

Checking the need for third-line therapy by careful re-assessment of the disease history, patient adherence, patient preferences and co-morbidities will benefit difficult-to-treat AIH patients. Applying the above recommendations should not only lead to better care of our patients, but should also form the basis for scientific progress in evaluating the utility and risks of third-line therapies in AIH.

Special recommendations for paediatric and adolescent patients with autoimmune hepatitis

While AIH is probably in general one and the same disease across all age groups, a number of special considerations for paediatric patients need to be stressed. Children and adolescents with AIH may differ from adult patients in several ways: they often present with a cholestatic variant of AIH, certain autoantibodies are more frequently found in children, such as anti-liver-kidney microsome antibodies (anti-LKM) or anti-liver cytosol type 1 (anti-LC1)[60], and the questions of growth, development, but also psychosocial aspects and questions of adherence, may present special challenges in paediatric care of AIH.

Up to about 50% of children presenting with features of AIH may later be found to have underlying cholestatic liver disease, termed by some autoimmune sclerosing cholangitis (ASC) or AIH/PSC overlap syndrome. Therefore, Sclerosing Cholangitis should be excluded by cholangiography (MRCP), and possibly by repeating liver biopsy.[21] As typical findings of sclerosing cholangitis may be subtle and can be missed by both methods, follow-up cholangiography and sometimes also follow-up liver biopsy may be required, and is strongly recommended for all children and adolescents failing

to reach remission. These children should also be screened for underlying inflammatory bowel disease (IBD) by measuring faecal calprotectin followed by a colonoscopy if elevated.[21] Other differential diagnoses must also be considered in an age-related fashion: In toddlers, careful evaluation of metabolic disorders must be undertaken. In both children and young adults, Wilson's disease must be excluded by measuring ferroxidase and 24-hour urine copper excretion, by slit-lamp examination for Kayser-Fleischer rings, by genetic analysis and, if in doubt, by measuring liver tissue copper content quantitatively in a liver biopsy sample.

Previous studies on the treatment of paediatric AIH include only one randomized trial.[57] That is why treating this patient group is mainly based on case reports, observational and retrospective studies and adult studies, and experience. Despite these difficulties, a paediatric scoring system for autoimmune liver disease (AILD) and treatment algorithm has been suggested by The European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN), which may help in the management of paediatric AIH.[21]

Paediatric patients have a comparable remission rate as adults on first-line therapy, with about 50% of patients achieving normal ALT levels 2-3 months after starting treatmen.[61,62] If remission is not achieved, monitoring of azathioprine metabolites should rather rely on the 6-MMP/6-TG ratio being below 4 than on sole 6-TGN levels. 6-TGN levels on their own appear to only poorly correlate with biochemical remission in paediatrics.[63,64] Although studies on the combination of allopurinol and low-dose azathioprine in children with AIH have not been published, it seems reasonable to use the same approach in children as in adults, with special attention to 6-MMP/6-TGN ratio.

Relapse during maintenance therapy in childhood and adolescence requires a special diagnostic and therapeutic approach: one reason may be accidental undertreatment, because as children grow, continued re-evaluation and adaptation of dosage per bodyweight is needed. Non-adherence is a challenge in any age group, but is much more commonly seen, and at the same time more complex in adolescence. It is probably the most common cause of relapse during this phase in life. Managing adherence well has to be considered a multidisciplinary task.[65] In smaller children, non-adherence may also occur, for example if the child dislikes the medication or cannot swallow the tablet. Furthermore, psychosocial problems of parenting may also be responsible for non-adherence. In type 2 AIH, representing about 10% of the paediatric AIH patients[61,66], recurrent relapse is not uncommon, and second- or third-line treatment is frequently needed.

As a consequence of the longer expected lifespan of a child with AIH, the long-term effects of immunosuppressive treatment should be taken into consideration in a paediatric AIH patient, and therapy may need to be adapted in different life phases accordingly. Intolerance and side effects of therapy may have a more severe impact on adherence and quality of life in paediatric patients. Studies on 6-MP in paediatric AIH are lacking, however, data from acute lymphoid leukaemia and inflammatory bowel disease (IBD) in childhood support the safety of 6-MP in this age group. Therefore, 6-MP can be an option also in children, if azathioprine is not tolerated. MMF is available as a liquid formulation, its use is well known for transplantation in children and its dosing can be easily regulated in a small child. In general, if monotherapy with prednisolone on a higher dose appears to be required for reaching and maintaining remission, other alternatives should be discussed with the parents and child, in particular already a teenager. Growth and bone mineralization should be monitored

closely, and third-line treatment may be considered, if side effects on standard therapy become worrisome, even if the child is in remission. Non-adherence due to the cosmetic side effects of prednisolone can be a major issue during adolescence, and can also be considered a form of drug intolerance leading to second- and third-line therapy. Patient involvement in the decision process seems important. Furthermore, as transition into adult life occurs, return to standard therapy should probably be attempted.

