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ABSTRACT

Background

Non-alcoholic steatohepatitis (NASH), a multifactorial disease, can progress to hepatic fibrosis and cirrhosis. The Peroxysomal Proliferator-Activated Receptors, PPARα, β/δ and γ, play a central role in the regulation of glucose and lipid metabolism and of the inflammatory and fibrogenic pathways in liver and in other organs that all contribute to NASH pathogenesis. Lanifibranor (IVA337), a panPPAR agonist, by acting on these three different PPAR isotypes, combines pharmacological effects that could address the different components of the disease as demonstrated in preclinical models.

Objectives

NATIVE study (EudraCT: 2016–001979-70, NCT: NCT03008070) aims to assess the safety and the efficacy of a 24-week treatment with lanifibranor (800 and 1200 mg/day) in adult non-cirrhotic NASH patients. The primary efficacy endpoint is a 2-point reduction in the activity part of the Steatosis Activity Fibrosis (SAF) histological score (combining inflammation and ballooning) without worsening of fibrosis.

Design

NATIVE is a Phase 2b randomised, placebo-controlled, double-blind, parallel-assignment, dose-range study. Eligible adult patients with a confirmed histological diagnosis of NASH should have a SAF Activity score of 3 or 4 (> 2) and a SAF Steatosis score ≥ 1. There is no specific criterion related to the fibrosis score except that patients with cirrhosis (F4) were excluded.

Summary

This study will evaluate the efficacy of a 24-week treatment of NASH with lanifibranor based on histological evaluations (SAF score) by biopsy. The number of responders according to the SAF Activity score-based definition from baseline to 24 weeks will be compared between groups and serves as primary endpoint.

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1. Introduction

Non-alcoholic Fatty Liver Disease (NAFLD) is defined by the presence of hepatic steatosis in the absence of significant alcohol consumption or causes other than the metabolic disorders constituting the metabolic syndrome. Non-alcoholic steatohepatitis (NASH), its more severe and potentially progressive subtype, is characterised by steatosis, lobular inflammation and liver cell damage, the latter histologically evidenced by ballooning at liver biopsy with or without associated hepatic fibrosis, which can lead to cirrhosis [1–4]. NASH harbours a risk for hepatocellular carcinoma and has extrahepatic consequences, including its strong association with cardiovascular disease and diabetes. The prevalence of NAFLD is estimated to be about 25% in the global adult population [3] and up to one fifth of NAFLD patients (around 21%) would have NASH [5].

The pathogenesis of NASH is complex with multiple pathogenic drivers for disease progression. Insulin resistance (IR) favours the pathologic evolution from normal to fatty liver [6] while many other factors contribute to the development of steatohepatitis along with inflammation and fibrosis [7–9]. Life-style interventions aimed at improving obesity and/or associated features of metabolic syndrome is widely recommended as the cornerstone of treatment of NAFLD/NASH. There are currently no FDA or EMA approved pharmacologic therapies licensed for the treatmentof NASH [2].

Peroxysomal Proliferator-Activated Receptors (PPARs) play an important role in glucose, lipid and energy metabolism and are also key regulators of inflammation and fibrogenesis [8,9]. Three PPAR isotypes have been identified—α, β/δ and γ—the expression and actions of which differ according to isotype, organ and intra-organ cell-type [10].

PPARα is predominantly expressed in tissues with a high rate of fatty acid oxidation [11]. PPARα regulates fatty acid transport, peroxisomal and mitochondrial β oxidation and lipolysis. PPARβ/δ plays an important role in glucose, lipid metabolism and inflammation [12]. It improves energy metabolism and IR in skeletal muscle. PPARγ, highly expressed in adipose tissue, is important in the regulation of adipocyte differentiation, adipogenesis and lipid metabolism [8]. PPARγ activation prevents the increased flux of free fatty acids and adipokines from the adipose tissue to other organs, especially to the liver [13].

NASH activity, which can fluctuate over time, influences the disease evolution. Fibrosis, a consequence of longstanding necroinflammation, is the strongest predictor of liver-related morbidity and mortality [14,15]. Besides their effects on metabolism, PPARs, through various mechanisms of action, may influence necroinflammatory and fibrogenic processes [10,16–19]. Therefore, combining PPARα, PPARβ/δ, and PPARγ activation may bring an innovative and efficacious therapeutic approach by targeting a large array of disturbances that contribute to the development, progression and consequences of NASH.

Lanifibranor is a non-thiazolidinedione (TZD), non-fibrate PPAR agonist that targets the 3 PPAR isotypes with a well balanced potency [20]. Lanifibranor exerts positive metabolic and anti-fibrotic effects in animal models [20,21]. These data, along with clinical data in healthy volunteers and patients with diabetes [22] gave rationale to the NATIVE study (EudraCT: 2016–001979-70, NCT: NCT03008070), a Phase 2b study, to test lanifibranor *versus* placebo for the treatment of patients with biopsy-confirmed NASH. This clinical study assesses the safety and the efficacy of a 24-week treatment of lanifibranor (800 or 1200 mg/day) in adult NASH patients without cirrhosis.

2. Methods

2.1. Study rationale

2.1.1. Role of the three PPAR isotypes in the pathophysiology of NASH

There is a complex inter-organ cross-talk implicated in NASH pathogenesis as such, but also in the set of intra- and extrahepatic consequences of NASH, which needs to be seen as part of a systemic disease [8,23]. Furthermore, dysmetabolic, inflammatory and fibrogenic processes are intimately linked. PPAR isotypes were reported to play directly or indirectly a role in those NASH-associated pathways.

The expression and actions of PPAR α,β/δ and γ differ according to isotype, organ and intra-organ cell-type, resulting in a complex system of nuclear receptor-mediated inter-organ crosstalk [9].

PPARα binds saturated fatty acids and is predominantly expressed in tissues with a high rate of fatty acid oxidation [11]. PPARα activation leads to reduction of triglyceride-rich lipoproteins and triglyceride accumulation in the liver, whereas plasma high-density lipoprotein cholesterol (HDL−C) increases. In the liver, PPARα is expressed mainly in hepatocytes but also in various other cell types including endothelial cells and to some extent in hepatic stellate cells (HSCs). In preclinical studies, PPARα deficiency leads to more severe NASH lesions whereas PPARα ligands are protective [24,25]. Conversely, PPARα expression in liver tissue of NASH patients is reduced with increasing NASH severity, and improvement of liver histology is associated with increased hepatic PPARα expression [26].

