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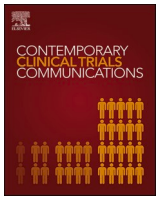

Design and rationale for a global novel non-invasive screening observational study using genetics and non-invasive methodologies to identify at-risk MASLD participants: The ALIGN study

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A B S T R A C T

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a common chronic liver disease that is heterogenous in nature with various drivers and modifiers such as metabolic dysfunction and genetic factors. MASLD and the progressive subtype, metabolic dysfunction-associated steatohepatitis (MASH) represent the most rapidly increasing cause of liver-related mortality. There are limited treatment options for patients living with MASLD and MASH, various treatments with an array of different targets are under investigation and one therapeutic has been approved since the initiation of this study. Clinical trials investigating treatments for MASLD and MASH are associated with a high screen failure rate, driven largely by the regulatory required histological inclusion criteria for clinical trial eligibility. Other available clinically utilized biomarkers, typically referred to as non-invasive tests (NITs), can assess both the presence of steatosis and the severity of liver fibrosis in patients with MASLD and MASH in the clinic but are not yet approved over histological changes as endpoints for pivotal trials. However, the use of NITs have been demonstrated to increase the likelihood of meeting clinical trial entry criteria. All-Liver Interventional Global Network (ALIGN) is the first described multi-centre global observational screening study aimed at identifying individuals with a high likelihood of MASLD/MASH interested in participating in therapeutic clinical trials using non-invasive methodologies and genetic testing. This study represents a valuable prototype for industry and academic groups looking to evaluate large populations for MASH eligibility and interest in clinical trial participation.

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously known as non-alcoholic fatty liver disease (NAFLD), is fast becoming one of the most common chronic liver disorder globally, estimated to have a prevalence of approximately 30 % in adults [1,2]. MASLD and the progressive subtype metabolic dysfunction-associated steatohepatitis (MASH) are heterogenous in nature and are characterised by the accumulation of lipotoxic fat in the liver [3,4]. The resultant metabolic inflammatory milieu drives the development of fibrosis, the severity of which is prognostic for liver-related outcomes and overall mortality [5]. MASLD/MASH represent one of the most rapidly increasing causes of liver-related mortality worldwide and an important cause of end-stage liver disease, primary liver cancer, and liver transplantation [6–9].

Despite increasing awareness of the long-term consequences and morbidity of MASLD/MASH, diagnostic barriers and a paucity of therapeutic options contribute to an underdiagnosis of MAFLD/MASH globally [10]. Standard practices for diagnosing MASH differ globally. According to international guidelines, a formal diagnosis of MASH requires a liver biopsy, which is usually reserved for patients with a higher burden of disease [11–13]. In some regions, non-invasive markers

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and/or liver imaging are used to assess MASLD/MASH disease burden in patients, and no formal diagnosis by liver biopsy is typically made [14]. In addition, primary care and other providers often manage MASLD/-MASH rather than refer patients to a hepatologist for diagnosis due to limited treatment options, meaning progressive MASLD might be unrecognised and underdiagnosed. Early diagnosis of MASLD/MASH may lead to improved outcomes, especially in patients at risk for disease progression.

Recent estimates suggest that there are more than 50 different investigational medicines across the drug development phases being evaluated as potential treatments for MASLD/MASH [15]. The FDA approval of resmetirom in 2024 as a treatment for adults with non-cirrhotic NASH was based on histological surrogate endpoints, but does not require a biopsy for clinical use [16]. Despite the important progress of a new treatment option available for MASH patients, there remains an unmet need for new therapies to address the different underlying drivers and improve patient treatment response [17,18].

Early phase clinical trials typically evaluate the safety, pharmacokinetics and target engagement across various spectra of disease, often including participants identified to have MASLD and/or MASH. Given the histological requirement to identify non-cirrhotic MASH with fibrosis exists for studies in phase 2 b and beyond, early development trials have an opportunity to evaluate participants identified through non-invasive methodologies who are considered to have a high likelihood of MASH with fibrosis [19,20]. Challenges exist in recruitment for MASH trials due to the high screen failure rates and often a reluctance to obtain biopsy. Thus, identifying methodologies whereby broader patient populations can be screened for high-risk MASH is important to advance the field of drug development[21].

