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Efficacy and safety study of cenicriviroc for the treatment of non-alcoholic steatohepatitis in adult subjects with liver fibrosis: CENTAUR Phase 2b study design

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ABSTRACT

Background: Non-alcoholic steatohepatitis (NASH) is often accompanied by liver fibrosis, which can progress to cirrhosis; C-C chemokine receptors type 2 and 5 (CCR2/CCR5), which mediate interactions driving inflammation and fibrosis, are promising treatment targets. Cenicriviroc (CVC), a dual-CCR2/CCR5 antagonist, has potent anti-inflammatory and antifibrotic activity in animal models; in HIV-positive subjects it reduced soluble CD14 levels, aspartate aminotransferase-to-platelet count ratio index, and non-invasive hepatic fibrosis risk scores; favorable tolerability was demonstrated in ~600 subjects. Efficacy and safety of CVC 150 mg for treating NASH with liver fibrosis are being evaluated over 2 years (primary endpoint at Year 1 [Y1]).
Design: Phase 2b, randomized, double-blind, placebo-controlled, multinational study (CENTAUR; NCT02217475). Adults with histological evidence of NASH, non-alcoholic fatty liver disease activity score (NAS) ≥ 4, and liver fibrosis (stages 1–3 NASH clinical research network system) enrolled. Subjects have increased risk of progression to cirrhosis due to ≥1 characteristic: type 2 diabetes; body mass index > 25 kg/m^2 with ≥1 feature of metabolic syndrome; bridging fibrosis and/or NAS ≥ 5. Liver biopsy evaluation at Screening, Y1, and Year 2 (Y2).
Objectives: Assess histologic improvement (≥2-point in NAS with ≥1-point improvement in >1 category) without worsening of fibrosis at Y1 (primary); evaluate complete NASH resolution without worsening of fibrosis at Y2 (key secondary).
Discussion: CENTAUR is the first prospective study evaluating an oral agent exclusively enrolling subjects with NASH and liver fibrosis, with increased risk of developing cirrhosis. It will compare shorter *versus* longer CVC treatment and assess correlations between decreased inflammation and fibrosis.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is commonly associated with obesity-related disorders (e.g. type 2 diabetes mellitus [T2DM] and metabolic syndrome) [1–5]. Approximately 10–20% of subjects with NAFLD have a progressive form, non-alcoholic steatohepatitis (NASH) [6], defined by the presence of steatosis, hepatocellular ballooning, and lobular inflammation. NASH is typically associated with inflammation and fibrosis [1,2,6], which can progress to cirrhosis, end-stage liver disease, and hepatocellular carcinoma [6–9]. Although fibrosis is not always present in NASH, fibrosis severity is linked to long-term outcomes [6,10]. NAFLD and NASH prevalences are increasing worldwide, associated with a rise in obesity-related disorders [6,11,12]. Globally, the prevalence rate of NAFLD is 6–35%, with a median of 20% [11]. Systematic reviews based on liver biopsy data suggest that approximately 3–5% of the US population have NASH [11]; comparable prevalences are expected in Europe and the Middle East [6,12]. Furthermore, a longitudinal study reported that 84.9% of patients with borderline or definite NASH in the USA, Europe and Thailand had liver fibrosis (stages 1–4) [10]. Despite its rising prevalence, there are currently no approved treatments for NASH.

The C-C chemokine receptor types 2 and 5 (CCR2 and CCR5), and their respective ligands, C-C chemokine ligand types 2 (CCL2/monocyte chemoattractant protein-1 [MCP-1]) and 5 (CCL5/RANTES), are

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implicated in liver inflammation and fibrosis [13–18]. In response to hepatocyte injury, resident liver macrophages (Kupffer cells) secrete CCL2; this promotes monocyte recruitment and migration to the liver, where they mature into pro-inflammatory macrophages [19,20]. Macrophages express pro-inflammatory cytokines, which activate hepatic stellate cells (HSCs) to promote their survival, while stimulating collagen production or fibrogenesis [21–24]. CCR2 and CCR5 mediate the intrahepatic immune-cell interactions that promote activation and migration of Kupffer cells and HSCs (Fig. 1) [13–16]. Activation of inflammatory cells and upregulation of several soluble inflammatory mediators, including CCL2 and CCL5, are features of NASH [25]. In animal studies of hepatic fibrosis, mice with a targeted deletion or pharmacological inhibition of CCR2 [16,17,19,20] or CCR5 [15,18] display lower immune-cell activation and reduced liver fibrosis; therefore, CCR2 and CCR5 are promising targets for treatment of NASH.

Cenicriviroc (CVC) is a novel, oral, dual CCR2/CCR5 antagonist with nanomolar potency against both receptors, and a long plasma half-life (30–40 h in humans). Phase 2 Studies 201 (NCT01092104) and 202 (NCT01338883), conducted in human immunodeficiency virus (HIV)-infected subjects, have also shown CCR2 blockade, associated with an increase in CCL2 levels, and CCR5 blockade, demonstrated by reduction in HIV-1 RNA levels [26,27]. Antifibrotic effects of CVC have been demonstrated in multiple animal models of liver, as well as in kidney, fibrosis [28,29]. A *post hoc* analysis of Study 202 revealed improvements in soluble cluster of differentiation 14 (sCD14) and fibrosis scores in subjects treated with CVC [27,30,31]. The proportion of subjects with aspartate aminotransferase-to-platelet count ratio index (APRI) score ≥ 0.5 and non-invasive hepatic fibrosis risk (FIB-4) score ≥ 1.45 decreased by 75% and 73%, respectively, between Baseline and Week 24; these decreases were maintained at Week 48 [32]. Additionally, the mean enhanced liver fibrosis (ELF) test index in subjects treated with CVC decreased from 10.53 ± 2.12 to 8.28 ± 0.88 ($p < 0.0001$) by Week 48 [31]. CVC has a favorable safety profile and was well tolerated in approximately 600 subjects, including those with mild or moderate hepatic impairment (Child–Pugh A and B) [26,27,33]. Therefore, CVC is an attractive candidate for the treatment of NASH and liver fibrosis by antagonism of CCR2/CCR5 receptors. CENTAUR (NCT02217475) will examine the efficacy, pharmacokinetics (PK), and safety of CVC for the treatment of NASH in adults with liver fibrosis, for which CVC has received Fast Track designation by the US Food and Drug Administration (FDA).

