Personalizing liver targeted treatments and transplantation for patients with alpha-1 antitrypsin deficiency

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ABSTRACT

Introduction: There is currently no specific treatment for liver disease due to alpha-1 antitrypsin deficiency (AATD) other than standard care and in severe cases liver transplantation. This review describes the personalised approaches to liver disease in AATD, and the current stage of development of new therapeutic agents.

Areas covered: We review the pathology, presentation and progression of AATD liver disease and the approaches being taken to understand the natural history of the disease to aide future therapeutic advances. Both peri- and post-transplant care is described and highlights the reasons that alternative approaches to avoid the need for liver transplantation are being explored. The role of patient selection for new therapies is addressed as this is likely to be a paramount importance to achieve a positive outcome.

Expert opinion: Treatments directed at both liver alone, and lung and liver combined, are now being trialed in patients with AATD. The next 5-10 years may determine a more reliable non-invasive measurement of liver fibrosis, together with predictors of who is most likely to progress and develop cirrhosis. Personalised approaches could optimize when and how to effectively manage an individual with a cost-effective treatment, thus avoiding the need for liver transplantation.

ARTICLE HIGHLIGHTS

- Liver disease in AATD can affect people of all ages and they are often asymptomatic until in the later stages and possibly requiring transplantation
- Importance of greater understanding of the natural history of AATD-related liver disease is recognized and there are several cohort studies ongoing in an attempt to address this
- Non-invasive monitoring methods are being established to avoid the need for high risk biopsy procedure and facilitate earlier detection and successful treatment
- New therapeutic agents are being developed to prevent AAT polymerization within hepatocytes and a personalized approach may hold the key to a cost-effective treatment and avoidance of liver transplantation

1. INTRODUCTION

Alpha-1 antitrypsin deficiency (AATD) is characterised by reduced levels, or reduced functionality, of the protein alpha-1 antitrypsin (AAT), a protease inhibitor encoded by the SERPINA1 gene.

AATD occurs due to genetically determined abnormalities in the structure of AAT protein, with over 100 different allelic variants of the gene being described. The Z and S alleles are the most commonly found in patients with AATD [1].

AATD is associated with early onset emphysema, chronic obstructive pulmonary disease (COPD), bronchiectasis, liver fibrosis and cirrhosis, vasculitis and panniculitis [2-6]. However, not all subjects develop liver disease and there is variation in clinical presentation which may be due to environmental factors, such as alcohol use, obesity and genetic modifiers [7].

Diagnosis of AATD is usually made following investigation of pulmonary or liver disease, but is influenced by local and national practice rather than when symptoms of disease develop. Family screening may also identify asymptomatic patients following the identification of a lung or liver index case.

AAT is synthesized in the liver and secreted into the circulation to protect lung tissue against the damaging effects of neutrophil elastase. Other targets of AAT include plasmin, thrombin, trypsin, chymotrypsin, and plasminogen activator.

Point mutations can lead to retention of abnormal protein in the liver which causes disease by toxic 'gain of function' and the resulting lack of circulating AAT predisposes to early-onset emphysema. The liver produces around 34mg of AAT protein per kilogram of body weight per day, with resultant plasma levels of 0.9 to 1.75mg per milliliter with a half-live of 3-5 days [8]. AAT is made within the endoplasmic reticulum (ER) and secreted by the Golgi apparatus. Severe deficiency caused by the Z allele does not affect the synthesis in the liver

but can cause approximately 70% of the mutant AAT to be degraded within hepatocytes, 15% to be secreted and 15% to form polymers [9].

AAT is normally secreted in a monomeric form but the Z-AAT protein has a point mutation (Glu342Lys) in the hinge region of the molecule that renders it prone to intermolecular linkage and loop-sheet polymerization [10]. These polymers aggregate in the ER of hepatocytes leading to liver damage, cirrhosis and hepatocellular carcinoma [11-15].

In some individuals, the intrinsic cellular mechanisms of the hepatocyte are sufficient to clear adequate amounts of the abnormally folded protein such that liver disease does not occur. In AAT deficient individuals who develop liver disease, environmental and other genetic factors stress the hepatocyte, and the normal cellular mechanisms that maintain homeostasis are disrupted, leading to liver disease.

AATD-related liver disease can affect people of all ages, although it is generally thought to have a bimodal distribution first in childhood and then in late adult life [16]. In adulthood AATD chronic liver involvement often remains undetected until a very late stage as patients may have no symptoms at all or they may be non-specific. Involvement of the liver is the second most common cause of decreased quality of life and life expectancy in patients with AATD [17], however, unlike the extensive efforts to avoid development and progression of lung disease there is currently no preventative care plan that is widely used other than lifestyle advice. While augmentation therapy can address the loss of AATD in the lung [18] there is no specific treatment for AATD-related liver disease.

Many potentially treatable conditions, such as viral hepatitis [19,20], excessive alcohol consumption [19,21] and diabetes, can cause liver damage, and these act as co-factors to

worsen prognosis in AATD liver disease, and have been the focus of preventive lifestyle advice to date in AATD. However with greater understanding of the pathogenesis of liver disease specific to AATD targeted drug treatment is becoming a possibility. In this article we review the pathology and progression of AATD liver disease, in order to demonstrate where personalized options are likely to be most appropriate both now and in the future. We go on to review the products in development for liver disease, and their place in potentially reducing progression to liver transplant.