Here we describe the design and rationale for ALIGN, All-Liver Interventional Global Network, which was designed as a multi-centre global observational screening study aimed at identifying individuals with a high likelihood of MASLD/MASH interested in participating in therapeutic clinical trials using non-invasive methodologies and genetic testing.

2. Methods

2.1. Overview

This observational screening study aimed to screen potential participants with a high likelihood of MASLD/MASH based on non-invasive biomarkers and imaging. Participants for this pilot observational screening study were recruited from 6 global sites. The clinical sites participating in this study included specialty endocrinology and hepatology centers, as well as general medicine sites. Participants were identified by a variety of approaches at different clinical trial sites and included media advertisements for community screenings during weekend open screening events, referrals from local family/general medicine clinics, and internal screenings from patients selected within the clinical trial database. The objectives of the study are listed in Table 1. The primary objective of the ALIGN study was to screen participants considered at risk for MASLD/MASH and identify those that were highly likely to meet the histological criteria for clinical trials investigating MASH experimental therapies using non-invasive tests.

Participants who met eligibility criteria were asked to participate in this study for up to one year. The study procedures included a screening visit, one or more optional follow-up visits at intervals of 3 months by teleconference or at site, and an end-of-study (EOS) visit by teleconference or at site (Fig. 1). Eligible participants who met the inclusion and exclusion criteria were invited to enroll in a therapeutic study (Fig. 1). If a participant was enrolled into a therapeutic study, their participation in ALIGN was completed, otherwise the participants remained in the study until approximately one year elapsed since their last screening visit (Fig. 1).

The study was approved by research ethics committees (supplementary file 1) and performed in accordance with ethical principles that have their origin in the Declaration of Helsinki, consistent with ICH/GCP and applicable regulatory requirements, and in accordance with national legislations and international regulations. No power size estimation was calculated for this observational screening study.

Table 1
Summary of the objectives for the study.

Primary Objective	Endpoint
To use non-invasive screening criteria to identify a pool of participants with a high likelihood of MASLD/MASH and who will be invited to participate in a therapeutic study.	Study disposition
Secondary Objective	**Endpoint**
To characterize phenotypic attributes associated with MASLD/MASH in participants with a high likelihood of MASLD/MASH.	Demographics, vital signs, clinical chemistry, liver stiffness and steatosis
Exploratory objectives	**Endpoint**
For participants with a high likelihood of MASLD/MASH, to evaluate genetic analyses associated with MASLD/MASH, by screening for genetic variants that are known or strongly suspected to play a role in disease progression including, but not limited to PNPLA3, HSD17B13, TM6SF2 and GCKR genes.	Prevalence of MASH related genetic variants
To evaluate the prevalence of familial medical history of liver and cardiorenal metabolic disease in participants with a high likelihood of MASLD/MASH.	Prevalence of the familial history of liver and cardiorenal metabolic disease (first, second, third degree relative status)
To evaluate interactions between the environment (eg, geography, demographics) and genetic risk in participants with a high likelihood of MASLD/MASH.	Interaction of environment and prevalence of MASH related genetic variants

2.2. Recruitment

Potential participants considered to be at-risk for MASLD/MASH or living with type 2 diabetes or living with 2 or more metabolic risk factors were targeted for screening for ALIGN. Participants were identified by health care professionals, family members, community screening initiatives, or other methods. They must have been between the ages $\geq$18 and $\leq$75 years and be informed about and willing to consider participation in a therapeutic study.