2. Methods

2.1. Study design

CENTAUR is a Phase 2b, randomized, double-blind, placebo-controlled, multinational study that will be conducted in adult subjects with NASH and liver fibrosis in 11 countries across the USA, Europe, and Asia-Pacific. The study was planned to recruit 252 adults; a total of 289 adults were enrolled due to increased screening efforts once all global study sites were activated. The study will evaluate the efficacy and safety of CVC 150 mg over 2 years of treatment, with a primary endpoint at Year 1. The study design is shown in Fig. 2.

2.2. Study rationale

CVC is expected to have anti-inflammatory and antifibrotic activities due to its dual antagonism of CCR2/CCR5 receptors. The mechanisms by which CVC may prevent inflammation and fibrosis in NASH are decreased recruitment, migration, and infiltration of pro-inflammatory monocytes to the site of liver injury induced by activated Kupffer cells, mainly via CCR2 antagonism. This consequently reduces the number

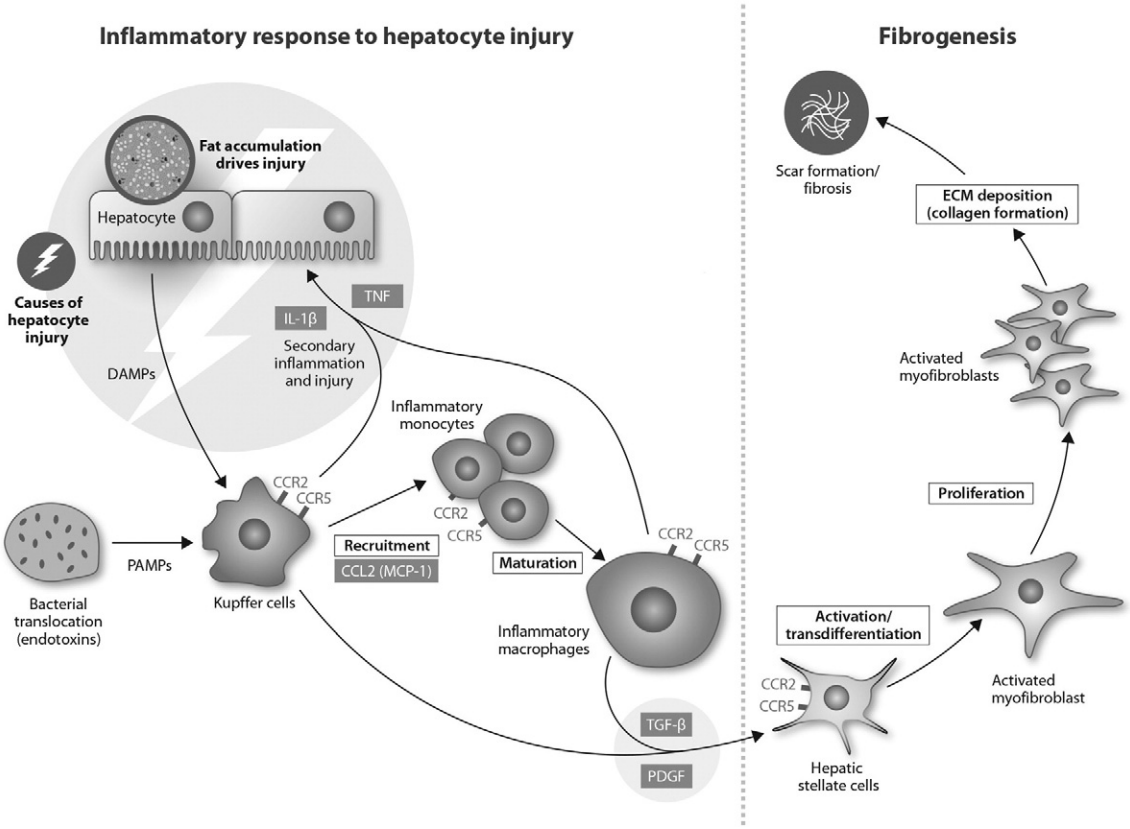

Fig. 1. Inflammatory response to hepatocyte injury leading to fibrogenesis [13–16,19–24]. CCL2, C-C chemokine ligand type 2; CCR2, C-C chemokine receptor type 2; CCR5, C-C chemokine receptor type 5; DAMPs, danger-associated molecular patterns; ECM, extracellular matrix; IL-1β, interleukin 1β; MCP-1, monocyte chemoattractant protein-1; PAMPs, pathogen-associated molecular patterns; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor beta; TNF, tumor necrosis factor.

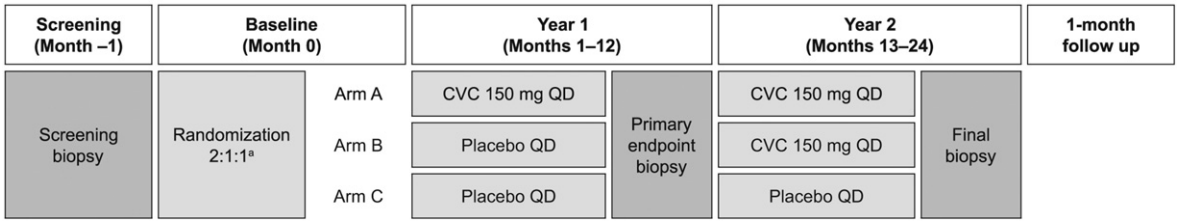

Fig. 2. Study design for CENTAUR. [a]$N = 252$, planned sample size; $N = 289$ enrolled. CVC, cenicriviroc; QD, once daily.

of pro-inflammatory macrophages in the liver, thereby decreasing chronic liver inflammation and downregulating the production of profibrotic cytokines, such as transforming growth factor beta 1 (TGF-β1). TGF-β1 promotes the transdifferentiation of HSCs to collagen-synthesizing myofibroblasts and the production of tissue inhibitors of metalloproteinases, which inhibit the metalloproteinase-mediated degradation of extracellular matrix components [34]. In addition, CCR5 and CCL5 mediate HSC migration and proliferation, as well as 'cross-talk' between HSCs and leukocytes during fibrogenesis [14]. Therefore, disruption of CCR2 and CCR5 signaling pathways is expected to provide anti-inflammatory and antifibrotic benefits.