PPARβ/δ is expressed in hepatocytes, sinusoidal endothelial cells, HSCs and Kupffer cells [27]. PPARβ/δ activates pathways of glucose utilisation and *de novo* lipogenesis in the liver [8]. In addition, it increases the production of monounsaturated fatty acids and protects against lipotoxicity and saturated fatty acid cytotoxicity by decreasing inflammasome pathway activity [28]. Targeting selectively PPARβ/δ caused an important dose-dependent increase in plasma HDL-C and decreased plasma triglycerides, low density lipoprotein cholesterol (LDL-C) and insulin levels in insulin-resistant middle-aged obese rhesus monkeys [29].

PPARγ is highly expressed in adipose tissue where it plays an essential role in the regulation of adipocyte differentiation, adipogenesis and lipid metabolism [8]. In metabolic syndrome and obese patients, there is a switch in gene expression within adipocytes to a pattern that more closely resembles that of macrophages, which promotes adipose tissue inflammation and an increased flux of free fatty acids and adipokines from the adipose tissue to other organs and in particular to the liver [13]. This results in ectopic triglyceride accumulation, increased synthesis of toxic lipid mediators and insulin resistance. In NAFLD, the severity of adipose tissue IR has likely an impact on hepatic steatosis severity [30]. The cross-talk between the adipose tissue and the liver plays a pivotal role in the development and progression of steatohepatitis [31]. PPARγ ligands such as TZDs, which improve adipose tissue biology, are beneficial in NAFLD given the dynamic cross-talk between the liver and adipose tissue [13,32].

PPARs play a positive role in the control of fibrogenesis through direct anti-fibrotic effects but also likely through their impact on inflammation and on dysregulated metabolism. Concerning the direct effects on fibrogenesis, PPARα decreases the expression of dermatopontin, which is a protein involved in fibrinogenesis and collagen deposition [17]. PPARγ maintains HSCs in quiescent state and its overexpression decreases their myofibroblastic character, resulting in reduced collagen production [19]. Inflammation is a trigger of fibrosis and PPARs have anti-inflammatory functions that may participate to their antifibrotic effects. PPARα has anti-inflammatory properties [16], mainly by transrepression of pro-inflammatory target genes [10]. PPARβ/δ modulates the expression of key genes involved in innate immunity and inflammation and also activates Kupffer cells toward a more anti-inflammatory phenotype, which results in less severe metabolic and hepatic disorders [18]. The upstream metabolic dysregulation leads to steatosis and possibly to fibrogenesis also. As mentioned before, PPARs are involved in the control of lipidic and glucidic metabolism.

2.1.2. Clinical data on PPAR ligands in NASH

PPAR ligands for each isotype have been tested in several animal models of steatohepatitis and fibrosis wherein they demonstrated the amelioration of histologic features of chronic liver injury through their intra- and extrahepatic effects. In a small pilot study in patients with

biopsy-proven NASH ($n = 16$), the PPARα ligand fenofibrate improved metabolic parameters and decreased ballooning but not steatosis, inflammation or fibrosis after 48 weeks of treatment [33]. Although the primary endpoint was not met, it was observed in *post-hoc* analyses that the dual PPARα,β/δ ligand elafibranor was superior to placebo in improving steatohepatitis in patients with high baseline NAFLD activity score (NAS ≥ 4) in the Phase 2b GOLDEN-505 study of 274 non-cirrhotic NASH patients [34]. Furthermore, patients with NASH improvement also improved fibrosis [34]. However GENFIT has announced recently that elafibranor fail to meet the primary endpoint of resolution of NASH without worsening of fibrosis during interim analysis on the intention-to-treat population in a Phase 3 trial [35]. No further details have been communicated or results published so the true impact of elafibranor on liver histology cannot be fully judged and and its eventual impact on clinical outcomes are continuing. Elafibranor is preferentially acting on PPARα with a potency about 10-fold higher than on PPARβ/δ receptors. This should be analysed in regards to the effect of seladelpar, a very specific and potent PPARβ/δ ligands on which Cymabay reported preliminary results showing dose-dependent increase in the percentage of patients with fibrosis improvement and NASH resolution in a one year study [36]. The PPARγ ligand pioglitazone, but not rosiglitazone, improved NASH with a trend toward fibrosis improvement as revealed by recent meta-analyses wherein pioglitazone significantly reduced mean fibrosis score in NASH according to aggregate data from three randomised controlled studies [37,38]. Pioglitazone also prevents the progression from prediabetes to diabetes, which affects many NAFLD patients and would delay clinical morbidity and mortality in these patients [39].

NASH patients have a higher risk of cardiovascular events [40]. PPARα ameliorates endothelial dysfunction and regulates multiple pathways involved in atherosclerosis [41]. PPARα agonism decreases LDL-C, Apolipoprotein B (ApoB), triglycerides and increases HDL-C in humans, an effect that is shared by PPARβ/δ [42]. In the liver, the activation of PPARα and γ receptors has shown protective effects on the development of portal hypertension and associated fibrosis in rodent models [43,44]. Several studies have reported the positive effects of PPARs on the cardiovascular risk in dyslipidaemic and diabetic patients. In diabetic patients, a significant prevention of microvascular events as retinopathy progression was reported in the FIELD study with fenofibrate [45] and a reduction of macrovascular events as stroke, coronary symptoms and myocardial infarction in the PROactive [46] and IRIS [47] studies with pioglitazone.

Positive therapeutic benefits from PPAR agonists in general need however to be balanced with their long-term safety and tolerability. Class effects on the increased risk of heart failure, fluid retention/oedema and bone fractures with TZDs were documented [48,49]. Pioglitazone is not widely used because of safety concerns such as risks of cardiac decompensation in patients with pre-existing myocardial dysfunction although overall cardiovascular prognosis improved [50]. Weight gain of 2–4% of body weight has been reported with TZDs in NASH and in T2DM patients, which was reversible upon treatment discontinuation. This weight gain was associated with a shift of fat from visceral adipose tissue and ectopic fat depots to the subcutaneous adipose tissue (hence resulting in a more healthy fat distribution pattern) [51]. Male T2DM patients treated with pioglitazone have been reported to be at a higher risk to develop bladder cancer [51]. This risk has, however, not been confirmed as other meta-analyses observed no link between long-term use of pioglitazone and bladder malignancies [52].