2.3. Inclusion/exclusion

Participants included in this study were required to have the presence of hepatic steatosis and the presence of a MASLD/MASH disease characteristic such as the presence of hepatic fibrosis or inflammation (Table 2). The criteria outlined in the first version of the protocol was designed to be as inclusive to the capabilities of the participating investigational sites as possible, as well as identify participants across the broad spectrum of MASLD/MASH disease (MASH with F $\geq$ 1) using various non-invasive modalities. The inclusion criteria were changed under protocol version 2 to be more stringent and increase the likelihood of identifying participants with MASH with active disease (NAS$\geq$4), and fibrosis (F $\geq$ 2).

Controlled attenuation parameter (CAP) is a parameter captured by vibration-controlled transient elastography (VCTE) which can be used to identify the presence of hepatic steatosis. CAP has been shown to be correlated to the level of hepatic fat content as determined by magnetic resonance imaging-proton density fat fraction (MRI-PDFF) [22]. The

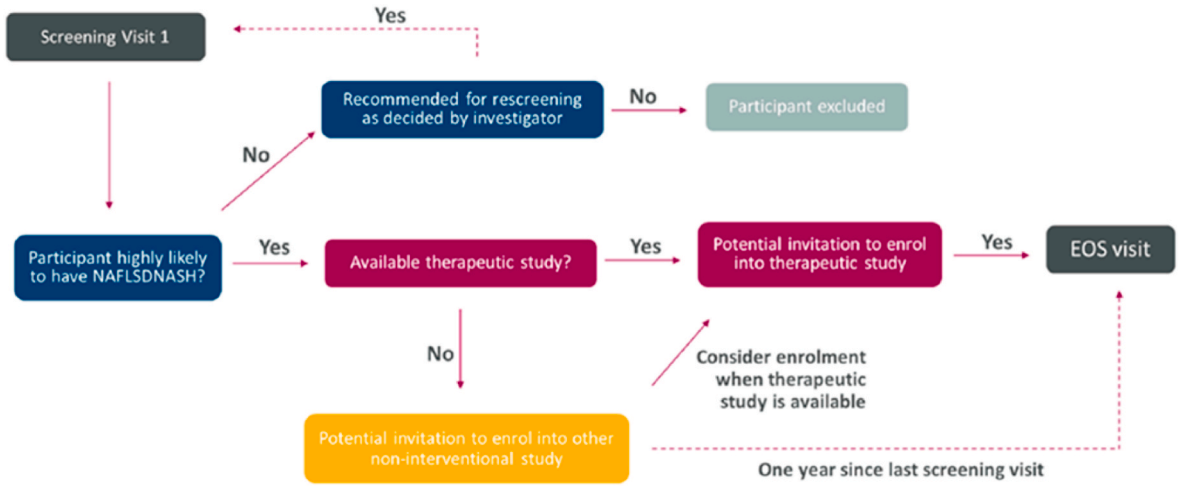

Fig. 1. Study flow chart detailing participation in the study.

Table 2
MASLD/MASH disease characteristics required for participation in study.

Protocol Version 1	Protocol Version 2
Participants must have at least one MASLD/MASH disease characteristic demonstrating evidence of hepatic steatosis, as follows:	
• CAP >285 dB/m within 6 months of screening, or • Fatty liver index ≥60, or • Ultrasound indication of hepatic steatosis within 6 months of screening	• CAP >285 dB/m within 6 months of screening
And at least one additional MASLD/MASH disease characteristic, as follows:	
• VCTE ≥4.9 kPa within 6 months of screening, or • Imaging indication of liver fibrosis within 6 months of screening, or • ALT > upper limit of normal or other relevant liver function test results (ie, ALT, AST, GGT) as judged by the investigator, or • FibroScan-AST score >0.35, or • Fibrosis-4 index ≥1	• Liver stiffness measurement (LSM) ≥ 8.0 kPa within 6 months of screening and ALT >40 IU/L or other relevant liver function test results (ie, AST, GGT) as judged by the investigator, or • Imaging indication of liver fibrosis within 6 months of screening, and ALT >40 IU/L or other relevant liver function test results (ie, AST, GGT) as judged by the investigator, or • FibroScan-AST score >0.35, or • Fibrosis-4 index (Fib-4) > 1.3 for participants under the age of 65 years, for participants 65 years or older Fib-4 > 2.

cutoff of >285 dB/m is optimized for both sensitivity and specificity for the detection of hepatic steatosis (histological grade ≥1)[23].