2.3. Endpoint rationale

A 2011 meeting sponsored by the American Association for the Study of Liver Diseases (AASLD), discussed key endpoints and specific trial design issues relevant for treatment trials for NASH [35]. It was concluded that the primary endpoint should be measurable, sensitive to change, clinically meaningful, and be able to be quantified consistently. Two histology-based primary endpoints were recommended: "a minimum of 2 point improvement in NAFLD activity score (NAS) with at least one point improvement in more than one category and no worsening of fibrosis" and "resolution of steatohepatitis with no worsening of fibrosis" [35]. More recently, a summary of a 2013 joint AASLD–FDA workshop proposed a similar endpoint to the latter: the "reversal (i.e. resolution) of steatohepatitis without progression to advanced fibrosis (stages 3–4)" [36]. This suggests that it could be an acceptable surrogate endpoint suitable for both Phase 2b and 3 trials that enroll patients with NASH and evidence of early fibrosis, and for FDA accelerated approval under Subpart H [36]. The advantage of NAS is that it is quantifiable and relatively more reproducible than the diagnosis of steatohepatitis, and remains a suitable endpoint to assess histological disease activity and provide robust evidence of early treatment effects. Importantly, it is expected that reversal of steatohepatitis would reduce the risk of developing cirrhosis [35,36]. This is supported by recent randomized clinical trials evaluating NASH agents, where antifibrotic effects have also been observed in addition to resolution of NASH [37,38]. The design of CENTAUR and its key endpoints are aligned with these recommendations. Specifically, the primary endpoint of CENTAUR, "drop in NAS by ≥2 with at least a 1-point improvement in more than 1 category and with no concurrent worsening of fibrosis stage (Year 1)" was also used in the PIVENS (NCT00063622) and FLINT (NCT01265498) studies [37,39]. The key secondary endpoint of CENTAUR will be "complete resolution of NASH (histopathologic interpretation of no fatty liver disease or simple or isolated steatosis with no steatohepatitis) with no

Primary efficacy endpoint	Key secondary efficacy endpoint
Hepatic histological improvement in NAS at **Year 1** relative to Screening biopsy (≥2-point drop in NAS with ≥1-point improvement in ≥1 category) with no concurrent worsening of fibrosis (Arm A *versus* Arms B and C)	Complete resolution of NASH at **Year 2** (histopathologic interpretation of no fatty liver disease or simple or isolated steatosis with no steatohepatitis) with no concurrent worsening of fibrosis (Arm A *versus* Arm C)

Other secondary efficacy endpoints
Complete resolution of NASH with no concurrent worsening of fibrosis at Year 1 (Arm A *versus* Arms B and C)
Hepatic histological improvement in NAS at Year 2 relative to Screening biopsy (≥2-point drop in NAS with ≥1-point improvement in ≥1 category) with no concurrent worsening of fibrosis (Arm A *versus* Arm C)
Improvement in histological fibrosis stage assessed using NASH CRN and Ishak scoring systems at Years 1 and 2
Change in CPA on liver biopsy to evaluate efficacy of CVC *versus* placebo at Years 1 and 2
Change in hepatic stellate cell activation marker (α-SMA) at Years 1 and 2
Change from Baseline in non-invasive scores and markers of hepatic fibrosis (APRI, FIB-4, hyaluronic acid, FibroTest [FibroSure], NFS, and ELF) at Months 6, 12, 18, and 24
Change from Baseline in biomarkers of hepatocyte apoptosis, assessed using CK-18 caspase-cleaved and total at Years 1 and 2

Tertiary efficacy endpoint
Change from Baseline in non-invasive liver imaging method (e.g. TE, MRE, and ARFI) at Months 6, 12, 18, and 24 (at sites where available)

Fig. 3. Efficacy endpoints for CENTAUR. α-SMA, α-smooth muscle actin; APRI, aspartate aminotransferase-to-platelet count ratio index; ARFI, acoustic radiation force impulse; CPA, collagen proportionate area; CRN, clinical research network; CVC, cenicriviroc; ELF, enhanced liver fibrosis test; FIB-4, non-invasive hepatic fibrosis risk score; MRE, 2-dimensional magnetic resonance elastography; NAS, non-alcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis; NFS, non-alcoholic fatty liver disease fibrosis score; TE, ultrasound transient elastography.

concurrent worsening of fibrosis stage (worsening defined as progression of NASH Clinical Research Network [CRN] fibrosis stage) at Year 2" (Fig. 3).

In addition, the consensus at the AASLD meeting was that trials assessing improvement in fibrosis should last at least 1–2 years, since it is assumed that improvement in fibrosis occurs only after other features of NASH are improved (e.g. hepatocellular ballooning and lobular inflammation) [35]. There are currently limited data to establish the optimal treatment duration of NASH. Findings from the PIVENS and FLINT studies suggest that improvements in the NAS score can be observed relatively early but that longer-term treatment may be required to achieve complete NASH resolution [37,39]. Furthermore, 24-week follow-up after treatment completion in these two studies suggest a return to baseline for hepatic and metabolic effects, indicating that chronic treatment is likely to be required for this condition. These considerations have been incorporated into CENTAUR study design and analysis, which will take place over a 2-year duration and include liver biopsies collected at Years 1 and 2. These two time points will allow for the assessment of the benefits of shorter *versus* longer treatment intervention for subjects on CVC, and provide natural history data for subjects on placebo. Several key efficacy endpoints will be assessed at Years 1 and 2 due to the differences in timing at which these endpoints are expected to be reached. Analysis of the primary endpoint will take place at Year 1, as it is expected that an improvement in NAS should occur relatively early during CVC treatment, due to the rapid and sustained blockade of CCR2 and CCR5 [26,27]. As improvement in fibrosis may be delayed in the setting of chronic liver disease [35], analysis of the key secondary endpoint will take place at Year 2 to assess longer-term effects on improvement in fibrosis and complete resolution of NASH.

At Year 1, all efficacy endpoints will compare results from Arm A (CVC 150 mg) with pooled results from Arms B and C (placebo; Fig. 2). After Year 1, subjects in Arm B will cross over from placebo to CVC, which will allow more patients to receive investigational treatment for NASH, thereby increasing their likelihood of receiving active drug during this 2-year study that requires a total of three serial biopsies. This is expected to increase motivation of subjects to participate and to remain in the study, while still maintaining placebo control throughout to evaluate the efficacy endpoints, and provide more robust evaluation of the efficacy and safety of CVC. Since Arm B subjects will cross over from placebo to CVC after Year 1, the main efficacy comparisons at Year 2 will include Arm A (CVC 150 mg) *versus* Arm C (placebo). Given that both Arms A and C will remain on assigned treatment for a 2-year duration, with liver biopsies obtained at Years 1 and 2, this will allow for evaluation of shorter *versus* longer CVC treatment (Arm A) even though the total number of placebo subjects at Year 2 will be reduced. Other comparisons pooling Year 2 results from Arms A and B (CVC 150 mg) to Arm C (placebo) will also be made to assess overall efficacy of CVC treatment.