2.1.3. Lanifibranor in NASH

Targeting simultaneously all PPARs can potentially contribute to a more efficacious treatment of NASH and fibrosis. Lanifibranor could be a promising treatment as compared to other dual or panPPAR agonists, lanifibranor activates the three receptors isotypes in the same range of concentrations and doses *in vitro* and *in vivo* in preclinical testing [20].

Lanifibranor, as a well-balanced panPPAR agonist, showed beneficial effects on lipid and glucid metabolism, on inflammation and fibrosis according to preclinical models [20]. Lanifibranor administered orally from 3 to 100 mg/kg showed activity in various rodent models of IR and diabetes as the genetic leptin receptor knock-out (KO) db/db mice and the Zucker diabetic fatty (ZDF) rat or the foz/foz mice model as well as in the inbred polygenic inherited metabolic syndrome WOKW rats and in the diet-induced IR mice, which all share common features withhuman metabolic disorders such as obesity, dyslipidaemia, impaired glucose tolerance and hyperinsulinism and diabetes.

Lanifibranor exerted preventive effects in rodent animal models of liver or other organ fibrosis and reversal effects were also observed in animal models with established liver fibrosis [20]. In the high fat diet foz/foz mice model, lanifibranor 30 mg/kg completely normalised fasting glycaemia, insulin adiposity index and serum triglycerides, increased adiponectin and dose-dependently reduced steatosis, ballooning, and inflammatory foci. In the methionine choline deficient diet (MCD) mouse model it prevented steatosis and inflammation while significantly reducing plasma alanine aminotransferase (ALT) levels. It also inhibited the induction of (pro)fibrotic genes, such as transforming growth factor beta (TGFβ), alpha smooth muscle actin (α-SMA), tissue inhibitor of metalloproteinase 1 (TIMP1) and collagen 1. Lanifibranor inhibited the carbon tetrachloride (CCl_4)-induced liver fibrosis in mice in a prophylactic and therapeutic model. It decreased the expression of (pro)fibrotic and inflammasome genes while increasing the expression of β-oxidation-related and fatty acid desaturation-related genes in both the MCD and the foz/foz models. In mice fed with a choline-deficient, amino acid-defined high-fat diet (CDAA-HFD), lanifibranor 30 mg/kg/day administered after 6 weeks significantly improved all histological features of steatohepatitis, including liver fibrosis, and reduced the hepatic triglyceride and hydroxyproline content. Infiltrating hepatic monocyte-derived macrophages (MoMF) and monocytes were reduced following treatment [53]. *In vitro* bone marrow-derived macrophages stimulation with palmitic acid induced the expression of pro-inflammatory and lipid metabolism genes. Lanifibranor treatment uncoupled these pathways, as lipid metabolic genes were upregulated and inflammation dampened. For the models wherein it was compared, lanifibranor displayed an antifibrotic efficacy superior to each selective PPARα, PPARβ/δ, or PPARγ agonists administered alone. In human primary HSCs, lanifibranor prevented fibrosis development, reversed fibrosis progression and inhibited proliferation and activation of human HSCs, the key cells driving liver fibrogenesis in NASH [20].

Lanifibranor also improved portal hypertension and hepatic fibrosis in an experimental rat model of advanced chronic liver disease [54]. Cirrhotic rats administered with Thioacetamide (TAA) receiving lanifibranor at 100 mg/kg showed significantly lower portal pressure than vehicle-treated animals with no significant changes in portal blood flow, thus indicating improved intrahepatic vascular resistance. In accordance with that, ascites was absent in most animals treated with lanifibranor. No effects on systemic haemodynamics were observed. In addition, lanifibranor-treated rats showed markedly reduced hepatic inflammation, improved phenotype of liver sinusoidal endothelial cells and HSCs and significant fibrosis regression.

This preclinical evidence of beneficial effects of lanifibranor on glucose and lipid metabolism, against the accumulation of fatty acid leading to steatosis, on the associated inflammation and on fibrosis clearly suggest that combined PPARα, β/δ and γ agonism with agents such as lanifibranor may be a promising approach for the treatment of NASH and warrant further study. Long-term toxicological studies including carcinogenicity studies have confirmed its good safety profile with no signs of cardiac and muscular safety issues as reported for some other PPARα and γ ligands.

2.2. Study design

NATIVE is a randomised, placebo-controlled, double-blind, parallel assignment, dose-range, multi-centre study. This Phase 2b study is the