2. PATHOLOGY OF AATD LIVER DISEASE

The ongoing processes in AATD liver disease are similar to those for non-AATD and include inflammation and fibrosis (Figure 1). Liver disease in AATD presents as a result of ongoing damage due to accumulation of abnormal AAT protein and ER stress. This may also be compounded by other drivers such as metabolic syndrome and genetic factors which are seen in non-AATD liver disease.

2.1. Fibrosis

Scar tissue forms in the liver as it tries to repair damage caused by any form of injury, including the ongoing inflammation and ER stress typically seen in AATD. Nodules form as dying hepatocytes are replaced by regenerating calls, causing the liver to become hard/stiff due to continuous damage and the accumulation of tough fibrous scar tissue. There are several semi-quantitative scoring systems available for the diagnosis of liver fibrosis [22]. The Ishak Score system has become widely used in clinical trials and uses 6 stages of fibrosis, with each one reflecting more scarring than the preceding stage. Clinicians and investigators assume that progression from one stage to the next represents progressive advancement of liver disease. The METAVIR scoring system has also been used in AATD-related liver

disease to assess histopathological inflammation and fibrosis with a 4 point activity grade and 5 point fibrosis stage from no fibrosis F0 to cirrhosis F4.

2.2. Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is a condition characterised by an accumulation of fat in the liver in people who drink little or no alcohol [23]. Its cause is not clearly understood but can be associated with obesity, insulin resistance, hyperglycaemia and high levels of fat in the blood. It does not usually cause symptoms and there is no specific treatment other than addressing the underlying conditions such as obesity. Theoretically AATD would not cause NAFLD, but fatty liver has certainly been observed in AATD cohorts [24], and whether this is related to co-factors or is a feature of AATD liver pathology is not certain.

2.3. Non-alcoholic steatohepatitis

A more serious form of NAFLD is non-alcoholic steatohepatitis (NASH) whereby the liver becomes persistently inflamed, with fibrosis around the liver and nearby blood vessels. NASH is thought to affect up to 5% of the UK population [25]. Risk factors for advanced fibrosis include being male, having metabolic syndrome and obesity. The precise effect of alcohol consumption remains to be determined but it is thought that it promotes disease progression. The prevalence of hepatic steatosis in an AATD cohort recruited from a number of patient registries in USA was found to be >40%, [26]. This was higher than expected for the general US population but the authors suggested this could be due to the study population having steatosis secondary to NAFLD. An alternative explanation is that AATD *per se* is a secondary cause of hepatic steatosis. Steatosis is commonly seen in liver biopsies taken from patients with AATD, further supporting the link between them [27].

2.4. Cirrhosis

Ongoing injury changes the ability of the liver to function effectively and can lead to cirrhosis which is the life-threatening consequence of many liver disorders and carries a poor prognosis [28]. Liver cirrhosis itself leads to many, often life-threatening, secondary disorders such as variceal bleeding or hepatocellular cancer. It is therefore crucial that liver disorders are diagnosed at an early stage, so as to prevent complications and to treat concomitant risk factors. In the case of a late diagnosis, the diverse complications of liver disease cannot be effectively prevented and transplantation is often the only option available.

3.0. PRESENTATION AND PROGRESSION OF AATD LIVER DISEASE

3.1. AATD in children

Children with AATD may have problems with their liver in early childhood, although this is usually temporary and most have normal liver function by the time they are in their late teens. In new-born babies, AATD can cause jaundice but this is generally managed safely and causes no lasting problems. However, AATD is one of the most common genetic causes of liver disease in children and is the second cause of neonatal cholestasis after biliary atresia [16]. A multicenter study has shown that during follow-up of 8 patients with a PiZZ genotype attending a paediatric hepatology unit one died, one developed cirrhosis, five developed chronic hepatitis and one was asymptomatic [29]. Although the prevalence is rare the study concluded that patients with liver disorders should have their AAT levels checked and receive long-term follow up.

Four genetic cholestatic liver diseases, including AATD, are currently being investigated in an ongoing 20-year observational prospective study (LOGIC) to provide a better understanding of their causes and effects (NCT00571272). The study enrolls participants from

infancy through to adults of up to 25 years of age who have, or are suspected of having, genetic cholestatic liver disease. Preliminary results from 269 subjects enrolled between 2008 and 2012 have shown that many patients with AATD presenting with elevated liver tests and jaundice improve spontaneously [30] and therefore may not need any therapeutic intervention. Four rare genetic liver disorders, Alagille Syndrome (ALGS), progressive familial intrahepatic cholestasis (PFIC), bile acid synthesis and metabolism defects and AATD account for approximately 20% to 30% of all infant cases of cholestasis [31]. This rare condition that involves a reduction or obstruction of bile flow from the liver to the small intestine can cause significant growth problems during childhood. If left untreated cholestasis can cause serious liver problems, the need for liver transplantation, and potentially death.

Approximately 11% of infants have an elevated serum bilirubin which returned to normal within 6 months and cholestatic jaundice developed in 10% of PiZZ infants. Clinical features of liver disease with the absence of jaundice occurred in 6% and symptoms usually resolved by age 2 but 15% had progression to juvenile cirrhosis. Longitudinal population-based cohort studies have also shown that in the first 12 months of life serum ALT level may be elevated but only 15% have persistently abnormal levels by age 12 [32,33]. The risk of death from liver disease in PiZZ children was 2-3% with no clinical symptoms remaining by the age of 12 in all of the survivors [34].