Other endpoints (Fig. 3) will include improvement in histologic fibrosis stage (NASH CRN and Ishak scoring systems); morphometric quantitative collagen (collagen proportionate area); HSC activation marker (α-smooth muscle actin [α-SMA]); biomarkers of hepatocyte apoptosis (CK-18 [caspase-cleaved and total]); non-invasive markers of hepatic fibrosis (APRI, FIB-4, hyaluronic acid, FibroTest [FibroSure], NAFLD fibrosis score [NFS], and ELF); and non-invasive liver imaging methods (e.g. ultrasound transient elastography [TE], 2-dimensional magnetic resonance elastography [MRE], acoustic radiation force impulse [ARFI]). All efficacy endpoints will be evaluated at Years 1 and 2, to allow comparisons between shorter and longer treatment intervals with CVC.

2.4. Dosing rationale

A dose of CVC 150 mg (new, improved single-tablet formulation) was selected for CENTAUR to ensure that plasma exposures are sufficient to provide near maximal antagonism of CCR2/CCR5. This was based on the CCR2/CCR5 concentration-dependent antagonism that had been observed in *in vitro* and *ex vivo* studies with CVC, as well as in extensive PK/pharmacodynamic (PK/PD) analyses from two Phase 2 HIV clinical studies of CVC. These two studies confirmed that doses of 100 mg and 200 mg (previous formulation) were effective and well tolerated, resulting in potent CCR2 and CCR5 antagonism [26,27]. Phase 1 studies have indicated that CVC plasma exposures may be higher in non-HIV-infected healthy subjects than in HIV-infected subjects [27,40]. PK data from these studies suggest that CVC 150 mg (new formulation) should provide exposures in subjects with NASH and liver fibrosis that are comparable to those of CVC 200 mg (previous formulation) in HIV-infected subjects. This choice of dose is further supported by PK findings from two multidose Phase 1 studies evaluating CVC 150 mg either in healthy subjects [41], or in patients with mild (Child–Pugh A) to moderate (Child–Pugh B) liver impairment [33].

2.5. Study objectives

The objectives of CENTAUR are shown in Table 1. The primary objective of this study is to assess hepatic histologic improvement in NAS after 1 year of CVC treatment *versus* placebo relative to Screening biopsy. This improvement is defined by a minimum 2-point improvement in NAS, with at least a 1-point improvement in more than one category. There must also be no worsening of NASH CRN fibrosis stage to be considered as improvement of NAS. The key secondary objective of this study is to evaluate the complete resolution of NASH with no concurrent worsening of fibrosis stage after 2 years of CVC treatment *versus* placebo.

2.6. Sample size

Based on a two-sided alpha of 0.05 and a 1:1 randomization, the planned sample size of 252 subjects (equal allocation of 126 subjects to the CVC treatment and placebo groups) was expected to provide at least 80% power to demonstrate superiority of CVC *versus* placebo with respect to the primary endpoint at Year 1 (Fig. 2). These sample-size calculations assumed a 20% response rate for placebo, based on results from the PIVENS and FLINT studies, and a 36% response rate for CVC treatment at the end of Year 1, using PROC POWER in SAS version 9.3 (SAS Institute Inc., USA) with the two-sample Fisher's exact test option. Due to a combination of high unmet need and investigator interest, the study over-enrolled by 15%, with a total of 289 subjects (approximately 145 subjects on CVC and placebo in Year 1). Hence, a revised power calculation table is provided, with 140 subjects receiving CVC and placebo during Year 1 (Table 2). Both updated and original sample size calculations assumed approximately 15% subject attrition over the 2-year duration, where a missing biopsy result is treated as non-response (by comparison, 13% of randomized subjects in PIVENS [39] were missing their post-treatment biopsy). Revised calculations were consistent with original powering assumptions, thereby supporting their robustness.

3. Study procedures

3.1. Recruitment and screening

CENTAUR will be conducted according to all applicable laws and regulations, and an ethics review was conducted according to the Declaration of Helsinki (October 2008), as described in the International Conference on Harmonisation E6 Guideline for Good Clinical Practice and/or local laws. Before enrollment, informed consent to participate in the study was obtained from each subject. The protocol and any information supplied to the subject to gain informed consent was reviewed and approved by a qualified Institutional Review Board (IRB)/

Table 1
CENTAUR study objectives and procedures.

Study objectives	Study timepoint (months)					
	3	6	12	15	18	24
Primary objective						
• Hepatic histological improvement in NAS at Year 1 relative to Screening biopsy (≥2-point improvement in NAS with at least a 1-point improvement in more than one category) with no concurrent worsening of fibrosis[a] (Arm A *versus* Arms B and C)			X			
Key secondary objective						
• Complete resolution of NASH[b] with no concurrent worsening of fibrosis[a] at Year 2 (Arm A *versus* Arm C)						X
Other secondary objectives						
• Complete resolution of NASH[b] with no concurrent worsening of fibrosis[a] at Year 1 (Arm A *versus* Arms B and C)			X			
• Assessment of CVC safety and tolerability over Years 1 and 2	Continuous					
• Plasma PK of CVC in a population PK analysis[c]	X	X	X	X	X	X
• Hepatic histological improvement in NAS at Year 2 relative to Screening biopsy (2-point improvement in NAS with at least a 1-point improvement in more than one category) with no concurrent worsening of fibrosis[a] (Arm A *versus* Arm C)						X
• Change in CPA on liver biopsy to evaluate efficacy of CVC *versus* placebo			X			X
• Improvement in histologic fibrosis stage assessed using NASH CRN and Ishak scoring systems			X			X
• Change in hepatic stellate cell activation marker (α-SMA)			X			X
• Change from Baseline in non-invasive scores and markers of hepatic fibrosis (APRI, FIB-4, hyaluronic acid, FibroTest [FibroSure], NFS, and ELF)		X	X		X	X
• Change from Baseline in biomarkers of hepatocyte apoptosis, assessed using CK-18 caspase-cleaved and total			X			X
• Change from Baseline in liver parameters and fasting metabolite parameters	X	X	X	X	X	X
• Change from Baseline in weight, BMI, waist circumference, waist–hip ratio, arm circumference, and tricep skinfold	X	X	X	X	X	X
Tertiary objectives						
• Change from Baseline in non-invasive liver imaging method (e.g. TE, MRE, and ARFI)[d]		X	X		X	X
• Change from Baseline in pro-inflammatory cytokines and biomarkers of inflammation	X	X	X	X	X	X
• Change from Baseline in eGFR	X	X	X	X	X	X
• Change from Baseline in biomarkers associated with bacterial translocation	X	X	X	X	X	X