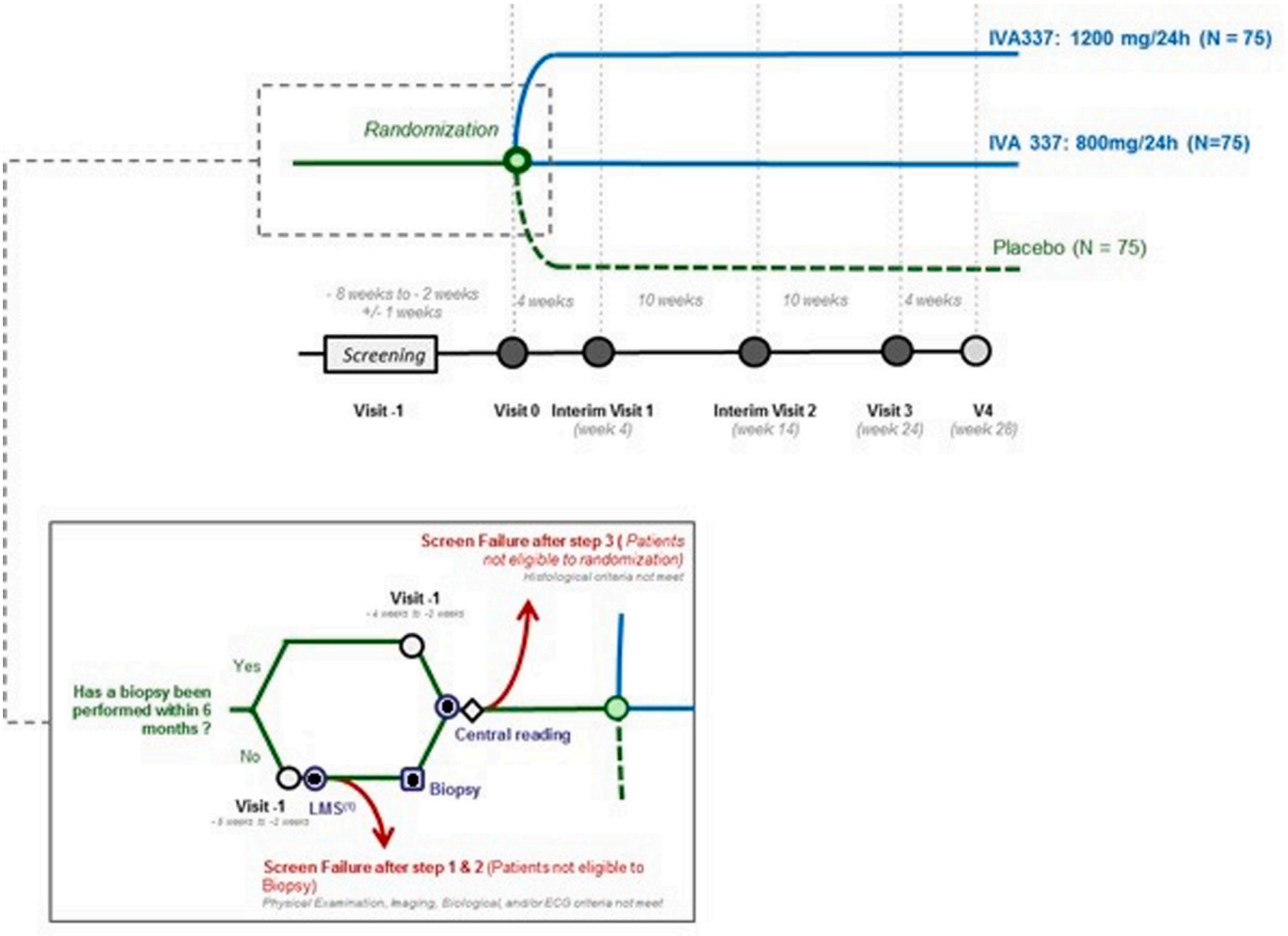

Fig. 1. Design, screening processes and conduct of NATIVE study.
IVA337: Lanifibranor; LMS: LiverMultiScan; , : Visits with physical examination, blood samplings, datarecords; : Visits with additional imagerie procedures performed (Fibroscan® and if available LiverMultiScan); : Visits wherein liver biopsy is also performed.

first trial exposing biopsy proven NASH patients to lanifibranor.

The randomisation ratio is 1:1:1 for placebo, lanifibranor 800 mg/day and 1200 mg/day arms. Type 2 diabetes mellitus (T2DM), an important risk factor contributing to NASH pathogenesis, is a stratification factor applied to balance the assignment of patients to the 3 arms (see Fig. 1). Placebo is chosen as comparator in the absence of approved and licensed therapy for NASH.

2.2.1. Choice of the primary endpoint

In NATIVE study, the primary endpoint is a decrease from baseline of at least 2 points in the activity (A: ballooning + inflammation) component of the SAF (Steatosis, Activity, and Fibrosis) score developed by the FLIP (Fatty Liver: Inhibition of Progression) consortium [4,55].

Hepatic enzymes ALT, AST and γGT and as well as effect on steatosis on imaging, which are frequently used as surrogate primary parameters of efficacy in proof of concept Phase 2 studies during the early development of different drugs, were not chosen as primary endpoints in NATIVE study as these surrogate endpoints are not always good predictors or representatives of disease activity in NASH [49–51]. In the absence of validated markers, only histological assessment through biopsy remains the gold standard for assessment of NAFLD severity and treatment efficacy [56]. Semiquantitative scoring of steatosis, ballooning, lobular inflammation and fibrosis captures the most discriminative histological characteristics. The SAF scores and NAFLD Activity Score (NAS) are considered clinically useful histopathological measures by the AASLD and the EASL.

The severity of hepatocellular damage and inflammation, and particularly the presence of ballooned hepatocytes, is a strong predictor for the presence of hepatic fibrosis and the risk for fibrosis progression (or regression) is correlated with the chronicity and severity of hepatic damage and inflammation [57,58]. Indeed, *post-hoc* analyses in the GOLDEN-505 study demonstrated that the subgroup of elafibranor-treated patients who had NASH resolution had significant reduction in fibrosis, whereas this was not the case in non-responders [34].

SAF activity scoring A, which is used here to assess lanifibranor efficacy in the primary endpoint definition, combines a score for ballooning and lobular inflammation, whilst steatosis is reported separately [55] contrarily to NAS score, which include steatosis in one global score together with lobular inflammation and ballooning [59]. A 2-point reduction in the latter NAS score is frequently used as histological endpoint. This score is a composite grade of 0–8 points with allowance of up to 3 points for severity of hepatic steatosis, a histologic features which does not characterise cell damage and inflammatory injury. SAF scoring reports steatosis separately (S) and the Activity (A) component of the score only combines ballooning and lobular inflammation. Furthermore, SAF Activity scoring attributes equal weight to inflammation and ballooning (score of 0 to 2 for each of them) while NAS scoring gives more importance to inflammation (score of 0 to 3) compared with ballooning (score of 0 to 2),. SAF scoring of hepatocyte

Table 1
. Comparison between SAF and NASH CRN on the grading of hepatocytes ballooning. Table 1 NAS: Non alcoholic fatty liver disease Activity Scoring, a scoring system developed by NASH CRN (or Non alcoholic steatohepatitis Clinical Research Network); SAF: Steatosis Activity Score, a scoring system proposed by the FLIP (Fatty Liver Inhibition of Progression) consortiumScreening steps are realized from week −4 to −2 before inclusion in the study if liver biopsy available.

	Scoring of hepatocytes ballooning	
	SAF scoring	**NAS scoring**
Grade 0	Normal hepatocytes with cuboidal shape and pink eosinophilic cytoplasm	No ballooned cells
Grade 1	Presence of clusters of hepatocytes with a rounded shape and pale cytoplasm usually reticulated. Although shape is different, size is quite similar to that of normal hepatocytes	Few ballooned cells
Grade 2	Same as grade 1 with some enlarged hepatocytes, at least 2-fold that of normal cells	Many ballooned cells, prominent ballooning

ballooning is based on objective size of ballooned as compared to nonballooned hepatocytes and not on an assessment by the pathologist of the number of ballooned cells noted under 20× light microscopy as done for the NAS score. Positive linear trends exist between NASH or severe disease and increasing BMI and HOMA-IR. There is a strong association between liver fibrosis and SAF-defined scores of activity [60]. As described in Table 1, the grading of hepatocytes ballooning is more precise with SAF scoring compared with NASH CRN scoring system. SAF scoring is believed to specifically provide an unbiased assessment of histological activity, with reduced intra- and inter-observer variability [54]. Of the available histopathological evaluations, the activity sub score of SAF scoring is considered a reliable main efficacy criterion as confirmed by European FLIP Pathology Consortium [4,50] and by US pathologists [61].

