Efficacy of Twin Precision treatment in patients with Non alcoholic fatty liver disease NAFLD - a multicentre, open label, parallel arm, randomised controlled trial

Protocol no TPT-03/2021 Version 1 dated 29 Sep, 2021

Sponsor details: Twin Health
Address: 420, 4th Cross Rd, HRBR Layout 2nd Block, Sena Vihar, Kalyan Nagar, Bengaluru, Karnataka 560043

Name & address of study site of the Principal investigator:

1. Dr. Shashsank Joshi, Lilavati Hospital, Mumbai
2. Dr. Paramesh Shamanna, Bangalore Diabetes Center
3. Dr. Nagesh, Dr. Sushruta Institute of GastroEnterology Science and Organ Transplant
4. Dr. Nageshwar Reddy, Asian Institute of Gastroenterology
5. Dr.Dinesh jothimani, Dr. Rela Institute & Medical Center, Chennai
6. Dr. SK Sarin, Institute of Liver & Biliary Centre Hospital, Delhi
7. Dr. Ashok, Chandana clinic, Bangalore
8. Dr. Samir Shah, Global Hospital / Dr. Chetan Bhat, Reliance Hospital
9. Dr. Praveen Mathew, Professor and Head of Medical Gastroenterology, Vydehi Institute of Medical Sciences and Research Centre. Bangalore
10. Dr. Jayashree Gopal, diabendoindia, Chennai.

Design synopsis

<table>
<tr><td>Type of trial: multicentric, open label, randomized control trial</td><td>Treatment groups: 2
Group A: TPT with standard of care
Group B: Standard of care</td></tr>
<tr><td>No. of Centers: 9</td><td>Randomisation: stratified block randomisation
Allocation concealment: Central randomization
Allocation ratio 1:1
Stratification factors: Diabetes.</td></tr>
<tr><td>Sample size: 194 (97 in each arm)

Study population:
Patients with NAFLD (either ELF score > 7.7 or MRE with kPa 2.5 to 5) or with a histological diagnosis of NASH (biopsy < 6 months old)</td><td rowspan="2">Primary outcome
1. Change in fibrosis score and hepatic fat content measured by MRE at 1 year
2. Change in ELF(enhanced liver fibrosis) scores from baseline to end of 1year</td></tr>
<tr><td>Study period
Run in period- 2 weeks
Intervention period -2 years
Post intervention follow up- 6 months</td><td>Other key outcomes
● ELF score
● Change in fibrosis score and hepatic fat content using Transient elastography (MRE and Fibroscan)
● Metabolic changes
● Non invasive markers for Fat and fibrosis
● PRO (CLDQ-NASH, EQ 5D)
● Microbiome status</td></tr>
</table>

Table of contents

Lists of tables & figures

Protocol signature page

Study title: Efficacy of Twin Precision treatment in patients with Non alcoholic fatty liver disease NAFLD - a multicentre, open label, parallel arm, randomised controlled trial

I have read this protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct the study as outlined therein, including all statements regarding confidentiality. I will make a reasonable effort to complete the study within the time designated. I will provide copies of this protocol and all pertinent information to the study personnel under my supervision. I will discuss this material with them and ensure they are fully informed regarding the device and the conduct of the study.
I will conduct the study in accordance with the protocol, the Declaration of Helsinki and applicable local requirements.

Principal Investigator name and Job title:

Institution:

Address:

Signature:

Date

List of abbreviations

APRI	AST to platelet ratio index
AUDIT	Alcohol Use Disorders Identification Test
CLDQ	Chronic liver disease questionnaire
CRN	Clinical Research Network
EQ-5D	EuroQol Five Dimension
ICH- GCP	International Committee on Harmonization of Good Clinical Practice
FSI	Framingham steatosis index
FLI	Fatty Liver index
HIS	Hepatic steatosis index
NAFLD	Non Alcoholic Fatty Liver Disease
NAS	NAFLD activity score
NASH	Nonalcoholic Steatohepatitis
VAI	Visceral adiposity index
WHO	World Health Organisation

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease in the general population. NAFLD is characterized by the accumulation of lipids within the hepatocytes exceeding 5% of the liver weight in the absence of excessive alcohol intake and secondary causes of liver diseases. NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) that can have different degrees of fibrosis and progress to liver cirrhosis and hepatocellular carcinoma.