α-SMA, α-smooth muscle actin; APRI, aspartate aminotransferase-to-platelet count ratio index; ARFI, acoustic radiation force impulse; BMI, body mass index; CPA, collagen proportionate area; CRN, clinical research network; CVC, cenicriviroc; ELF, enhanced liver fibrosis test; eGFR, estimated glomerular filtration rate; FIB-4, non-invasive hepatic fibrosis risk score; MRE, 2-dimensional magnetic resonance elastography; NAS, non-alcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis; NFS, non-alcoholic fatty liver disease fibrosis score; PK, pharmacokinetics; TE, ultrasound transient elastography.

[a] Worsening defined as progression of NASH CRN fibrosis stage.

[b] Histopathologic interpretation of no fatty liver disease or simple or isolated steatosis with no steatohepatitis.

[c] Plasma samples for population PK analysis will be collected on Day 1 (Baseline) and Months 0.5, 3, 6, 12, 15, 18, and 24.

[d] At sites where available.

Independent Ethics Committee (IEC) prior to subject enrollment. All IRB/IEC requirements will be followed during the study and after its completion.

3.2. Eligibility process

The subjects recruited in this study will need to satisfy the following histological criteria, read by a central pathologist: presence of steatohepatitis (NASH), NAS ≥ 4, and presence of fibrosis Stages 1–3 according to the NASH CRN system. They will also be at increased risk of disease progression due to one or more of the following: documented evidence of T2DM, high body mass index (BMI > 25 kg/m^2) with at least one of the criteria of the metabolic syndrome, as defined by the National Cholesterol Education Program (NCEP), bridging fibrosis (NASH CRN Stage 3) and/or definite NASH (NAS ≥ 5). The detailed inclusion and exclusion criteria for subject eligibility are shown in Tables 3 and 4, respectively.

Table 2
Sample size prediction calculations.

Placebo, N	CVC treatment, N	Placebo response (%)	CVC treatment response (%)	Difference in response (%)	Power[a] (%)
140	140	16	36	20	96
140	140	18	36	18	91
140	140	20	36	16	82
140	140	20	38	18	90
140	140	20	40	20	95

CVC, cenicriviroc.

[a] Power calculated using Fisher's exact test with response for placebo ranging from 16 to 20% and for CVC treatment from 36 to 40%, and differences in response from 16 to 20%.

3.3. Prior and concomitant therapy

CVC is a known substrate of CYP2C8, CYP3A4, and P-glycoprotein, and a weak inhibitor of CYP3A4; therefore, the following classes of medication are disallowed/excluded:

- CYP3A4 — inhibitors/inducers
- CYP2C8 — inhibitors
- sensitive CYP3A4 substrates (e.g. drugs that should not be co-administered with weak CYP3A4 inhibitors, such as CVC)
- P-glycoprotein and breast cancer resistance protein – substrates and inhibitors/inducers
- other medications that may have confounding effects on CVC efficacy.

Pioglitazone, rosiglitazone, vitamin E > 400 IU/day, S-adenosylmethionine, pentoxifylline, and ursodiol are disallowed due to possible confounding effect on efficacy.

Other disallowed medications that are commonly administered to subjects with NASH include the antidiabetic agent saxagliptin, the lipid-lowering agents gemfibrozil and rosuvastatin, and the antihypertensive agents aliskiren, captopril, carvedilol, diltiazem, verapamil, and felodipine.

Medications that are CYP3A4 substrates (e.g. the lipid-lowering agents atorvastatin, simvastatin, pravastatin, and lovastatin) can be used as concomitant treatments in this study, but clinical monitoring and dose titration are recommended to achieve the desired clinical response: CVC is a weak CYP3A4 inhibitor and, therefore, may increase exposure of these medications. Other medications that can be used with caution are intravenous midazolam, alfentanil or fentanyl, and systemic corticosteroids. Antacids, H_2-receptor antagonists and proton-pump

Table 3
CENTAUR inclusion criteria.

Criteria type	Description of inclusion criteria
Sex and age	Males and females; 18–75 years of age
Histological evidence of NASH	Based on Screening biopsy, with a NAS of ≥4 with at least 1 point in each component of NAS
Histological evidence of liver fibrosis	Based on Screening biopsy, defined as NASH CRN system stages 1–3 inclusive
Increased risk of disease progression	Meeting any of the following criteria: • documented evidence of T2DM • high BMI (>25 kg/m^2) with at least one criteria of the metabolic syndrome[a] • bridging fibrosis (NASH CRN stage 3) and/or definite NASH (NAS ≥5)
AST and ALT levels	≤5× ULN
Serum albumin levels	≥3.5 g/dL
eGFR	≥50 mL/min/1.73 m^2 (according to the MDRD equation)
Platelet count	≥100 000/mm^3
Informed consent	Ability to understand and sign a written informed consent form
Use of concomitant medications	Subjects need to be on stable therapy for 30 days prior to the Baseline visit

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRN, clinical research network; eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease; NAS, non-alcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis; T2DM, type 2 diabetes mellitus; ULN, upper limit of normal.
[a] Criteria of the metabolic syndrome, as defined by the National Cholesterol Education Program, are: central obesity, waist circumference ≥ 102 cm or 40 in. (male), ≥88 cm or 35 in. (female); dyslipidemia, triglyceride ≥ 1.7 mmol/L (150 mg/dL); dyslipidemia, high-density lipoprotein-cholesterol <40 mg/dL (male), <50 mg/dL (female); blood pressure ≥ 130/85 mm Hg (or treated for hypertension); fasting plasma glucose ≥6.1 mmol/L (110 mg/dL).

inhibitors should be administered at least 2 h after administration of study drug, as CVC absorption is enhanced with low gastric pH [42].