The secondary endpoints include effects on steatosis and fibrosis, the percentage of patients with NASH resolution (a key regulatory endpoint for Phase 3 trials in NASH) and 2-point decrease of NAS score. This will allow to fully draw the therapeutic effect of lanifibranor on the target disease.

Finally, including patients with high activity would provide a better chance to avoid enrolling patients with mild disease who have been shown to more frequently regress spontaneously, hereby decreasing the placebo effect. By giving an equal weight to both lesions and not including steatosis into this score, a grade of activity A ≥ 3 results in uniform diagnosis of definite NASH and enriches for the presence of hepatic fibrosis [4].

All liver biopsies were read centrally by an experienced liver histopathologist blinded to treatment allocation and timing of the biopsy in relation to initation or completion of treatment. The biopsies were systematically graded and staged according to NASH CRN and SAF scoring systems.

In summary, main inclusion criterion in NATIVE study is a SAF Activity score ≥ 3 (out of a total of 4) and the primary efficacy endpoint is a SAF Activity score reduction ≥2 points without progression of fibrosis. Given the fact that the regression of steatohepatitis is anticipated to occure more readily than regression of fibrosis, the primary endpoint of this 24 weeks study was focused on improvement in the activity of the disease, with improvement in fibrosis assessed in secondary endpoints.

2.3. Study duration

Most studies in biopsy-confirmed NASH patients with primary endpoint defined by improvement in histologic disease activity have a duration of one year and more [34,62,63]'. Phase 2b studies, such as that with pegbelfermine (BMS-986036), a pegylated fibroblast growth factor (FGF) 21 analogue for NASH patients with Stage 3 Liver Fibrosis (FALCON 1) [64], had a shorter duration (24 weeks). Aldafermin, a FGF19 analogue improved the histological NAS score in a dose-related manner in as little as 12 weeks [65]. Further, liver fibrosis improved by one stage or more in 25% and 42% with the doses of 1 or 3 mg, respectively, suggesting that liver remodelling may occur within 6 months. Pioglitazone, a PPARγ agonist, combined with hypocaloric diet permitted histologic improvements (in steatosis, ballooning and necroinflammation) in patients with biopsy-proven NASH and T2DM after 6 months, compared with placebo [66].

Metabolic effects of PPARs reach maximal efficacy after 3 months but are already measurable between 2 and 4 weeks post-treatment. The time course of their effects on anti-inflammatory pathways is not known. In the Phase 2 GOLDEN-505 study [34] with the dual PPAR α,β/δ agonist elafibranor, a 48-week biopsy driven study, hepatic enzymes as ALT, AST and γGT decreased significantly after 2 months of treatment. In patients with prediabetes or T2DM, pioglitazone 45 mg administered during 6 months was associated with improvement in ballooning and inflammation when histologically assessed and steatosis measured by magnetic resonance spectroscopy [16].

These studies indicated that therapeutic benefits of PPAR agonists, either on blood biomarkers or on liver histological features, could be observed after 6-month treatment and support the administration of lanifibranor for 24 weeks in NATIVE study.

2.4. Dosing rationale

In humans, lanifibranor has shown a favorable safety profile in 237 healthy volunteers treated in Phase I trials up to 3000 mg and up to two weeks. A total of 47 diabetic patients were treated in a Phase 2a study at 3 doses (400, 800 and 1400 mg /day) for 4 weeks. There was no safety concern identified from clinical, biological and electrocardiographic examinations in these patients. PanPPAR agonist activity was confirmed on key metabolic markers as triglycerides decrease (PPARα and β/δ mediated), adiponectin increase (PPARγ mediated) and HDL-C increase (PPARα and β/δ mediated) with an acceptable safety profile, supporting thus the dose selection for the Phase 2b study NATIVE. Lanifibranor is administered with food at a daily dose of 800 mg or 1200 mg for 24 weeks.

2.5. Study objective

The study objective is to assess the safety and the efficacy on the activity of NASH as measured by the SAF histological scoring system of a 24-week treatment with two doses of lanifibranor (800, 1200 mg/day) in adult NASH patients without cirrhosis.

2.6. Ethics

Before its initiation, the NATIVE study was approved by Ethics Committee and by the Competent Authorities of the countries involved in. The study is carried out in accordance to the approved protocol and with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice issued by the International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human use and all relevant applicable regional or local requirements and laws.

Before the entry in the study, information on the study (goals,

analyses performed, potential benefits and risks, insurance, *etc.*) are documented and explained to any potential participant; then, if they accept to participate in the study, a documented consent form with their signature is collected.

2.7. Eligiblity criteria

The following eligibility criteria have been defined: adult patient (age ≥ 18 years) with a liver biopsy performed within 6 months of screening confirming the presence of NASH [concomitant presence of steatosis (any degree ≥5%), lobular inflammation of any degree and liver cell ballooning of any amount)] without cirrhosis (< stage 4 fibrosis) according to the definition from the European Association for the Study of Liver (EASL) [2] and the American Association for the Study of Liver Disease (AASLD) [3]. Biopsies were read centrally. Patients should also have a SAF Activity score of 3 or 4 (> 2), SAF Steatosis score ≥ 1.

Furthermore, weight stability between the time of liver biopsy and inclusion in the studyl is required, defined by no more than a 5% loss of initial body weight.

Patients should have no other causes of chronic liver disease including, but not limited to autoimmune hepatitis, primary biliary cholangitis, hepatitis B virus, hepatitis C virus (patients with sustained viral response with negative Hepatitis C RNA since > 3 years are eligible), Wilson's disease, α-1-antitrypsin deficiency, and haemochromatosis. Diabetic patients must have a stable T2DM, defined as haemoglobin A_{1c} (HbA_{1c}) ≤ 8.5% and fasting glycaemia < 10 mmol/L (180 mg/dL) with no introduction of new anti-diabetic medication in the previous 6 months and no new symptoms associated with decompensated diabetes in the previous 3 months. Patients with a clinically relevant alcohol consumption, defined as follows, were excluded from the study: the daily alcohol intake is limited to ≤30 g/day (less than 3 drinks per day) for males and ≤ 20 g/day (less than 3 drinks per day) for females in the year of the pre-treatment biopsy.