Liver stiffness measurement (LSM) by VCTE has demonstrated high correlation to the severity of histological fibrosis [23]. Furthermore, LSM shows similar prognostic ability to histological fibrosis to predict liver related outcomes [24]. Under protocol version 1, a LSM cutoff of ≥4.9 kPa was used to identify participants with histological fibrosis stage F ≥ 1 [23]. Under protocol version 2, the LSM cutoff was increased to ≥8.0 kPa to increase the specificity for advanced fibrosis [25]. Additionally, elevated liver function tests added to the criteria to reflect active liver disease and those likely to progress [26].

The FAST score is a composite score derived from LSM, CAP and AST aimed to identify MASH with active disease (NAS≥4), and fibrosis (F ≥ 2). The FAST cut-off of 0.35 has a high sensitivity and accuracy in ruling out the presence of advanced fibrosis [27]. Meta-analysis has shown that the FAST score is an accurate and useful methodology to identify patients with fibrotic MASH [28].

FIB-4 is a simple composite score composed of routinely assessed variables and has a high NPV for ruling out advanced fibrosis [29]. The inclusion criteria was changed at protocol version 2 to reflect age dependent cut-offs (Table 2) [30].

Fatty liver index (FLI≥ 60) is a blood based composite score that has shown value as a screening tool to identify those with hepatic steatosis [31]. Similarly, hepatic ultrasonography has been demonstrated to accurately detect moderate to severe hepatic steatosis [32]. Both parameters were removed from protocol version 2.

Key exclusionary criteria for the study is detailed in Table 3 ensuring that participants included in the study had chronic liver disease which was highly likely due to MASLD/MASH.

2.4. Study procedures

The assessments and procedures included in the study were designed to be minimal for the patient and to minimize investigator time and burden (Table 4). Screening assessments at visit 1 were permitted to be conducted over a 60-day window. These assessments consisted of gathering medical history, vital signs, demographic data as well as blood sampling for parameters such as clinical chemistry, hematology and virology. Additionally, liver imaging was conducted using VCTE to obtain both CAP and LSM measurements. If the participant consented, an optional blood sample was collected for genetic analysis to determine the participants genotype for genes associated with MASLD/MASH (including but not limited to PNPLA3, HSD17b13, TM6SF2 and GCKR). Genetic testing was performed either using centralized testing or local testing depending upon the participating sites preference. The optional and EOS visits consisted of updating medical history, concomitant mediations, collecting any serious events that may have occurred as well as discussing the participant's options for participating in available therapeutic studies (Table 4).

3. Discussion

ALIGN was a global screening study which aimed to identify participants with a high likelihood of MASLD/MASH based on non-invasive biomarkers and genetics to pre-identify candidates for therapeutic interventional studies. Furthermore, the study aimed to characterize the phenotypic, genetic and familial attributes associated with MASLD/MASH. As previously described, MASH clinical trials are associated with a high screen failure rate and slow recruitment [21]. This study is a valuable prototype for industry and academia looking to evaluate large numbers of patients that could be interested in therapeutic clinical trials, in disease areas with a predicted high screening failure rate. To our knowledge, ALIGN is the first described screening study for MASLD/-MASH with the aim of identifying participants for multiple

Table 3
Exclusion criteria common to both protocol versions.