3.4. Randomization

Eligible subjects will be assigned to either CVC treatment or placebo at Day 1 (Baseline) using permuted block randomization stratified by NAS at Screening (4 or ≥5) and fibrosis stage (≤2 or >2). Subjects will be randomized in a 2:1:1 ratio to CVC 150 mg once daily (QD) for 2 years (Arm A), placebo QD for 1 year then CVC 150 mg QD for 1 year (Arm B), or placebo QD for 2 years (Arm C), as shown in Fig. 2.

3.5. Study drug administration and blinding

Subjects will be instructed to take one tablet (CVC or matching placebo) every morning with food for 2 years of treatment. The subjects, sponsor, investigators, and personnel involved in the study will be blinded to the individual assignments of CVC and placebo until all subjects have completed the 2-year study and the database has been locked for all study data. The sponsor will provide CVC and matching placebo that are visually indistinguishable in order to ensure blinding of these products.

4. Study procedures

The schedule of assessments for the study procedures is summarized in Table 1.

Table 4
CENTAUR exclusion criteria.

Criteria type	Description of exclusion criteria
Liver co-morbidities	• History of cirrhosis and/or hepatic decompensation including ascites, hepatic encephalopathy, or variceal bleeding • HBsAg positive • HCVAb positive, with two exceptions if all other eligibility criteria are met. Subjects previously treated for viral hepatitis C with at least a 1-year period since documented sustained virologic response at Week 12 (post-treatment) Subjects with the presence of HCVAb but negative hepatitis C virus RNA without treatment (i.e. spontaneous clearance)
Other co-morbidities	• Other known causes of chronic liver disease, including alcoholic liver disease • Prior or planned liver transplantation • Clinically significant cardiovascular or cerebrovascular disease within the past 3 months, including, but not limited to, myocardial infarction, acute coronary syndrome, revascularization or ischemic stroke, or implanted defibrillator or pacemaker • Any Grade ≥ 3 laboratory abnormality[a], with exceptions unless clinical assessment foresees an immediate health risk Subjects with pre-existing diabetes or with asymptomatic glucose elevations Subjects with Grade ≥ 3 dyslipidemia with triglyceride or cholesterol elevations Subjects with asymptomatic Grade ≥ 3 creatine kinase elevations
Lifestyle	• HIV-1 or HIV-2 infection • History of malignancy within the past 5 years or ongoing malignancy other than basal-cell carcinoma, or resected non-invasive cutaneous squamous-cell carcinoma • Active, serious infections that require parenteral antibiotic or antifungal therapy within 30 days prior to Screening visit • Alcohol consumption > 21 units/week for males or >14 units/week for females • Positive urine screen for amphetamines, cocaine, or opiates at Screening, with the exception of medical treatment with opiates or amphetamines for co-morbidities
Concomitant treatments	• Weight reduction through bariatric surgery in the past 5 years or planned during the conduct of the study • Current or anticipated treatment with radiation therapy, cytotoxic chemotherapeutic agents or immunomodulating agents • Receiving ongoing therapy with any disallowed medication from Screening onwards • Receiving any experimental medications within 30 days prior to Screening or anticipated use during the trial
Other	• Females who are pregnant or breastfeeding • Allergy to the study drug or its components • Participation in any other clinical trial at Screening without approval from the Sponsor • Any other clinically significant disorders or prior therapy that would make the subject unsuitable for the study or unable to comply with the dosing and protocol requirements

HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; HIV, human immunodeficiency virus.
[a] As defined by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.03 Toxicity Grading Scale.

4.1. Screening procedures

At Screening, informed consent will be obtained and eligibility for the study will be confirmed. Eligibility will be analyzed by obtaining demographic information, medical history, and a liver biopsy, undertaking safety evaluations (as described in safety assessments), performing a urine drug screen and drawing blood to determine any presence of HIV-1 or -2 antibodies, hepatitis B surface antigen and/or hepatitis C virus antibody. The liver biopsy will be read by a central pathologist, blinded to treatment, and used to assess NAS, histologic fibrosis stage (NASH CRN and Ishak scoring systems), collagen morphometry (collagen proportionate area [CPA]) and α-SMA. An historical biopsy (obtained ≤180 days prior to Screening) can be used if the subjects were metabolically stable following the procedure and have had no new therapeutic intervention for NASH. Year 1 and 2 biopsies will be performed within 1 month prior to the end of the respective year, and all efforts will be made to obtain a Year 1 liver biopsy for each study subject to allow comparison with the Year 2 biopsy. In the event that obtaining a liver biopsy at Year 1 is not feasible, subjects will be allowed to proceed to Year 2 and will subsequently undergo liver biopsy at the end of Year 2. Subjects with missing post-baseline biopsies will be imputed as non-responders for Year 1 and 2 primary and key secondary efficacy endpoints. Additional imputation methods will be utilized to perform sensitivity analyses. These biopsies will be centrally read by the same expert pathologist blinded to treatment, for assessment of primary and secondary histological outcomes.

4.2. Safety assessments

The following safety evaluations will be undertaken for every subject visit from Screening until 1-month follow-up (shown in Table 1): assessment of adverse events (AEs) and concomitant medication use, physical examination, vital-signs measurements, hematology and serum chemistry laboratory tests, urinalysis, and urine pregnancy tests. BMI determination and estimated glomerular filtration rate (eGFR) will also be assessed at Screening and in Months 0 (Baseline), 3, 6, 12, 15, 18, and 24. The following safety evaluations will also be undertaken for Months 0, 3, 6, 12, 15, 18, and 24: 12-lead electrocardiogram (ECG), measurement of biomarkers of hepatocyte apoptosis (CK-18), inflammation and bacterial translocation, and fasting metabolic parameters.

4.3. Physical assessments, measurements, and laboratory tests

A complete physical examination will be undertaken at Screening and Baseline. Furthermore, a symptom-directed physical examination will be performed as needed for every subject visit from Screening until 1-month follow-up. Vital signs will be performed with the subject in the seated position, after 5 min of rest, and before any blood draws.

Table 5
Classifications of AE intensity [43].
AEs will be graded according to the criteria in this table, which is from the NCI CTCAE version 4.03 Table for Grading the Severity of Adult Adverse Events.

Grade	Description
Grade 1 (mild)	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2 (moderate)	Minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL
Grade 3 (severe)	Medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
Grade 4 (life-threatening)	Life-threatening consequences; urgent intervention indicated
Grade 5 (death)	Death related to AE

ADL, activities of daily living; AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events; NCI, National Cancer Institute.