Nonalcoholic steatohepatitis (NASH), the aggressive form of NAFLD, can progress to cirrhosis and is associated with an increased risk of cardiovascular mortality and type 2 diabetes mellitus. Cirrhosis due to NASH increases the risk of hepatocellular carcinoma and NASH contributes substantially to the population burden of hepatocellular cancer.

The high prevalence of obesity and type 2 diabetes and the improved management of chronic viral hepatitis has resulted in NAFLD becoming a leading cause of chronic liver disease and a major health concern. Though several studies have been conducted, currently there is no approved pharmacological therapy for NAFLD.

Management of NAFLD and NASH

Lifestyle modifications

Lifestyle modification with focus on diet and increasing physical activity is the cornerstone in the treatment of NAFLD. While increased consumption of refined carbohydrates, fructose, sucrose, and processed meat etc are associated with increased risk of NAFLD; dietary fiber and unsaturated fats may be beneficial in NAFLD. Though most of the guidelines recommend a Mediterranean style of diet, there is no general consensus regarding the composition on dietary modification

Pharmacotherapy of NAFLD/ NASH

Pharmacological treatment is recommended only for patients with high risk of progression (such as age above 50 years, presence of diabetes, metabolic syndrome or raised liver enzymes) or those with progressive NASH (having bridging fibrosis and cirrhosis). However, since no drugs have been approved by FDA or European Medicines Agency, any medication used for treatment would be considered as off label treatment.

Metformin is not recommended to specifically treat NAFLD due to inconclusive efficacy on improving histological features. Though the PIVENS trial has shown efficacy of Pioglitazone in improving histological features (except for fibrosis) compared to placebo, its use is restricted due to concerns on adverse effects. Vitamin E, another drug investigated in the PIVENS trial (800 IU/d of α?tocopherol for 96 wk) also showed improvement in histological features and liver enzymes. However, there are concerns about increased risk of overall mortality with vitamin E use. Thus more evidence is required before its recommendation for NAFLD/NASH. Similarly there is inadequate evidence supporting efficacy of incretins for NAFLD/NASH.

2. Study rationale

Though several studies have been conducted, currently there is no approved pharmacological therapy for NAFLD. Treatment strategies are largely focused on lifestyle modifications such as change in dietary habits and improvement in physical activity. However, the same dietary approach may not be effective and acceptable by all individuals. Twin Precision Treatment is a personalized form of dietary intervention based on data collected from individuals using sensors and analyzed using computer technology.

The Twin Health platform uses a Whole-Body Digital Twin, powered by artificial intelligence and Internet of Things technology, to precisely understand the metabolic impairment in the patient's body, which is unique to the patient. This strategy is successfully being implemented in patients with diabetes. Our current ongoing studies in diabetic patients (unpublished data) have shown significant improvement in parameters that are implicated in NAFLD such as body weight, fat content and insulin resistance.
Thus we hypothesize that a precision based approach using Twin Health platform on lifestyle modifications can help in reducing disease progression in patients with NAFLD or NASH.

3. Study objectives

Primary objective

1 . In patients with NAFLD, to compare the change in fibrosis score and liver fat fraction measured using MR elastography between the 2 groups at the end of 1 year
2. To compare the change in ELF(enhanced liver fibrosis) scores from baseline to end of 1year between the 2 groups

Secondary objective

1.In patients with NAFLD, to compare the **change in fibrosis score and liver fat fraction** measured using MR elastography between the 2 groups at the end of 2 years
2. To study the change in ELF scores from baseline to end of 2years

3.To compare the change in **fibrosis score and liver fat fraction (measured using Transient Elastography)** from baseline till 2 years (every 3 months)
4.To study the improvement in **metabolic parameters** from baseline till 2 years between the 2 groups Fasting plasma glucose and insulin, insulin resistance, hemoglobin A1c, lipid profile, adiponectin.
5.To study the change in various **non invasive markers for Fat and fibrosis** every 3 months till end of study between the 2 groups
Fat scores will include Framingham steatosis index (FSI), Fatty Liver index (FLI), NAFLD liver fat score
Fibrosis scores will include AST to platelet ratio index (APRI), Fibrosis-4 score, NAFLD fibrosis score.
6. To study the change in **quality of life** from baseline till 2 years between the 2 groups as assessed using

- Chronic Liver Disease Questionnaire (CLDQ)-NASH
- EuroQol Five Dimension (EQ-5D)