Exclusion Criteria
History of liver transplantation.
History or known evidence of other known forms of known chronic liver disease such as hepatitis B, non-treated hepatitis C, primary biliary cirrhosis, primary sclerotic cirrhosis, autoimmune hepatitis, Wilson disease, iron overload, alpha-1-antitrypsin deficiency, drug-induced liver injury, or HCC.
Evidence or confirmation of medically unstable cirrhosis or decompensated cirrhosis as judged by the investigator.
Any positive result for HIV infection.
Unsafe levels of alcohol consumption as determined by local guidance (eg, in Europe and the United States of America, >21 units [1 unit = 14 g pure alcohol] per week [21 standard drinks per week] for men and >14 units per week [14 standard drinks per week] for women, on average), within 2 years prior to screening, or evidence of alcohol dependence as assessed by the AUDIT questionnaire at screening.
Participation in another clinical study with an investigational product administered within 3 months prior to screening. After enrolment in this study, participants are permitted to enrol in a therapeutic study.

interventional trials.

Pre-screening is an acknowledged cornerstone of participant recruitment for clinical trials by decreasing the screen failure rate. High screen failure rates often have negative implications on study conduct, including but not limited to participant burden, site and investigator motivation, study duration, and cost [33]. Pre-screening methodologies are varied, in its most simplistic form, it typically consists of site staff checking internal records for potential candidates [33]. MASH is a disease area with broad research interest, demonstrated by the increasing number of pharmaceutical and academic clinical trials that have been listed in clinicaltrials.gov databases over the past 5 years. Consequently, the desire to identify trial participants has increased, highlighting the need for efficient non-invasive pre-screening processes that can be utilized by a variety of different clinical trial centers. Pre-screening using tools such as VCTE and blood-based biomarkers have been successfully utilized for MASH recruitment and have demonstrated success as non-invasive tests with high predictive probabilities [17,34,35]. However, no data exists regarding the combination of these tests as a separate study supporting recruitment through a global network of sites. Examples of interventional trials that have included non-invasive testing as a prescreening method include the FASCINATE-2 trial which investigated the safety and efficacy of denifanstat in biopsy proven MASH patients. Cut offs for AST and CAP were added to the protocol via an amendment which then successfully reduced the screen-failure rates from 96 % to 80 % [21]. Similarly, the SYNERGY-NASH trial introduced the composite score FAST (≥0.35) and AST (>23 IUL) as eligibility criteria, resulting in a decrease in the proportion of patients who screened failed according to histological criteria in those who underwent a per protocol biopsy [35]. The addition of NITs to the eligibility criteria was found to have the greatest benefit among non-specialist clinical sites, again highlighting the value of NITs in pre-evaluation of suitable patients for MASH trials [35].

Participant recruitment is a crucial and challenging step for clinical trial conduct and success, research into clinical trial recruitment has found that 48 % of clinical trial sites enroll one or fewer participants into a given trial leading to delays, inefficiencies and potential increases in costs [36,37]. With this in mind, ALIGN included and concentrated resources at a small number of high-volume centers, rather than spreading across a large number of centers some of which recruit very few patients.

The ALIGN study actively recruited participants in a patient centric manner. In keeping with the values outlined by Yeoman et al., this trial aimed to support a variety of clinical trial centers with different specialties to evaluated participants eligibility, personal needs and interest in participating in various therapeutic clinical trials that were available to them at their respective sites [38]. Furthermore, the design of the ALIGN study ensured that common reasons for interventional study screen failures such as exclusionary medical history and/or concomitant medications could be identified by ALIGN to reduce interventional study screening volume and reducing the burden of assessments on patients and sites [21].

MASH is a heterogenous disease with various drivers and modifiers, such as metabolic dysfunction, age and genetic factors among others, collectively influencing disease progression [4,39]. Genome-wide association studies have identified single nucleotide polymorphisms (SNP) in genes such as PNPLA3, TM6SF2, GCKR, MBOAT7 and HSD17B13 that are associated with MASH development and progression [40]. The best described disease modifying genetic variant for MASH is the SNP in PNPLA3 known as rs738409 c.444C > G p. I148M [41,42]. Patients living with MASLD that are homozygous for PNPLA3 I148M are at increased risk for liver-related mortality [43–46]. The global prevalence of PNPLA3 I148M allele is estimated to be 26 % in the 1000 Genomes Project phase 3 combined population, with the prevalence higher in Latin American countries [47]. AZD2693 is an antisense oligonucleotide investigational treatment that silences hepatic PNPLA3 mRNA and is currently under investigation in a phase 2 b study to evaluate the efficacy and safety in patients with MASH that are homozygous for PNPLA3 I148M (NCT05809934). ALIGN is a unique screening study with the inclusion of genetic testing for known risk alleles for MASH, such as PNPLA3 I148M, facilitating the pre-identification of participants who may be eligible for and interested in participating in clinical trials investigating precision medicines such as AZD2693.