Urinalysis will be undertaken and analyzed using standard procedures by a central laboratory, and will include determination of urine albumin/creatinine ratio and urine β_2-microglobulin. Urine pregnancy tests will be undertaken using a dipstick method. Hematology and serum chemistry laboratory tests will be performed by a central laboratory using standard procedures, and any abnormal results that are possibly related to study drug treatment will be reported weekly until the abnormality is resolved or is otherwise explained. At Screening, the height and weight of subjects will be measured to calculate BMI, and subsequently the waist circumference, hip circumference, arm circumference, and triceps skinfold will also be measured. The eGFR will be calculated by the central laboratory using the Modification of Diet in Renal Disease formula, the Cockcroft-Gault equation, the Chronic Kidney Disease Epidemiology Collaboration formula (based on cystatin C [mg/L] and serum creatinine), and adjusted for age, sex, and race. ECGs will be performed with the subject in the supine position, after 5 min of rest, and before any blood draws. Biomarkers of hepatocyte apoptosis, inflammation and bacterial translocation, and fasting metabolic parameters will be undertaken by a central laboratory.

4.4. Adverse events

All AEs during the study will be recorded in the electronic case-report form, with information about the date of onset and end date (if applicable), severity and seriousness of the AE, investigator's opinion of the relationship to CVC treatment, action taken regarding CVC usage and treatment for AE, cause of event (if known), and information regarding the resolution or outcome. AEs classified as serious will be recorded on a serious-adverse-event reporting tool and reported to the sponsor. The intensity of an AE will be graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.03, which includes the classifications of AE intensity shown in Table 5.

4.5. Pharmacokinetic analyses

Plasma samples for population PK analysis will be collected according to the schedule stated in Table 1. For each study visit, all subjects will take their daily dose during the visit under witnessed dosing. At Months 0.5, 3, and 15, one pre-dose sample and one post-dose sample will be collected; on all other visits one pre-dose sample will be collected. A plasma sample for PK will also be taken, preferably within 24–48 h of the last dose of study drug if a subject discontinues the treatment early due to an AE.

4.6. Other study procedures

For the study visits at Months 0, 3, 6, 12, 15, 18, and 24, subjects must have fasted for at least 8 h prior to the visit. Those who have not fasted will be required to return after fasting within 72 h and before blood is drawn for metabolic assessments. Subjects who discontinue study treatment before the 2 years will attend an early discontinuation visit within 48 h of withdrawal. Follow-up evaluations will be conducted on subjects approximately 1 month after their last dose of study drug, including those who discontinue the treatment early. A liver biopsy will be performed within 1 month of discontinuation, if possible, from subjects who have received treatment with the study drug (CVC or placebo) for at least 6 months during Year 1 or Year 2.

4.7. Criteria for discontinuation of study treatment

Study treatment must be discontinued in cases of suspected drug-induced liver injury, unacceptable toxicity, acute viral hepatitis types A, B, C, D, or E, autoimmune or alcoholic hepatitis, hypoxic or ischemic hepatopathy, biliary tract diseases, or pregnancy. Treatment would

also cease if the subject requests discontinuation or if the study is discontinued.

5. Outcome measures

5.1. Efficacy evaluation

The efficacy endpoints for this study are shown in Fig. 3.

5.2. CVC PK evaluation

The population PK endpoints in this study include the characterization of the population PK of CVC in subjects with NASH, and prediction of plasma CVC exposure over the duration of the study. A further population PK endpoint to be evaluated is the covariates that impact CVC PK (e.g. age, sex, weight, BMI, race, fibrosis stage).

5.3. Safety evaluation

The safety and tolerability of CVC will be evaluated over the 2 years of treatment in subjects with NASH. This will include the evaluation of AEs, clinical laboratory tests, physical examination, vital signs, and 12-lead ECG. The clinical laboratory tests will include liver and fasting metabolic parameters. Liver parameters will include alkaline phosphatase, alanine aminotransferase, serum albumin, aspartate aminotransferase, direct bilirubin, total bilirubin, and gamma-glutamyl transferase.

5.4. Statistical analyses

The primary efficacy endpoint will be analyzed by comparing CVC (treatment Arm A) *versus* placebo (combined treatment Arms B and C) treatment groups at the end of Year 1 (Fig. 2) for all randomized subjects who received at least one dose of study drug and had a measurable Screening biopsy. The sample size for the primary endpoint was based on the primary binary endpoint comparing treatment Arm A *versus* combined treatment Arms B and C. This comparison will be performed using logistic regression, with the Cochran–Mantel–Haenszel test used for sensitivity analysis, and stratification will be based on the randomization strata. Treatment differences will be assessed and include estimates and 95% confidence intervals for the stratification adjusted difference between the response rates for CVC and placebo and the stratification adjusted odds ratio for the comparison between CVC and placebo. Subjects with missing post-baseline biopsies will be imputed as non-responders for Year 1 and 2 primary and key secondary efficacy endpoints. Additional imputation methods will be utilized to perform sensitivity analyses. However, subjects that discontinue early who have had >6 months of treatment in Year 1 or 2 will be included in the efficacy analysis if a biopsy can be obtained within 1 month of discontinuation. A sensitivity analysis will be performed for all subjects with available biopsy data. If the primary endpoint is achieved with statistical significance, the key secondary endpoint will be tested using logistic regression, with a similar Cochran–Mantel–Haenszel test used for sensitivity analysis. Analysis of endpoints at Year 2 will mainly compare subjects who are randomized to and treated with CVC or placebo for 2 years (i.e. Arms A and C; Fig. 4).

Secondary efficacy endpoints will be described using both descriptive statistics and two-sided 95% confidence intervals for observed data.

A population PK analysis of CVC will be performed using pre-dose and random PK samples collected throughout the study. Measured plasma CVC concentrations will be used to conduct non-linear mixed effects modeling of population PK. Subject covariates will be analyzed and exploratory exposure-response (PK/PD) analyses may be conducted on efficacy and/or safety endpoints of interest.

The occurrence of treatment-emergent AEs will be summarized by treatment group using Medical Dictionary for Regulatory Activities preferred terms, system-organ classifications, and severity. Clinical laboratory tests will be presented as descriptive summaries by study visit. Laboratory abnormalities will be graded according to NCI CTCAE version 4.03. The number and percentage of subjects that experience treatment-emergent graded toxicities will be summarized by treatment group and severity grade. Laboratory toxicity shifts from Baseline to post-Baseline assessments and changes from Baseline in laboratory tests will be summarized for each treatment group. Any abnormal findings considered clinically significant will be recorded as AEs, or noted as medical history if already present at Screening. Results of ECGs will be reviewed for clinically notable abnormalities according to predefined criteria, and patients exhibiting Grade 3 or 4 PR or QTc interval will be summarized. The number and percentage of subjects taking concomitant medications will be summarized using World Health Organization preferred terms and drug classifications.