The number of paediatric studies on third-line therapy is very low and the number of patients included small (supplementary table 1). This highlights the need for multicentre studies and close collaborations together with adult hepatologists. Two observational paediatric studies on calcineurin inhibitors indicate that remission can be achieved in about 75% of cases within 6 months and that well-monitored usage of tacrolimus is safe in children.[61,67] However, we lack long-term data, which due to the possible toxic effects are urgently needed. Other third-line drugs (supplementary table 1) in paediatrics can only be supported by case reports or adult data. The lack of data in this area underlines the need of prospective databases and multicentre paediatric studies.

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Author names in bold designate shared co-first authorship.

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Table 1 Third-line therapy in autoimmune hepatitis: Expert opinion and suggestions for standards

Therapy	Dose - Adults	Dose - Children	Comments
Tacrolimus	0.1 mg/kg twice daily, or prolonged-release formulation of tacrolimus in lower dose. Serum trough levels < 8ng/ml	0.05 mg/kg/day. Initial serum trough levels 6-8 ng/ml, tapering to 3-5 ng/ml after full biochemical remission has been achieved.	The best studied alternative. Variable and generally lower doses have been applied in retrospective and prospective studies (e.g. 0.5-6 mg/day or 2-3 mg twice daily). Several of these studies have reported a significant, but insufficient effect (full remission was not achieved). Hence, by expert opinion, we recommend standardisation of the dose at a higher serum trough level as given in this table and tapering the trough levels after remission has been achieved. Renal function (eGFR) should be assessed prior to and during treatment; consider dose reduction in cases of eGFR reduction >25%.
Ciclosporin	2 mg/kg twice daily; Serum trough levels < 120 ng/ml	4 mg/kg twice daily to initial trough levels at 200-250 ng/ml, tapering to trough levels < 120 ng/ml after full biochemical remission has been achieved.	Doses of 2-5 mg/kg/day were assessed in a small open-label clinical trial. A dose of 2.5 mg/kg twice daily is frequently advised for non-transplant indications (e.g. nephrotic syndrome) and tapering the trough levels after remission has been achieved. Renal function (eGFR) should be assessed prior to and during treatment; consider dose reduction in cases of eGFR reduction >25%.
Infliximab	5 mg/kg/day; at week 0, 2, 6, and every 4-8 weeks thereafter	No data	The proposed dose corresponds to standard recommendations for other indications (e.g. inflammatory bowel disease, ankylosing arthritis). Maintenance therapy in AIH appears to usually require a 4-week interval contrary to most IBD patients
Rituximab	1000 mg at week 0 and 2, to be repeated whenever transaminases rise (e. g. after 6-12 months)	375 mg/m^2	Surveillance of CD20+ B-cells is recommended. Supplement with immunoglobulins may be necessary. Paediatric data limited to single case reports.
Methotrexate	7.5-15 mg per week	10 mg/m^2 per week	Data are scarce and limited to case series. Some case reports associate development of autoimmune hepatitis with ongoing methotrexate therapy for other indications.

Cyclophosphamide	1-1.5 mg/kg/day or pulse therapy 1 g i.v. every 4 weeks	No data	Data are scarce and limited to case series.
Everolimus	0.75-1.5 mg/day Serum trough level 3-6 ng/ml	No data	Data are scarce and limited to case series.

eGFR, estimated glomerular filtration rate; IBD, inflammatory bowel disease.

Figure legends

Fig. 1. Treatment algorithm for AIH patients with insufficient response to first-line treatment

In AIH patients with insufficient response under first-line treatment with steroids and azathioprine, the active metabolite of azathioprine, 6-TGN (6-thioguanine nucleotide), should be measured. Insufficient response in AIH patients is defined by not achieving full remission. Biochemical remission is defined as normal transaminases as well as normal IgG levels and histological remission is defined as a hepatitis activity index (HAI-score) of up to 3 out of 18. Patients with 6-TGN levels below 220 pmol per $8x10^8$ red blood cells should be assessed for incompliance. After exclusion of incompliance, optimization of 6-TGN levels, either by increasing azathioprine dosage or by the combination of low-dose azathioprine and allopurinol, should be performed. In AIH patients not achieving full response and with 6-TGN levels above 220 pmol per $8x10^8$ red blood cells, alternative or concomitant diagnoses to AIH must be considered before steroid and azathioprine treatment is intensified or third-line treatment is started.