The Polygen DEFI-ALPHA study (NCT01862211) in a cohort of French children compared the allele frequencies of polymorphisms in the SERPINA1 gene between those AATD patients with and without hepatic symptoms (portal hypertension and its complications, severe liver failure leading to transplant or not, or an indication for liver transplantation) and results showed that AATD patients presenting with neonatal cholestasis were likely to develop severe liver disease [35]. Some patients with non-homozygous ZZ genotype (PISZ and M variants) can also develop severe liver disease when associated with predisposing factors and

further genetic studies are needed to identify other factors involved in the development of liver complications.

Studies in children with AATD suggest that about 10% will develop cirrhosis and require liver transplant [16]. This is a relatively small proportion of individuals and it is unknown why they do not develop significant liver disease despite the presence of polymerogenic AATD. It may be due to differences in the ability of hepatocytes to transport polymers out of the ER for degradation or variation in the hepatocyte autophagic degradation pathways may play a role [36].

While liver biopsy is often used in the initial diagnostic evaluation of children with liver disease, subsequent surveillance liver biopsy is rarely performed in children or adults because of its inherent invasiveness and risks. Therefore, understanding of the natural history of fibrosis progression in children is limited. The patchy nature of fibrosis in many important pediatric liver diseases, e.g. biliary atresia (BA) and cystic fibrosis liver disease (CFLD), limits the utility of sequential liver biopsy even if it were to be employed in clinical practice in pediatrics. Thus, non-invasive means of assessing liver fibrosis throughout the liver would be highly desirable and clinically useful in pediatric hepatology, and AATD is no exception.

Definitive NASH was present in only one biopsy in this cohort. As such, significant fibrosis is more likely related to AATD than to the presence of NAFLD or NASH in the study population.

Where children have progressive disease which does need liver transplantation they generally do well and experience no recurrence of liver disease and do not develop AATD-related lung disease later in life [37].

3.2. AATD in adults

It is well recognized that usual tests to examine liver function do not accurately detect liver injury and fibrosis. Measurement of liver enzymes such as ALT has a low sensitivity to identify AATD adults with liver disease [38] and ALT and GGT have been shown to be similar to patients without liver disease and lower than in patients with NAFLD [39]. One study found that 35.1% of asymptomatic PiZZ individuals with AATD-related lung disease also had significant underlying liver fibrosis not detected by routine liver function test monitoring and only detectable with additional elastography and biopsy [26]. Accumulated abnormal AAT, portal inflammation and hepatocellular degeneration were associated with clinically significant fibrosis and transient elastography was found to be an adequate method to detect METAVIR ≥ F2 fibrosis stage.

Given the severity of liver disease in AATD, with approximately 10% of affected patients developing liver cirrhosis and a subgroup of those patients in need of liver transplantation, and lack of an effective treatment that addresses the toxic hepatic "gain-of-function" mechanism [40], there is an urgent unmet medical need to develop a therapy that can help in this particular patient population. A cross-sectional biopsy study showed 35% of PiZZ adults had clinically significant liver fibrosis and large European analysis using non-invasive assessment showed liver fibrosis in 20 to 36% [24, 26]. With progressive disease little can be done except for liver transplantation and this has led to research into the mechanism of liver damage and trials of investigational products.

Liver transplants are generally very successful and most recipients live for more than 10 years post-transplant, with some even surviving up to 20 years or more so the prognosis is good. Systematic review suggests that outcomes in AATD are similar to other causes of transplant post procedure [4]. AATD patients having a liver transplant have an 86% chance of one-year

survival with ten-year survival rate of 72% for PiZZ and 91% and 79% respectively for PiSZ [37]. Liver transplantation cures cirrhosis caused by AATD and restores normal AAT production. However, there are limited reports on post-transplantation pulmonary outcomes in AATD. Data collected from patients treated at 3 large transplant centres in UK between 1987 to 2012 found that despite survival being excellent the FEV_1 continued to decline unexpectedly post-transplant in some PiZZ and PiSZ patients [41]. Interestingly, additional underlying causes of liver disease beyond the pathology of AATD itself was present in only 8% of PiZZ patients compared to 40% of PiSZ and 90% in PiMZ.

A promising alternative to liver transplantation for metabolic diseases and acute liver failure is hepatocyte transplantation and a review of the current status and latest developments has been produced [41]. The clinical application of this technique is currently restricted by shortage of quality liver tissue, early cell loss post infusion and reduced cell engraftment. However, published data is encouraging and ex-vivo genetic correction could enable the use of autologous cells in future personalised medical approaches.

4.0. HOW CAN WE PERSONALIZE TREATMENT NOW?

4.1. General preventative measures

The Model for End-stage Liver Disease score (MELD), a number ranging from 6 to 40 based on creatinine, bilirubin, INR and serum sodium levels, can be used as a guide to severity of liver disease and need for liver transplant [42]. This can be used to help decide when to start managing the various aspects of liver cirrhosis in individual patients in a more proactive way.

4.2. Lifestyle approaches

Liver disease is generally caused by obesity, hepatitis infection or alcohol misuse and patients with AATD-related liver disease may also have an element of disease caused by these other

factors. This adds to the complications of effective treatment but education on how to reduce risk by lifestyle changes to maintain a healthy weight and avoid excess alcohol consumption can help. Vaccination against hepatitis A and B should also be considered.