2.8. Prohibited and concomitant medications

Certain drugs treating comorbidities and risk factors are allowed in the study whether they were at stable dose before the entry in the study. Concomitant intake of T2DM drugs is allowed providing that their dosage is stable for at least 6 months prior to screening. Statins at stable doses in the last 3 months prior to the pre-treatment biopsy are the only anti-hyperlipidaemic drug allowed.

Medications that may influence significantly the disease evolution or interefere with the safety evaluation of lanifibranor are prohibited to eliminate confounding factor. Other PPAR agonists such as TZDs (PPARγ agonists), fibrates (PPARα agonists) and drugs tested in NAFLD/NASH such as vitamin E (alpha-tocopherol) or Glucagon like peptide-1 receptor agonists are prohibited. Phytosterols, fish oils, ezetimibe and bile salts chelators that could change the lipidic/cholesterolaemic status of patients are not allowed. Anticoagulants (including warfarin, dabigatran, rivaroxaban, apixaban) are prohibited because the results of the Drug-Drug Interaction studies with lanifibranor were not available at the time of the study start. Insulin is prohibited due to its potential risk of oedema when administrated concomitantly with a PPARγ agonists. Oral corticosteroids are also prohibited as they potentially induce steatosis.

2.9. Conduct of the study

The visits and procedures planned in NATIVE study are summarised in Fig. 1 and in Table 2. During screening processes, review of medical history and previous/concomitant treatments and new biological check up are firstly peformed for each patient. For any potential participant who has no diagnosis of NASH confirmed by a biopsy within 6-month time frame, Fibroscan® is performed to exclude the presence of cirrhosis based on the measure of transient elastography (TE) that predicts the absence of cirrhosis for values ≤12 kPa. In some selected sites, a LiverMultiscan, a non-invasive MRI to measure the levels of fat, iron and fibro-inflammatory disease is also performed. In a perspective of inclusion in the study, a liver biopsy is proposed to a potential participant at the discretion of the investigator if cirrhosis is excluded according to Fibroscan® and if liver inflammation and fibrosis (LIF) is ≥2 and MRI proton density fat fraction (MRI-PDFF) is > 5% according to LiverMultiScan.

Each participant randomised into one of the treatment groups has 6 visits scheduled: a screening visit (V-1, 8 weeks to 2 weeks before randomisation), randomisation visit (V0), first visit under therapy (V1, 4-week on-treatment), second visit under therapy (V2, 14-week on-treatment), end-of-treatment visit (V3, 24-week on treatment) and follow-up visit (4 weeks after end-of-treatment visit). The measures planned in each visit are summarised in Table 1.

2.10. Safety assessments

The safety and tolerability of the investigational drug is assessed through the occurrence of any adverse event, any significant change of laboratory parameters performed at each study visit including haematology, blood chemistry (creatinine, urea, albumin, creatine phosphokinase, ALT, AST, γGT, alkaline phosphatase and total bilirubin), International normalised ratio and search of haematuria by dipstick. The following safety parameters are evaluated at randomisation, visit V0 and end-of-treatment visit V3: blood bone biomarkers (beta-crosslaps, osteocalcin) and cardiac toxicity markers (N-terminal pro-hormone of brain natriuretic peptide (NT-proBNP)). Twelve-lead electrocardiogram (ECG) is performed at screening and after 4-week (visit V1) and 24-week (visit V3) treatment.

An independent committee, the Data Safety Monitoring Board constituted by clinicians and biostatistician, assesses regularly the safety of patients participating in the study, before and during the study course.

2.11. Efficacy assessments

2.11.1. Primary efficacy endpoint

A decrease of at least 2 points of the SAF Activity score without fibrosis progression [2,3] (any stage increase of fibrosis), from baseline to week 24 (visit 3). If no post-treatment biopsy is available, the patient is considered as non-responder.

2.11.2. Main secondary efficacy endpoints

Include percentage of NASH improvers (2-point decrease of NAS with no fibrosis worsening), percentage of patients with NASH resolution (defined as normal liver or steatosis with or without mild inflammation (≤1 according to NASH CRN), no ballooning and no fibrosis worsening at week 24), proportion of patients with improvement in each histological param

eter (steatosis, ballooning, inflammation, activity, CRN-fibrosis, Ishak-fibrosis, EPoS staging system) [67], from baseline to 24-week treatment. These endpoints are evaluated in the diabetic and non-diabetic subgroups of patients.

Changes from baseline to 24-weeks of inflammatory markers (fibrinogen, high sensitivity C-reactive protein, alpha2 macroglobulin and haptoglobin levels), glucose metabolism (fasting glucose and insulin, HOMA-IR and HbA_{1c} in patients with T2DM) and main plasma lipid levels (total cholesterol, HDL−C, calculated LDL-C, triglycerides and Apolipoprotein A1) are also evaluated.

2.12. Other assessments

Changes from baseline to 24-weeks of the following parameters are also assessed:

Table 2
. Schedule of procedures and measures performed in NATIVE study (a) Screening steps are realized from week −4 to −2 before inclusion in the study if liver biopsy available in the last 6 months. These steps are realized from week −8 to −4 if no liver biopsy available in the last 6 months. (b) Central reading on biopsy within the 6 months prior to screening to confirm NASH diagnosis. (c) Only patients having consent for genetic testing are concerned. APO A1, B and C3: Apolipoprotein A1, B and C; FFA: Free fatty acid; HbA1C: Haemoglobin A1C; HDL-C: High density lipoprotein cholesterol; HOMA: Homeostasis model accessment of insuline resistance; hs-CRP: High sensitivity C-reactive protein; IL (−6, −13, −17A, 1B): Interleukin (−6, −13, −17A, 1B); IFNg: Interferon gamma; IVA337: Lanifibranor; LDL-C: Low density lipoprotein cholesterol; MMP(−2, −9): Matrix metalloproteinase (−2, −9); P3NP: Procollagen-3 N-terminal petide; PNPLA3: Patatin-like phospholipase domain-containing protein 3; pro−C3: N-terminal propeptide of type 3 procollagen; T2DM: Type 2 diabetes mellitus; TC: Total cholesterol; TG: Triglycerides; TIMP (−1, −2): TIMP metallopeptidase inhibitor (1,2); TNFα: Tumor necrosis alpha.