Fig. 2. Treatment algorithm for AIH patients with side-effects under first-line treatment

AIH patients who are intolerant to standard treatment with azathioprine due to side effects, should be treated with 6-mercaptopurine or mycophenolate mofetil before starting third-line treatment. Insufficient response in AIH patients is defined by not achieving full remission. Biochemical remission is defined as normal transaminases as well as normal IgG levels and histological remission is defined as a hepatitis activity index (HAI-score) of up to 3 out of 18.

Fig. 3. AIH treatment algorithm

In selected AIH patients being intolerant to second-line treatment with 6-mercaptopurine and mycophenolate mofetil, steroid mono-therapy can be an option. All AIH patients requiring third-line treatment, should first be discussed in an expert panel, such as the clinical patient management system (CPMS) for patients with rare liver diseases, hosted by the European Reference Network on Hepatological Diseases (ERN RARE-LIVER). Before starting third-line treatment, inclusion of the patient into a clinical trial should be considered. Insufficient response in AIH patients is defined by not achieving full remission. Biochemical remission is defined as normal transaminases as well as normal IgG levels and histological remission is defined as a hepatitis activity index (HAI-score) of up to 3 out of 18.

Fig. 1. Treatment algorithm for AIH patients with insufficient response to first-line treatment

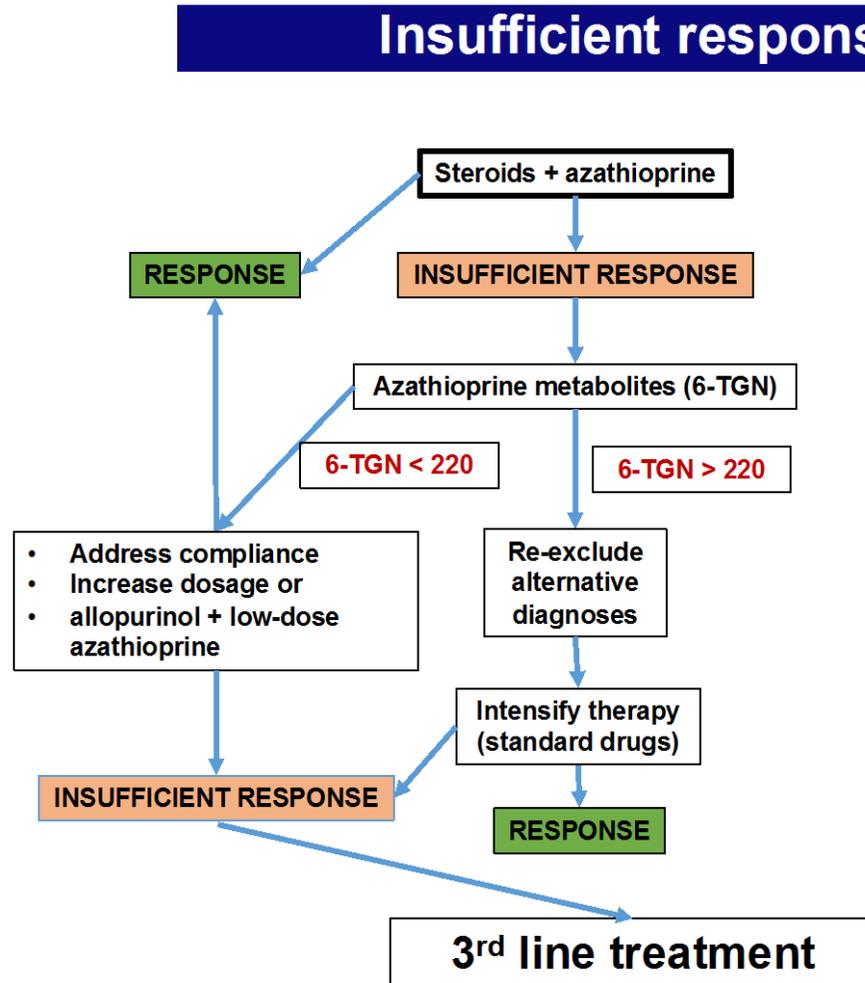**Fig. 2. Treatment algorithm for AIH patients with side-effects under first-line treatment**