4.3. Peri-transplant care in AATD

Liver transplant involves initial assessment (usually takes around 5 days and may require hospital stay), being placed on the transplant waiting list (average waiting time in UK is 135 days for adults) and then surgery if a suitable donor organ has been found. The transplant process can be long and difficult both physically and emotionally for the recipient and also presents a huge burden on the health system so being able to prevent progression to this stage has multiple benefits. A personalised approach to treatment would be beneficial as individuals with AATD-related liver disease may also have lung disease (treatments for liver damage may have negative effects on lung disease) and/or liver damage due to non-AATD mechanisms. Those patients also having lung disease will have increased risk associated with surgery and long-term immunosuppressant medication may increase the risk of infection. Thus practitioners may need to modify anaesthetic and ITU care in AATD patients to account for these factors.

Outpatient liver transplant assessments are usually carried out in 2 phases (Figure 2). Phase 1 involves comprehensive testing including risk assessment scoring using model for end stage Liver disease (MELD) or United Kingdom model of end-stage liver disease (UKELD). Additional tests are carried out for special considerations such as bubble echo to rule out cardiac shunting, lying standing blood gas tests for Hepatopulmonary Syndrome (HPS) evaluation. Successful completion of phase I leads to phase II of assessments. This involves assessments by the multidisciplinary team (MDT) members, including detailed review of pulmonary status. Pulmonary complications associated with liver cirrhosis due to any etiology

include Portopulmonary hypertension (POPH), HPS and Hepatic hydrothorax. These require in depth anaesthetic assessment and medical management prior to listing patients for liver transplant [43]. Patients with AATD lung disease also require joint care with the respiratory team to optimise the medical therapy for AATD during phase I of transplant assessments.

The successful candidates are then discussed at a transplant listing meeting and they are then listed on the active transplant list.

The perioperative transplant management of patients with AATD is no different from other indications for liver transplantation. The selection for transplantation means that baseline lung function for liver transplantation alone is usually good. An uncomplicated transplant is generally brought back to ICU with the endotracheal tube in place. The patient is warmed if necessary and the cardiovascular system managed with judicious fluid and vasopressors. Lung protective ventilation is a general standard in ICU so that tidal volumes of 6-8 ml/kg are used and a mild hypercarbia is a result. Adequate analgesia is instigated and sedatives are withdrawn once any residual acidosis and lactataemia remaining after reperfusion has settled and any bleeding has been assessed. Patients with significant bronchiectasis may have an extended course of pre-emptive antibiotics. It is usual in our unit to manage liver transplant patients in ICU with pre-emptive anti-fungal therapy with an azole.

There are currently no treatments that modify the inflammatory response and although the patient will have received methylprednisolone and an initial dose of adjunctive immunosuppression (usually tacrolimus), some patients do become hyperinflamed. This is usually attributed to a reperfusion response; whilst theoretically AATD patients may be more at risk given the anti-inflammatory properties of AAT this does not seem to be a significant issue in our practice. The addition risk of nosocomial infection means that patients may be managed with hydrocortisone alone and low dose tacrolimus whilst in ICU.

Immunosuppression does not prevent peri-operative inflammation, and adjustments to immunosuppression specific to AATD are not generally considered.

The evidence on the utility and long-term outcomes of combined liver and lung transplant (CLLT) is sparse. There are a few reports on single centre experiences of CLLT [44, 45] with variable survival rates and post-operative complications. CCLT is complex and an infrequently performed surgery and hence, requires a large database analysis to improve the understanding of longterm outcomes. Scarcity of donor availability and an increasing demand for solid organ transplants also impacts the organ allocation when considering multi organ transplant for a single recipient.

4.4 Post-transplant care

There are currently no disease specific indications for consideration of liver transplantation in patients with cirrhosis secondary to AATD. Hence, the indication for referral and post-transplant management is carried out in line with the recommendations and protocols for chronic liver disease due to any etiology. Standard immunosuppression for most cases includes triple therapy including maintenance Tacrolimus, Prednisolone and azathioprine. Basiliximab is an alternative to tacrolimus in the immediate post op period for patients with significant renal impairment.

There are no reports of long-term effects of immunosuppression specific to AATD. General approach in post-transplant follow up includes MDT approach to minimize modifiable risk factors that could result in graft loss and poor outcomes for the patient.

5.0. PERSONALISING TREATMENT IN THE FUTURE

Approaches to treat liver disease associated with the accumulation of mutant AAT have been reviewed previously [46] and there are several potential mechanisms currently being explored. The main approaches include modifying proteostasis networks activated by Z AAT polymers, stimulating autophagy, blocking polymerisation using small interfering RNA and small molecules and the ultimate aim of correcting the underlying genetic defect using stem cell technology. Reduction of polymer burden in the liver, either through reduced protein formation or improved polymer clearance, is a potential therapeutic route which has also been highlighted in a recent review of experimental and investigational drugs to treat AATD [47].

There are currently a number of clinical trials in AATD-related liver disease listed on ClinicalTrials.gov as summarised in Table 1. In addition to trials of new therapeutic agents there are several observational studies in both children and adults. At present there is no reliable way of determining progression of AATD-related liver disease and therefore research is needed on genetic modifiers and biomarkers and/or prognostic scoring.