Study period	**Screening**			**Treatment**				
Visit	**V-1**			**V0**	**V1**	**V2**	**V3**	**V4**
Screening steps	1	2	3					
Weeks (target ± 3 days, referred to V0)	-4 to – 2 or − 8 to − 4 (a)			0	4	14	24	28
Informed consent	X							
Inclusion/exclusion criteria evaluation	X			X				
Biopsy available in the last 6-month time frame								
FibroScan™ (if available)	X						X	
Central Reading of liver biopsy to confirm NASH diagnosis	X(b)							
Biopsy not available in the last 6-month time frame								
FibroScan™	X						X	
LiverMultiScan™ (in selected sites)		X					X	
Liver biopsy			X				X	
Primary efficacy evaluation: Inflammation and ballooning SAF	X						X	
Liver biopsy at end of treatment							X	
Secondary efficacy evaluation								
NAS score and other liver histology indices	X						X	
Inflammatory markers levels (*IL-6, IL-13, IL-17A, IL1B, TNF-α, INFg)*				X			X	
Fibrinogen, hs-CRP, alpha2-macroglobulin, haptoglobin				X			X	
Glucose metabolism								
including *Fasting glucose and in subjects with T2DM: HbA1c*	X			X	X	X	X	X
Insulin, HOMA, Peptide C, Fructosamine				X			X	
Lipid metabolism								
including *Lipids: TC, HDL−C, calculated LDL-C and TG*	X			X	X	X	X	X
FFA, adiponectin, Leptin and apoA1				X			X	
Chemistry *Plasma Iron, Transferrin, Ferritin*	X						X	
Fibrosis markers: *TIMP-1, TIMP-2, Cytokeratin K18, hyaluronic acid, P3NP, FGF 21*				X			X	
Exploratory criteria Biobank								
Other biomarkers *APO B, APO C3, MMP2, MMP9, ProC3 (non-exhaustive list)*				X			X	
Genotype *PNPLA3, TM6FS2*				X(c)				
Safety assessments								
Laboratory measures	X			X	X	X	X	X
Adverse events				X	X	X	X	X
Pharmacokinetics: IVA337 (+ metabolites) trough sampling					X		X	
Administration of study treatment				X	X	X		
Compliance check					X	X	X	

(i) fibrosis markers including TIMP-1, TIMP-2, cytokeratin K 18 (CK18), hyaluronic acid, procollagen-3 N-terminal petide (P3NP), matrix metalloproteinase (MMP) 2, MMP9, N-terminal propeptide of type 3 procollagen (pro−C3),
(ii) markers of lipids and glucid metabolism,
(iii) inflammation markers including interleukins (IL)-6, IL-13, tumor necrosis factor alpha (TNFα),
(iv) markers of bone remodelling,
(v) TE and controlled attenuation parameter (CAP)
(vi) genotype signatures such as patatin-like phospholipase domain containing protein 3 (PNPLA3) and TM6SF2.

Pharmacokinetic assessments requiring a fasting blood sampling after 4-week (visit V1) and 24-week (visit V3) treatment are also be conducted.

If deemed relevant, immunohistochemistry evaluating the change from baseline to 24-week of ballooning and HSC activation may be performed.

2.13. Statistical analysis and sample size calculation

The primary efficacy endpoint is a binary variable (responder *versus* non-responder, definition of responder provided in efficacy assessments). The rate of responders at the end of the treatment (24 weeks) is compared between the 3 arms by the means of Cochran Mantel Haenzel test (stratified on diabetes). The two comparisons of interest are each of the two lanifibranor doses *versus* placebo. In order to take into account multiplicity of tests, the ascending Hochberg procedure is applied.

For the main secondary efficacy endpoints, the following changes from baseline to 24-week of treatment will be compared between the 3 arms: percentage of NASH improvers (2-point decrease of NAS without fibrosis worsening), percentage of patients with NASH resolution with no worsening of fibrosis, percentage of patients with SAF score change (steatosis: −1 point, lobular inflammation: - 1 point, ballooning: −1 point), percentage of patients with at least 1-point improvement of fibrosis score on a 4-point scale (SAF) without worsening of NASH, using the same methodology as the primary efficacy endpoint.

Those analyses will be run on full analysis dataset (FAS, all randomised patients receiving at least one dose), randomised patients dataset (all randomised patients whether taking or not at least one dose), evaluable patients dataset (all randomised and treated patients and with end-of-treatment biopsy readable in addition to patients having stopped prematurely the study treatment due to safety concerns) and per protocol set (all randomised and treated patients with end-of-treatment biopsy readable, free from other major protocol deviations that can bias the estimate of the treatment effect).

2.13.1. Sample size calculation

It was hypothesized that the rate of responders (according to the definition of the primary endpoint) would be 10% for placebo and an excess rate of responders of 20% for any of the two doses of lanifibranor considered clinically significant. The sample size required to reach a statistical power of at least 80% under these conditions is 72 patients per arm with a two-sided alpha of 0.025 (adjustment for multiplicity). It was decided to round up to 75 patients per arm the number of patients to be randomised. In total 225 patients needed thus to be randomised in this study.

3. Discussion

The NATIVE study is assessing the safety and efficacy of lanifibranor, a pan-PPAR (α,β/δ,γ) agonist in NASH patients. Lanifibranor, by its balanced action on the 3 PPAR isotypes, could also be considered as a combination therapy, albeit in one molecule, combining beneficial effects of each isotype with potentially also compensating for some of the side effects of the individual agonists. The idea of combination therapy in NASH is increasingly popular, as pathophysiology is complex and single agents have so far failed or have shown positive results but with a rather small effect size, resulting in many non-responders even in trials that have met their primary endpoint. In that context, combining drugs with a different mechanism of action aiming at obtaining an additive or even synergistic effect, is attractive. A few combination treatments are investigated although the efficacy of the individual treatment has not always been established [68–70]. In such context, lanifibranor has hence theoritically an advantage as it targets all of the 3 PPAR isotypes compared to single or dual agonists. Moreover, lanifibranor, thanks to its panPPAR activity, would tackle simultaneously the dysregulation of glucid and lipid metabolisms, which are considered to be the metabolic drivers of the disease, as well as the inflammatory pathways and the processes of fibrogenesis directly. The overall expected effect will result from these direct effects on these different pathways in NASH pathophysiology on the one hand, and indirect effect on the other hand, meaning that beneficial effects on inflammation and cell damage (that drive fibrogenesis) might subsequently have a favorable impact on fibrosis. The preclinical data comparing lanifibranor to other single and dual PPAR agonist support this potential, although this remains of course to be demonstrated clinically in NASH patients.