Treatment intolerance

Steroids + azathioprine

RESPONSE

INSUFFICIENT RESPONSE

INTOLERANCE

6-Mercaptopurine

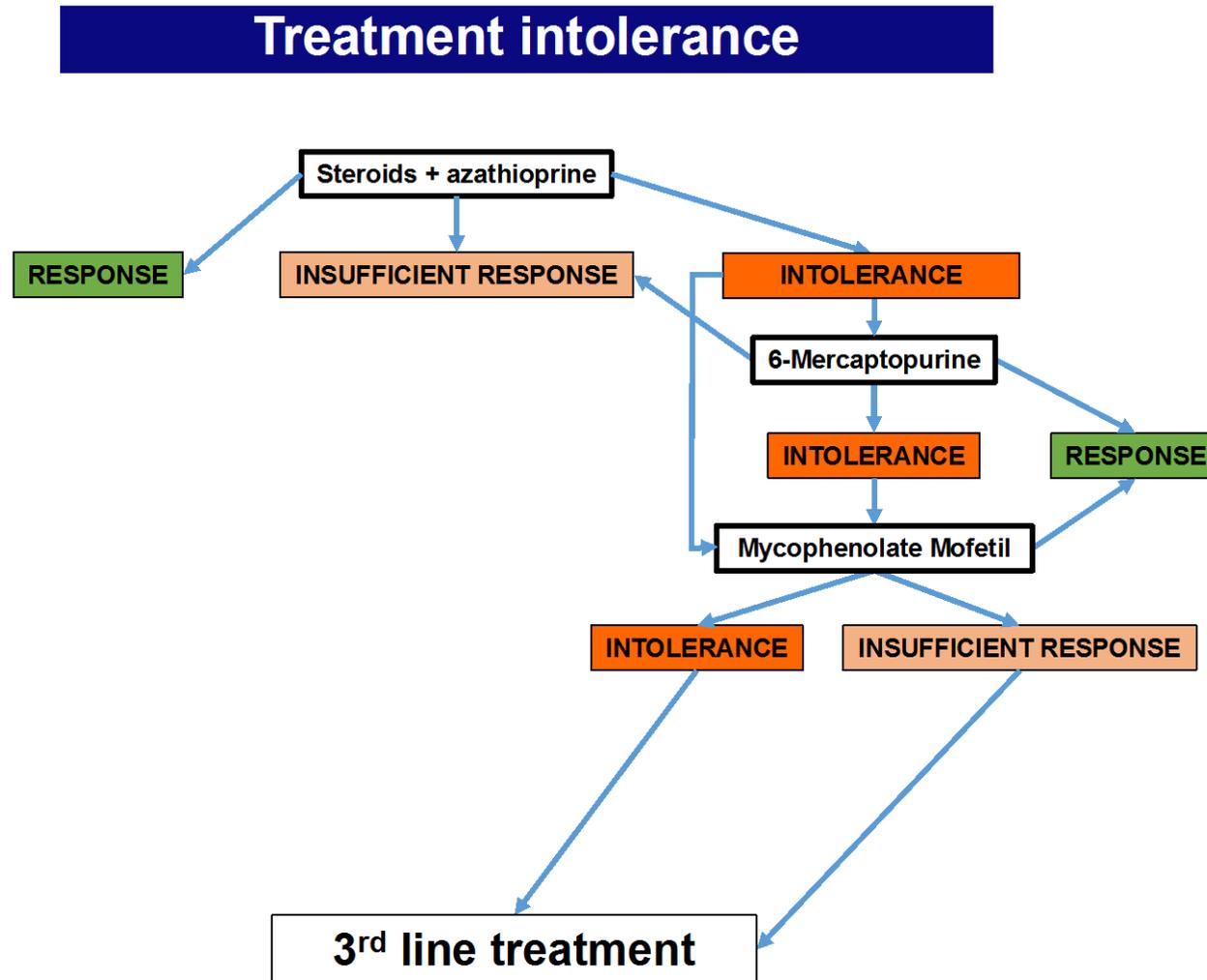INTOLERANCE

RESPONSE

Mycophenolate Mofetil

INTOLERANCE

INSUFFICIENT RESPONSE

3rd line treatment

Fig. 3. AIH treatment algorithm

AIH treatment algorithm

Steroids + azathioprine

RESPONSE | INSUFFICIENT RESPONSE | INTOLERANCE

6-Mercaptopurine

Azathioprine metabolites (6-TGN)

INTOLERANCE | RESPONSE

6-TGN < 220 | 6-TGN > 220

- Address compliance
- Increase dosage or
- allopurinol + low-dose azathioprine

Re-exclude alternative diagnoses

Mycophenolate Mofetil

INTOLERANCE | INSUFFICIENT RESPONSE

Intensify therapy (standard drugs)

INSUFFICIENT RESPONSE

RESPONSE

Steroid monotherapy

- Include in trial, if possible
- Discuss with experts, e.g. in CPMS of ERN RARE-LIVER

3rd line treatment

ERN on hepatological diseases
(ERN RARE–LIVER)