Autophagy contributes to the degradation of mutant protein and therefore may help to reduce the impact of liver disease in AATD. Carbamazepine (a known autophagy enhancer) has shown the strategy to be effective in reducing liver damage by stimulating proteasomal and autophagy pathways to eliminate intracellular AAT polymers [48]. A current Phase 2 study of carbamazepine versus placebo in 30 participants with AATD-related severe liver disease is using a starting dose of 400mg/day with weekly increases until a stable therapeutic concentration is achieved (NCT01379469). The study aims to determine whether therapy leads to a significant reduction in the hepatic accumulation of Z-AAT. The study is due to be completed in in January 2021 and in addition to safety of carbamazepine treatment it will also

Table 1

ClinicalTrials.gov Identifier	Title	Summary	Status
CLINICAL TRIALS			
NCT04174118	A study of DCR-A1AT in healthy adult volunteers and patients with A1ATD-associated liver disease	60 participants Ascending dose study of subcutaneous DCR-A1AT	Phase 1 / 2 interventional study Active - estimated completion June 2022
NCT03767829	A study of ALN-AAT02 in healthy participants and participants with PiZZ type alpha-1 antitrypsin deficiency liver disease	96 participants recruited Double-blind, placebo-controlled study of subcutaneous injection of ALN-AAT02 in single-ascending and multiple doses	Phase 1 / 2 interventional study Recruitment ended
NCT03946449	Assessment of changes in a novel histological activity scale in response to ARO-AAT	12 participants Pilot open label, multi-dose study	Phase 2 interventional study Active - estimated completion November 2020
NCT04167345	Evaluation of the efficacy and safety of VX-814 in subjects with the PiZZ genotype	50 participants Double-blind, placebo-controlled study of 3 dose levels of VX-814	Phase 2 interventional study Completion expected November 2020
NCT01379469	Carbamazepine in severe liver disease due to alpha-1 antitrypsin deficiency	30 participants Carbamazepine versus placebo	Phase 2 interventional study Active - estimated completion January 2021
NCT03945292	Safety, tolerability and effect on liver histologic parameters of ARO-AAT (SEQUOIA)	120 participants Placebo-controlled multi-dose study of subcutaneous injection of ARO-AAT	Phase 2 / 3 interventional study Active - estimated completion May 2023
NCT02503683	Study of investigational ALN-AAT in healthy adults and PiZZ type alpha-1 antitrypsin deficiency liver disease	26 participants Single-blind, placebo-controlled single ascending and multiple dose of subcutaneous injection of ALN-AAT	Phase 1 interventional study Terminated in January 2018

ClinicalTrials.gov Identifier	Title	Summary	Status
NCT02900183	Safety, tolerability and effect of ARC-AAT injection on circulating and intrahepatic alpha-1 antitrypsin levels	0 participants recruited	Phase 2 interventional study Withdrawn November 2016
NCT00067756	4-PBA: Will it increase the level of alpha 1-antitrypsin (AAT) in persons with AAT deficiency?	12 participants Open label study	Phase 2 interventional study Completed October 2003
COHORT STUDIES			
NCT02929940	Liver disease in patients with alpha1-antitrypsin deficiency	500 participants recruited from multiple centres across Europe	5 year prospective cohort study Active - estimated completion December 2020
NCT02014415	Alpha-1 antitrypsin deficiency adult liver study	At least 120 PiZZ adults enrolled to 3 study arms - no previous history of liver disease - known moderate-severe liver disease - post liver transplant	5 year prospective cohort study Active - estimated completion date of December 2021
NCT02922751	FibroScan in pediatric cholestatic liver disease (FORCE)	458 participants Case-only, cross-sectional study	Observational study Completion expected Dec 2022
NCT00571272	Longitudinal study of genetic causes of intrahepatic cholestasis (LOGIC)	1675 participants Involves 20 year follow-up	Observational prospective cohort Estimated completion May 2024
NCT01455298	Liver disease in adults with alpha-1 AT deficiency (LIDIA-A1AT)	33 participants Study of transient elastography and fibrotest	Interventional study Completed March 2012
NCT01862211	Polygen Defi-Alpha: genetic polymorphisms study in children with alpha-1 antitrypsin deficiency, included in the DEFI-ALPHA cohort (Polygen)	296 participants recruited	Interventional study Completed November 2017
NCT01810458	Liver fibrosis in alpha-1 antitrypsin deficiency (Liver AATD)	109 recruited Investigate clinical predictors and epigenetic markers for liver fibrosis	Observational prospective cohort Completed in September 2019

assess whether it reduces hepatic fibrosis in AATD patients with severe liver disease, reduces portal pressure and leads to stabilization in disease severity as measured by the MELD scores.

Rapamycin (sirolimus) has also been shown to upregulate autophagy and reduce polymer accumulation and hepatocellular injury in a PiZ mouse model [49]. Many licensed drugs are known to influence autophagy, hence it is an attractive proposition for treatment as we could move rapidly to trials of other drugs known to be safe, such as lithium and valproate. However there are no current interventional studies other than of carbamazepine.

The use of chemical chaperones has been suggested as a way to increase secretion of AAT and a previous study looked at 4-phenyl butyrate (4-PBA) in experimental cell culture models of AATD (NCT00067756). The aim was to determine whether 4-PBA would significantly increase serum Z-AAT levels in individuals with and without evidence of hepatocellular injury and to assess its effects on liver injury. Results presented by Gonzalez-Peralta *et al* at American Association for the Study of Liver Diseases conference in 2006 reported that the short-term use of up to 40 g/d 4-PBA did not meaningfully increase AAT levels and was associated with significant adverse events including headache, nausea, vomiting, drowsiness and altered vision and smell [50]. It also led to premature discontinuation from the study in 2 of 11 subjects.