7. To study the effect of Twin Precision treatment on gut microbiome in patients with NAFLD/NASH
8. To study the safety of Twin Precision treatment in patients with NAFLD/NASH

Exploratory objective

1. In patients with NASH (with available follow up liver biopsy), to compare the proportion of patients showing resolution of NASH (by histopathology) between control group and intervention group at 1 year and 2 years
2. In patients with NASH (with available follow up liver biopsy), to compare the proportion of patients showing **reduction in NAFLD activity score (NAS) of ≥2** (from two different histological categories) without any worsening of fibrosis at the end of 1 year and 2 years
3. To compare the proportion of patients showing progression or worsening of liver disease between the 2 groups at the end of 2 years
4. To study the incidence of major adverse cardiovascular events (MACE) such as MI, stroke, cardiovascular death between the 2 groups at the end of 2 years

4. Study population:

Patients diagnosed with NAFLD (radiological diagnosis) or NASH (based on liver biopsy) will be included based on eligibility criteria

4.1. Eligibility criteria

4.1.1 Inclusion criteria

1. Patients with NAFLD (either ELF score > 7.7 or MRE with kPa 2.5 to 5) or with a histological diagnosis of NASH (biopsy < 6 months old)
2. Age ≧ 18 years of age of either gender
3. BMI 19 kg/m2 and above
4. Non diabetic or diabetic (HbA1c < 9%) on stable dose of antidiabetic medications for the last 3 months
5. Willing to provide written informed consent and comply with the study protocol

Criteria for NAFLD:

Patients with evidence of hepatic steatosis (HS), either by imaging, in the absence of secondary causes of hepatic fat accumulation such as significant alcohol consumption, long term use (3 months or more) of a steatogenic medication, or monogenic hereditary disorders (Chalasani N) Frequency of alcohol consumption will be assessed using AUDIT questionnaire.

MR elastography will be used for assessing liver stiffness (kPa) and liver fat content. Relation between fibrosis stage and liver stiffness will be assessed as mentioned in Table-1 Safa Hoodeshenas et al

	F0	F1	F2	F3	F4
NAFLD (KPa)	< 2.5	2.5-3.4	3.5-3.9	4.0-4.9	>5.0

Including NASH patients and criteria for diagnosis

Patients with a diagnosis of NASH based on available biopsy can be considered for the study if he/she meets the other eligibility criteria. No liver biopsy will not be done as part of study. However data will be collected of any liver biopsy done during the study period (as part of clinical indication) for exploratory purpose

Clinical research network (CRN) Criteria for diagnosis of NASH. (For patients with available biopsy report) NASH activity score (NAS) greater than or equal to 4 with at least 1 point each in inflammation and ballooning along with a NASH Clinical Research Network (CRN) fibrosis score greater than stage 1 fibrosis but less than stage 4 fibrosis.

4.1.2 Exclusion criteria

Patients with any of the following criteria will be excluded

1. Those with a history of significant alcohol consumption. Significant alcohol intake is considered when alcohol consumption > 7 standard drinks/week (70 g ethanol) in women and > 14 standard drinks/week (140 g ethanol) in men (according to Asia-Pacific Guidelines)
2. AUDIT score > 8 indicating harmful alcohol consumption
3. Patients with a diagnosis liver disease due to other etiologies such as alcohol or drug abuse, medication, chronic hepatitis B or C, autoimmune, hemochromatosis, Wilson's disease, α1-antitrypsin deficiency
4. Those with clinical evidence of hepatic decompensation such as history of ascites, esophageal bleeding varices, or spontaneous encephalopathy.
5. Those with evidence of portal hypertension such as low platelet counts (< 1.5 lakhs per microlitres), esophageal varices, ascites, history of hepatic encephalopathy, splenomegaly (moderate, severe)
6. Child Pugh class B/C
7. ALT and AST elevation greater than five times the upper limit of normal (ULN)
8. Alkaline phosphatase more than 2 ULN (less than 250–300 271 U/L)
9. Severe hypertension either treated or untreated. (defined as SBP > 180 mm Hg or DBP > 100 mm Hg)
10. Patients with a history of clinically significant heart disease (NYHA Class greater than grade II), peripheral vascular disease (history of claudication), or diagnosed pulmonary disease
11. History of bariatric surgery or intestinal bypass surgery within the 5 years prior to randomization or planned during the conduct of the study.
12. Change in body weight more than 5% in the last 3 months
13. History of malignancy in the last 5 years
14. Active, serious medical disease with a likely life expectancy <2 years

15. Participation in an investigational new drug trial in the 60 days or 5 half-lives, whichever is longer, prior to randomization.
16. Patients on supplements for weight loss.
17. Pregnant and lactating women and postpartum up to 2 yrs
18. Those with contraindications for MR elastography.
19. Any other condition which, in the opinion of the Investigator, would impede compliance, hinder completion of the study, compromise the well-being of the patient, or interfere with the study outcomes.