6. Discussion

The key endpoints of CENTAUR are based on those initially proposed by the AASLD in 2011, and later discussed in a joint meeting between the AASLD and the FDA in 2013, and those used in the PIVENS, FLINT and GOLDEN studies [35–37,39,44]. Liver histology endpoints, such as complete resolution of NASH, are considered surrogates for preventing cirrhosis (i.e. they are thought to predict clinical benefit, but are not direct measures of it) [36]. However, considering that morbidity and mortality are endpoints that would require a large time frame and sample size to determine, these surrogates provide short-term methods that are reasonably likely to predict clinical benefit in preventing cirrhosis and liver-related death. The primary endpoint, "drop in NAS by ≥2 with at least a 1-point improvement in more than 1 category and with no concurrent worsening of fibrosis stage (Year 1)", has the advantage of being measurable, sensitive to change and treatment effect, and can be quantified consistently [35,36]. However, the degree to which

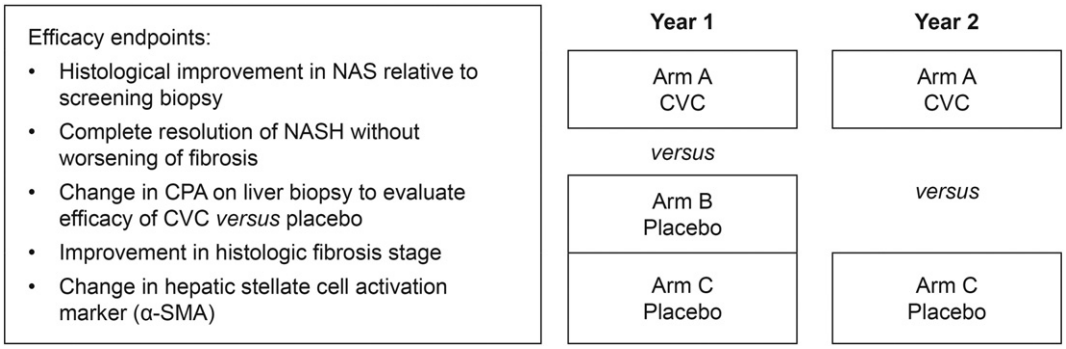

Fig. 4. CENTAUR treatment group comparisons in Years 1 and 2. The main efficacy endpoints are listed in the left panel. The treatment groups that will be compared for these efficacy endpoints in Years 1 and 2 are shown in the right panel. α-SMA, α-smooth muscle actin; CPA, collagen proportionate area; CVC, cenicriviroc; NAS, non-alcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis.

improvement in NAS correlates with a clinically significant decrease in the risk of cirrhosis or mortality, or the degree to which different components of NAS affect the development of cirrhosis, remain unknown [35]. The key secondary endpoint, "complete resolution of NASH (histopathologic interpretation of no fatty liver disease or simple or isolated steatosis with no steatohepatitis) with no concurrent worsening of fibrosis stage (worsening defined as progression of NASH CRN fibrosis stage) (Year 2)", is expected to be associated with reduced risk of cirrhosis, making it an approvable surrogate endpoint [35,36]. Although there are limited data to confirm that reversal of steatohepatitis prevents cirrhosis, studies suggest that steatohepatitis is associated with a substantial long-term risk of developing cirrhosis [7,35,36].

A disadvantage of using histological methods for assessing clinical benefit is that liver biopsies are invasive, painful, subject to sample variability, and can occasionally lead to serious complications [36]. Currently, the non-invasive markers that are used to assess NASH progression are not robust enough to replace liver biopsy, but may be useful for supporting efficacy [35,36]. Various non-invasive markers of NASH progression (Table 1) will be included as secondary endpoints to allow comparison against the histological endpoints.

The study population in CENTAUR has been enriched to select for subjects with NASH and liver fibrosis (i.e. at higher risk of progression to cirrhosis), and there is broad consensus that those with the greatest risk of cirrhosis are particularly important to target [35]. Therefore, the inclusion criteria contain presence of fibrosis and risk factors for fibrosis progression, including presence of steatohepatitis, T2DM, and high BMI (Table 3) [7,35,36,45]. Since the majority of NASH patients will not progress to cirrhosis, it is important to reduce the heterogeneity of the study population, yet have a large enough histological spectrum to allow the findings to be relevant for a wide range of subjects with NASH [36]. This disadvantage of a heterogeneous study population was a feature of the GOLDEN study, where subjects with early NASH had an unexpectedly high rate of NASH resolution in the placebo group, and the primary endpoint of the study could only be met after correcting for baseline severity [44]. Using a high-risk population with early-stage NASH and fibrosis in CENTAUR will reduce the heterogeneity in the study groups, allowing the detection of a greater difference between CVC treatment and placebo. In contrast, the PIVENS, FLINT and GOLDEN studies included subjects mostly based on the presence of steatohepatitis and NAS score, and neither required liver fibrosis for eligibility nor enriched for other risk factors associated with fibrosis progression [37,39,44]. CENTAUR will also allow the assessment of the safety and tolerability of CVC in a larger population of subjects with NASH. CENTAUR is the first study that has specifically targeted subjects at higher risk of cirrhosis, who are likely to benefit most from improvement in NASH and prevention of fibrosis progression.

7. Summary

CENTAUR is a Phase 2b, randomized, double-blind, placebo-controlled, multinational study for the treatment of NASH and liver fibrosis. The study will evaluate the efficacy and safety of CVC 150 mg over 2 years with a primary endpoint at Year 1. The primary endpoint of this study is the histological improvement in NAS at Year 1 relative to Screening biopsy, with no worsening of fibrosis stage. The key secondary endpoint, which is the complete resolution of NASH with no concurrent worsening of fibrosis stage, a suitable endpoint for accelerated approval, will be analyzed at Year 2. Of 812 subjects screened, 289 were enrolled in 11 countries (166 in the USA, 94 in the European Union, 29 in the Asia-Pacific region). Results of this study will allow the comparison of the benefits of shorter *versus* longer treatment with CVC, the correlation between improvement in inflammation and fibrosis, and evaluation of the progression of NASH at Year 1 and 2 in the placebo group.

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