The burden of AAT polymers within the liver can be reduced as a result of limiting production of AAT by interfering with its mRNA using antisense or RNA interference. ARC-AAT, a short interfering RNA (siRNA) therapeutic developed by Arrowhead Pharmaceuticals Inc. targeting liver production of AAT produces sustained knockdown of AAT with no safety issues observed in clinical trials but use of the IV preparation was discontinued due to the excipient molecule being thought to be responsible for off target effects [51]. A newer subcutaneous product (ARO-AAT) which is more convenient for the repeated dosing needed in chronic liver disease, has been approved to progress to a phase II/III study with the primary

outcome based on liver fibrosis appearance within biopsy samples (NCT03945292). The product has been tested in 45 healthy volunteers resulting in 90% knockdown of AAT and no safety concerns.

Studies in PiZ mice have demonstrated that RNAi targeting AAT mRNA prevented the accumulation of nascent polymer and also reduced pre-existing polymer [52]. Sustained treatment reduced hepatic Z-AAT polymer, restored ER and mitochondrial health, normalized expression of disease-associated genes, reduced inflammation and prevented tumor formation, thus holding promise for the effective treatment of patients with AATD-related liver disease.

ARO-AAT has recently been given in single and multiple doses to a small number of normal healthy volunteers and results showed a dose response in the reduction of serum AAT and duration. The lowest single dose of 35mg reduced serum AAT by 79%, the 100mg dose by 87% and 200mg and 300mg doses reduced the level to below the limit of quantitation [52]. AAT serum levels remained reduced but gradually began to increase 12 weeks after dosing but in the 200mg and 300mg groups they had only increased slightly by 16 weeks. ARO-AAT was well tolerated at doses up to 300mg given up to 3 times and no severe adverse events (AE) were reported. The most common AEs were upper respiratory tract infection (39%) and headache (32%) and no statistically significant adverse difference in FEV_1 from baseline between those receiving placebo and active drug.

In the ongoing Phase II/III study patients with AATD will also receive single or multiple subcutaneous doses of ARO-AAT at varying dose levels. The primary outcome for patients receiving a single dose is the number achieving a 2-point improvement in a histologic grading scale of AATD associated liver disease and no worsening of liver fibrosis based on Ishak Score system. The primary outcome measures in patients receiving multiple doses is the percentage change from baseline in soluble and insoluble liver Z-AAT, percentage change from baseline in serum AAT levels and number of participants with AEs possibly or probably

related to treatment. The study has an estimated completion date of May 2023 and it is hoped that the results could indicate an exciting advancement in the treatment of AATD-related liver disease.

A further study to evaluate the effect of ARO-AAT injection on a histological liver disease activity scale in AAT-associated liver disease over time is also currently being run in a small number of participants with AATD (NCT03946449). Participants will receive multiple subcutaneous doses of ARO-AAT after undergoing a pre-dose biopsy, with a repeat at the end of the dosing period to determine change from baseline over time in a histological liver disease activity scale. The change in Ishak Fibrosis Score, percentage change in soluble and insoluble liver Z-AAT levels, percentage change in serum AAT levels, change over time in hepatic SERPINA1 mRNA expression, changes over time in liver fibrosis gene expression and number of participants with adverse events possibly or probably related to treatment.

A Phase I study of a different siRNA (ALN-AAT) was terminated due to transient liver enzyme rises in some patients (NCT02503683). However, a newer version of this product (ALN-AAT02) is being tested in a phase I/II study (NCT03767829) and results are expected once the study ends in 2021. The aim of the study is to evaluate the safety and tolerability of single or multiple doses of ALN-AAT02 in healthy participants, and then multiple-ascending doses in PiZZ participants with biopsy-proven AATD-associated liver disease by comparing percentage of treatment emergent AEs.

RNAi therapy is a promising treatment area for AATD-related liver disease and Dicerna Pharmaceuticals Inc. have also submitted an application to conduct a study to evaluate the safety and tolerability of their new orphan drug, DCR-A1AT (NCT04174118). Prior to initiation of the study in late 2019 this experimental drug had not been previously tested in humans so will include people who do not have AATD to determine the dose that has an acceptable safety profile for testing and then what dose is safest and most effective in AATD.

Like the Arrowhead Pharmaceuticals ARO-AAT molecule DCR-A1AT is a subcutaneously administered RNAi molecule designed specifically to treat liver manifestations by silencing the genes that cause AATD-related disease and Dicerna expects to dose the first patient with AATD in early 2020.

Molecular approaches to block polymerization in the liver have potential to reduce the chance of liver disease in AATD, whilst also allowing AAT to enter the circulation and reduce the impact of deficiency in the lung. Precise inhibition of polymerization of Z-AAT can be achieved by annealing peptides to the reactive loop of β sheet. Smaller peptides are both specific and effective at blocking polymerization, however dissociation from AAT is required in the circulation, otherwise the bound molecule prevents anti-NE activity and would be helpful to the liver but not add any protection to the lung. Development of small peptides targeting a lateral hydrophobic cavity in AAT has been carried out, but the strategies have been limited due to lack of maintained secretion of AAT, of failure to adequately block/reverse polymerization. Targeting a different area of the AAT protein with peptides in hepatocyte models resulted in a reduction in intracellular aggregation of Z-AAT, alongside secretion of active AAT.