In summary, the ALIGN study is a valuable prototype for industry and academics looking to evaluate large numbers of patients that could be available and interested in participating in clinical trials investigating investigational treatments for therapy areas with high screen failure rates. The use of an overarching pre-screening study including non-invasive testing combined with imaging, family history, and genetics provides a novel approach to accelerate drug development pathways and support global clinical trials in MASH.

CRediT authorship contribution statement

Samuel J. Daniels: Writing – review & editing, Writing – original draft, Project administration, Conceptualization. **Karin Nelander:** Writing – review & editing, Conceptualization. **John Eriksson:** Writing – review & editing, Conceptualization. **Lutz Jermutus:** Writing – review & editing, Project administration, Funding acquisition. **Jelena Saillard:** Writing – review & editing, Project administration, Conceptualization. **Stephanie Oyesola:** Writing – review & editing, Project administration. **Federica Tavaglione:** Writing – review & editing, Project administration. **Marco Arrese:** Writing – review & editing, Investigation. **Alma Laura Ladrón de Guevara:** Writing – review & editing, Investigation. **Umberto Vespasiani-Gentilucci:** Writing – review & editing, Investigation. **Naim Alkhouri:** Writing – review & editing, Investigation. **Jenny E. Blau:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal

Table 4
Schedule of assessments for the study.

Visit	Visit 1	Visit 2[a,b] (optional)	Visit 3[a,b] (optional)	Visit 4[a,b] (optional)	Visit 5[a,c]
Study Period	Screening	Follow-up			EOS
Day (Window, days)	Day 1–60	Month 3	Month 6	Month 9	Month 12 or earlier
Informed consent	X				
Verify eligibility criteria	X	X			
Demographic characteristics	X				
Assess willingness to participate in other studies	X				
Medical and surgical history, and comorbidities (complete)	X				
Medical and surgical history, and comorbidities (updated)		X	X	X	X
Smoking history, and alcohol history assessed using AUDIT questionnaire	X				
Vital signs (height, weight, BMI calculation, waist and hip circumference, BP, pulse, and body temperature)	X				
Concomitant medications	X	X	X	X	X
SAEs	X	X	X	X	X
Blood sample for genetic testing, (optional)[d]	X				
Blood assessments	X				
Vibration-controlled Transient Elastography (VCTE)	X				
Discuss screening results[d], and options for participating in available therapeutic studies or other non-interventional studies; if applicable	X	X	X	X	X
Invite participants with a high likelihood of MASLD/MASH to enrol in therapeutic study[a], or non-interventional study (ies), if applicable[b]	X	X[b]	X[b]	X[b]	X

AUDIT = Alcohol Use Disorders Identification Test; BMI = body mass index; BP = blood pressure; EOS = end of study; MASLD = Metabolic dysfunction-associated steatotic liver disease; MASH = Metabolic dysfunction-associated steatohepatitis; SAE = serious adverse event.

[a] By teleconference or at site.

[b] If a site identified a suitable therapeutic or other non-interventional study after the screening visit, they may have arranged an unscheduled clinic visit or telephone call before the next optional follow-up visit to invite the participant to enrol in the identified study.

[c] The EOS visit occurred either approximately when the participant was randomised into a therapeutic study or when approximately one year elapsed since the last screening visit.

[d] Only applicable to sites who performed genetic testing.

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Appendix A. Supplementary data

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