The NATIVE study differs from most of the paired-biopsy Phase 2b studies in NASH so far by its duration, its main inclusion criteria (based on the use of SAF instead of NAS score and hence separating disease activity (lobular inflammation and ballooning from steatosis) and its primary endpoint (SAF Activity score reduction of ≥2 [again exclusive of the effect on steatosis] without fibrosis progression from baseline to week 24). Secondary enpoints include, however, classical endpoints such as NASH improvers (2-points decrease of NAS with no fibrosis worsening), NASH resolution and change in histological parameters (steatosis, ballooning, inflammation, activity and fibrosis), from baseline to 24-week treatment.

Patients eligible to NATIVE study have by definition high disease activity and are hence at high risk of disease progression. Limiting the time of exposure to placebo or drugs with an efficacy under evaluation appears thus justified for these patients. Of course, if the results are positive, exposing patients to lanifibranor for a longer period in future studies, including Phase 3, is needed to determine the sustainability of the clinical effect over time. Furthermore, several studies have shown the feasibility of obtaining a benefit on liver histology in a 6-month time frame. Although the exact kinetics of the changes on histology are not known, the fact that several non-invasive markers tend to show improvements in the first months of treatment and then subsequently plateau after a few months, further support the concept of re-assessing disease activity after a 6-month period, especially as disease activity might be subject to rapid changes [57].

As liver fibrosis is generally considered to run a slower course, NASH trials with paired biopsy usually have a treatment duration of 1 year or longer in order to capture antifibrotic activity. It is well known that liver stifness improves rapidly after viral eradication in chronic Hepatitis C patients [71], but whether this reflects fibrosis regression, is unknown as biopsies were not performed in that early setting. Furthermore, whether this would apply to NASH patients after a reduction of disease activity has not been studied so far. The Belfort trial with pioglitazone already showed a significant reduction in the mean fibrosis score of patients receiving active drug during 6 months whereas placebo-treated patients showed no changes [66]. Aldafermin also showed a significantly higher proportion of fibrosis reponders (fibrosis reduction ≥1 point) over placebo starting on 12-week treatment [65]. These studies illustrate that changes in fibrosis can be observed within 6-month of treatment with drugs that are sufficiently powerful. This rapid effect on fibrosis can be understood in light of the finding that in NAFLD patients extracellular remodelling rates are high and positively correlate with the severity of NASH and fibrosis [72]. It presumably follows that drugs that efficaciously reduce disease activity, which is the driving force of fibrogenesis, will quite rapidly result in fibrosis regression as fibrogenesis slows down whilst fibrolytic mechanisms are still active. It is therefore to be anticipated that lanifibranor, with its combined action on metabolic and inflammatory drivers of the disease activity by several PPAR-mediated pathways as well as direct effects on fibrogenetic mechanisms, will also result in antifibrotic effects that migh be observed within 24 weeks of treatment.

The most challenging point in the care of NASH consists in the ability of a therapy to inhibit disease activity through the reduction of lobular inflammation and hepatocyte ballooning, both being the histological features that differentiate NASH from isolated steatosis. A primary endpoint addressing only these specific features of NASH is potentially more powerful in showing clinically relevant benefit rather than a composite endpoint comprising steatosis. Changes in NAS can be driven by changes in steatosis and do not necessarily reflect a decrease in disease activity and *a fortiori* not resolution of NASH. In the FLINT study [63], for example, a clear positive result was obtained on the primary endpoint of ≥2 points NAS reduction but it did not reach statistical significance in terms of NASH resolution.

The statistical hypothesis of NATIVE study for sample size calculation relies on a placebo effect of 10% for the primary endpoint of SAF Activity score reduction. A 2-point decrease of SAF activity score from a baseline score of 3 or 4 can indicate NASH resolution in a substantial proportion of patients. Consequently, the 2-point decrease of SAF Activity score without worsening of fibrosis ressembles thus greatly the regulatory endpoint of NASH resolution without worsening of fibrosis reported in several previous studies and required in Phase 3. The placebo effect for the NASH resolution endpoint from these studies ranged from 5 to 13%: 5% with aramchol in ARREST study [73], 6% with cenicriviroc in CENTAUR study [74], 6% with resmetirom in a Phase 2 study [75], 12% with elafibranor in GOLDEN-505 study [34] and 13% and 8% with obethicholic acid in FLINT and REGENERATE studies, respectively [63,76]. These data support the determination of 10% as placebo effect for the 2-point decrease of SAF Activity score in this study. The placebo effect for the NASH resolution endpoint (which is largely implied by the endpoint of 2-point decrease of SAF activity) is much lower than the placebo effect based on a ≥ 2 reduction in NAS score, which was estimated at 25% by a meta-analysis of 39 randomised controlled studies involving 1463 placebo-treated NASH patients [77]. NAS score was also significantly dependent on the variation of body mass index (BMI) in that meta-analysis. As outlined before, NAS score encompasses steatosis in contrast to SAF Activity score and this could explain why it was significantly related to BMI, which in return is known to be very sensitive to lifestyle changes. It is well-known that patients involved in a clinical study, being aware that they are tightly monitored, pay naturally more attention to their lifestyle behaviour and tend to decrease their BMI during the course of a study, even those

receiving placebo (the so-called Hawthorne effect). It is thus not surprising that the placebo effect in the NAS-defined endpoint (25%) exceeds by 2-times that observed in the NASH resolution endpoint (5 to 13%). Finally, it has become clear that the placebo effect is the highest in patients with milder disease and lower with increasing disease severity [34]. Given the fact that NATIVE only includes patients with more active disease, placebo rates can be anticipated to be low, further substantiating the estimates used for the placebo effect in power calculation.

In summary, the NATIVE study testing the efficacy by histological endpoints and the safety of lanifibranor in histopathologically confirmed non-cirrhotic NASH patients with high disease activity is designed to detect the therapeutic effect of lanifibranor in an adequate and sufficient period of exposure (6 months). The primary endpoint assesses particularly the disease activity, namely hepatocyte ballooning and lobular inflammation, regardless of the effect on steatosis, and this by using the SAF Activity score. Additional endpoints such as total SAF and NAS score, NASH resolution, individual components of SAF and NAS scores as well as fibrosis score will fully draw the efficacy profile of lanifibranor.

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