An oral small molecule corrector is currently being developed by the biopharmaceutical company Vertex Pharmaceuticals. This family of protein correctors aims to address the underlying cause of AATD by facilitating proper folding of the Z-allele protein (Z-AAT) and thus preventing intracellular protein polymerization and increasing secretion of functionally active protein [10]. The corrector therapy is designed to restore the body's ability to produce its own AAT, increasing production as needed and has the potential to treat both lung and liver manifestations of AATD. There is currently limited data in the public domain around this molecule but Vertex report that Phase 1 evaluation of single and multiple ascending doses of VX-814 has been completed and has now advanced into the next stage of development.

The Phase 2 study of VX-814 is designed to assess the change in plasma levels of functional AAT and its safety and tolerability in AATD (NCT04167345). A total of 50 PiZZ subjects will be randomized to active drug or placebo and clinical data is anticipated in late 2020. Vertex has also advanced a second molecule, VX-864, into Phase 1 development and whether it will be possible to fully correct AAT levels remains unclear.

The role of gene therapy in AATD has been reviewed comprehensively with respect to treating all aspects of disease [53]. Induced pluripotent stem cells (IPSCs) have potential to generate unlimited cells for autologous cell-based treatment to correct the PiZZ mutation in AATD patients by differentiation into functional hepatocyte like cells. IPSCs have been transplanted successfully into a murine model of liver injury but this has a risk of other mutations arising during prolonged IPSC culture, so careful screening is essential for safe use in clinical practice

5.1. Patient selection for targeted therapies

Whether only those with a high risk of progressive liver disease should be selected for treatment or if everyone should receive prophylactic therapy is an important consideration. Not everyone with AATD has liver disease and research to understand the natural history and progression is vital. A systematic review of AATD and associated liver disease highlighted that the clinical course remains poorly understood [4] and research is clearly needed to identify those at risk.

An ongoing multi-center study is focused on identifying biomarkers for the progression of liver disease (NCT02014415) to help understand what causes liver disease in some patients, but not others, and also how liver disease progresses in this population. This 5-year

prospective cohort study is looking at over 120 PiZZ adults with either no previous history of liver disease, known moderate-severe liver disease or post liver transplant. Blood samples collected for genetic testing, including Induced Pluripotent Stem Cells (iPS cells), microRNA and DNA will be analysed, and a sub-group of patients will also undergo a liver biopsy and FibroScan at enrollment and Year 5.

It is essential to be able to determine who would be most likely to benefit from personalized treatment and therefore establishing reliable detection methods are critical. A recent study has shown that FibroScan elastography is an easy and repeatable method to screen for the presence of significant liver fibrosis in PiZZ AATD (NCT01455298) [54]. Fibrosis was considered significant in case of liver stiffness > 7.2 kPa. Cirrhosis was defined by liver stiffness > 14 kPa and/or ultrasound signs of cirrhosis (hepatic dysmorphy with splenomegaly) [55].

A comprehensive longitudinal assessment of the utility of FibroScan-specific elastography, liver stiffness measurement (LSM), as a measure of hepatic fibrosis in children with serious chronic cholestatic liver disease (NCT02922751) is ongoing and due for completion at the end of 2022. This observational study aims to address an unmet and critical need for noninvasive monitoring of liver fibrosis in the clinical management of children as monitoring by use of liver biopsy is avoided due to its inherent invasiveness and risks.

An observational study currently being conducted across Europe aims to clarify how liver function is modified in patients with AATD (NCT02929940). The study uses modern ultrasound-based scanners for non-invasive measurement of the degree of liver scarring along with measurement of specialized liver parameters in blood samples to gather information on any existing liver disorders. Preliminary data from PiZZ adults without pre-existing liver

disease have been reported and showed significantly higher serum liver enzymes compared to controls [24]. Non-invasive tests revealed likelihood of significant fibrosis to be 9- to 20-fold more common in PiZZ individuals than in controls (adults without the PiZ mutation) and severe steatosis was detected in 39% compared to 31% of controls. The final results will hopefully help with therapeutic advances and early detection mechanisms which are crucial to prevent the complications of advanced liver disease and treat concomitant risk factors.

6.0. CONCLUSION

Movement toward products capable of targeting the liver with the potential to act as preventative treatments against cirrhosis if used early enough is exciting.

Targeted therapy using RNA interference (RNAi) mechanisms and small molecules aimed to prevent Z-AAT polymerization within the liver are probably those with the greatest potential. siRNA technology and agents that are able to enhance pathways involved in clearance of polymers or block their formation will be important in preventing AATD-related liver disease

It is clear that cost-effectiveness and patient selection needs to be carefully considered. Education at a very early stage of AATD diagnosis can play a part in terms of avoiding excessive alcohol consumption and excessive weight gain which predisposes to fatty liver. Do we need to distinguish liver disease caused by AATD *per se* from that caused by other factors such as alcohol and weight as will it change management or treatment? It may be equally important to give guidance regarding lifestyle, ie. abstain from consuming alcohol and maintain a healthy weight, as already given to patients being assessed for liver transplant before being considered for new treatments.

As discussed early detection to prevent progression and target specific treatment at an early stage in the disease process is vital. It would be useful to have an understanding of who will get AATD-related liver disease and why not everyone does and a reliable way to diagnose

liver disease is key to this. Some AATD-related liver disease may not progress so whether or not treatment is required also needs to be considered. Treatment may be complicated by the fact that many patients with AATD also have chronic lung disease and where treatment for liver disease reduces AAT levels, such as RNAi mechanisms, then this may further affect lung disease so may need to simultaneous administer augmentation therapy to reduce the risk of worsening lung disease.