5. Study design: Multi center**,** Open label, parallel arm, randomized controlled trial containing 2 groups

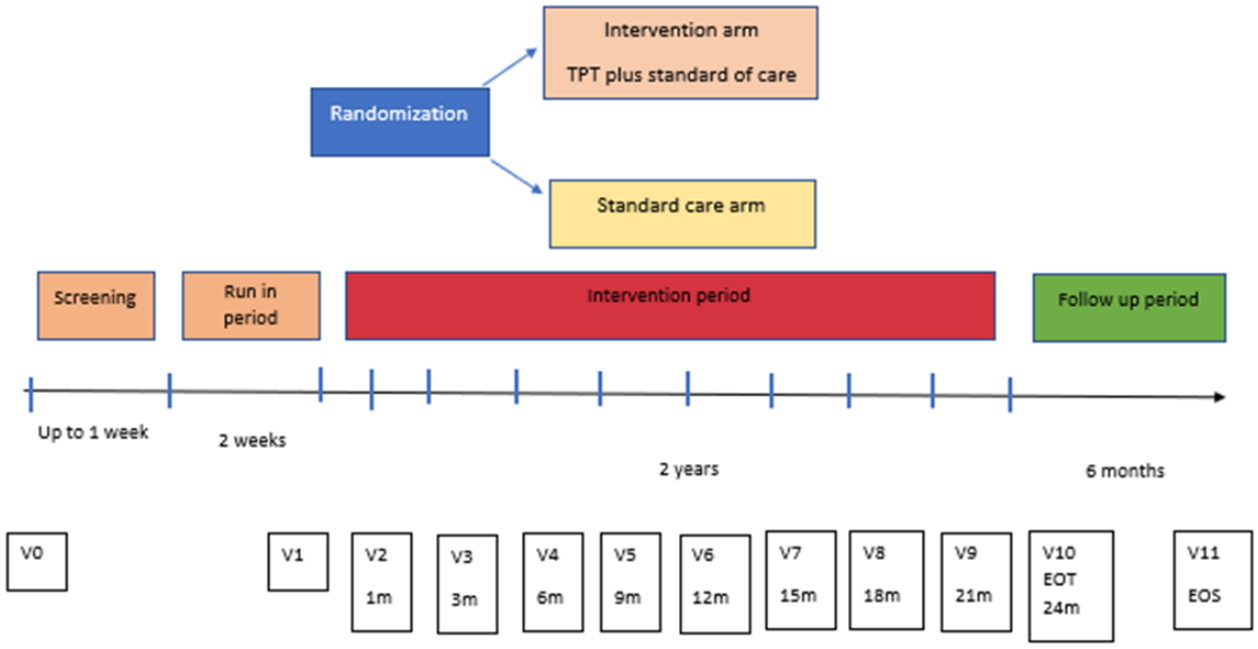


Figure-1 study design

Study period:
Run in period- 2 weeks
Intervention period -2 years
Post intervention follow up- 6 months