Treatment to prevent polymerization and therefore increase circulating AAT may help both liver and lung disease. New therapies are likely to be expensive but possibly more cost-effective than transplant and long term follow-up and anti-rejection drugs so it is critical to have a robust strategy of who to treat to ensure they are approved for use in AATD and avoid issues that have been seen with expensive augmentation therapy to treat lung disease. More research on rare liver diseases is necessary to develop a scientific basis for improvement in diagnostic techniques to facilitate early diagnosis and treatment.

7.0. EXPERT OPINION

7.1. Recent advances and impact on diagnosis and treatment

The parallels between lung and liver research in AATD are striking, and there is much that can be brought from pulmonologists to the field as a result. Thirty years ago whilst it was known that AATD leads to emphysema, and that protease imbalance was a key factor in pathogenesis, such that correcting this by AAT augmentation ought to be a valid way to manage patients, the rate at which emphysema progressed, and even how best to measure it was unknown. Choosing outcome measures for definitive drug trials of augmentation was therefore difficult, as was trial duration and patient selection. The liver field is now in a similar position – we know that AATD causes cirrhosis and that this is driven by polymer

accumulation, however we know less about how best to measure fibrosis or what duration trials will need to be to detect an effect of a drug that alters polymer accumulation.

In pulmonology, large cohorts have been established [32, 56-60], which have largely answered the questions of how disease progresses and which outcomes are appropriate for trials; lung function, whilst it clearly measures emphysema, is a relatively poor way to detect early lung disease, and is inappropriate as a primary outcome measure for AATD trials, because it is relatively insensitive to decline over time. We have detected factors which relate to decline over time [61,62], thus aiding patient selection for trials that seek to detect differences in decline, by enriching for those most at risk of rapid deterioration. Density scanning of the lung (CT densitometry) has been developed as a way to quantitatively measure emphysema severity and progression [63-65], detecting disease earlier, and change much more quickly than lung function. Densitometry has been successfully used in registration level trials – however a key lesson for liver trials is that this was difficult, and reimbursement has not always followed. Early work to demonstrate that quantitative measures of liver fibrosis (surrogate outcomes from the point of view of drug regulators and reimbursement agencies) relate to hard outcomes – such as mortality or quality of life (QOL) may be required. Some of the cohort studies in table 1 could answer questions for the liver of quantifying decline and describing factors which associate with rapid decline, which may aid later phase trial design, reimbursement or both. The importance of QOL in trials has also been prominent in lung disease [66]; considering which measures to include in liver trials for this, and in particular ways in which these can then be modeled to give an indication of quality adjusted life years gained from a treatment may also be key to adoption of new agents, by enabling an intervention to show its importance to patients, and its cost-effectiveness respectively.

7.2. What are the key areas for improvement?

One difference in liver disease is that the major outcome measure currently being used in phase II trials is invasive – liver biopsy – unlike the lung function measures used in emphysema. Invasive measures for efficacy of augmentation therapy (eg. measurement of AAT level in bronchoalveolar lavage) have typically only been used in phase I trials. However the principle of showing that a quantitative imaging measure relates to an accepted measure of disease still holds, as does the idea that a blood biomarker could add value. Elastography, or MRI-elastography may have promise in the imaging arena for the liver, indeed current phase II work (NCT03945292) uses adaptive design to try and reduce the need for biopsy if imaging is sufficiently reflective of biopsy. In emphysema desmosine, a marker of elastin breakdown, has been shown to change with augmentation therapy [67], and a number of phase II studies are now looking at this as an outcome; whether it will be suitable for use in phase III is not yet known, but the potential for combination with an imaging measure to further aid patient stratification or selection is interesting.

Over the next 5 years the field is likely to evolve to have selected a more sensitive, less invasive measure of fibrosis progression than biopsy of the liver, and to phase III trials of several agents that could reduce polymer burden. Trial design is likely to become more efficient through enhanced understanding of progression and the factors that drive it, such that those at risk of getting worse can be selected for trials (or ultimately routine clinical use) of an agent to reduce polymer burden. Selecting a liver only treatment using siRNA such as ALN-AAT and ARO-AAT or potential lung and liver treatment using the VX series of small molecules may also become a reality, and will require detailed phenotyping and follow up of both organs to select the most clinically and cost-effective treatment for an individual.

7.3. Strengths and limitations of current research

The strength of current research programmes in AATD is the appreciation that surrogate markers are needed to aid sensitivity in clinical trials. With respect to AATD-related lung disease it has taken many years to establish CT lung density as a valid marker to determine disease progression [68]. The use of elastography is looking promising to be able to detect and monitor fibrosis in AATD-related liver disease rather than the current invasive and high risk biopsy. In recent years a number of international networks have been established, including a pan-European observational study in patients with AATD (EARCO Registry) [69] and the European Reference Network (ERN) "Rare Liver" and the European Association for the Study of the Liver (EASL) registry group "Alpha-1 Liver" to further the understanding of the pathogenesis of AATD and hopefully provide the evidence needed to work with regulatory agencies on future trial design.

The limitations of research in the field stem mainly from the fact that AATD is a rare disease hence recruitment to trials is difficult. A non-invasive marker that is both sensitive and specific to detect liver fibrosis and its progression is also needed to determine who is at high risk of liver disease.

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