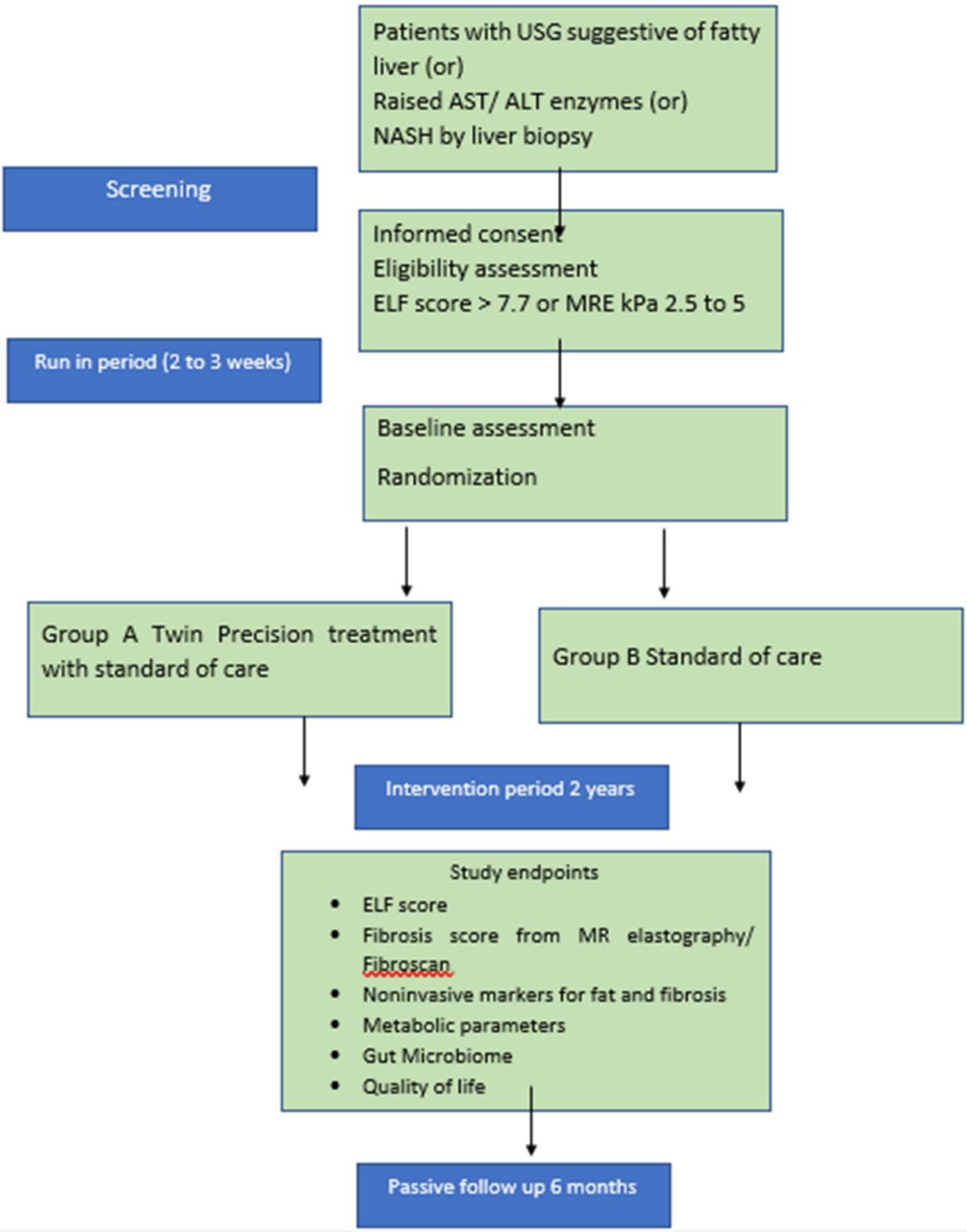
End of treatment is defined as the period until when study intervention will be given that is till 2 years (24 months)
End of study will be the period until when the subjects will be followed up that is 6 months after end of treatment or at the time of withdrawal from study

6. Methodology

Study will be initiated after obtaining ethics committee approval and the trial registered in CTRI prospectively. All study activities will be conducted in accordance with the protocol and ICH-GCP guidelines.

6.1. Patient screening

Figure 2 Scheme for patient screening and study enrollment


Patients with USG suggestive of fatty liver (or)
Raised AST/ ALT enzymes (or)
NASH by liver biopsy
Screening
Informed consent
Eligibility assessment
ELF score > 7.7 or MRE kPa 2.5 to 5
Run in period (2 to 3 weeks)
Baseline assessment
Randomization
Group A Twin Precision treatment with standard of care
Group B Standard of care
Intervention period 2 years
Study endpoints
ELF score
Fibrosis score from MR elastography/ Fibroscan
Noninvasive markers for fat and fibrosis
Metabolic parameters
Gut Microbiome
Quality of life
Passive follow up 6 months

Patients will be screened from the Gastroenterology OPD and those with a diagnosis suggestive of NAFLD (USG or raised liver enzymes); The study will include both patients with NAFLD as well as those with a diagnosis of NASH with liver biopsy done within 6 months prior to enrollment. Patients with ELF score >7.7 or MRE kPa 2.5 to 5 and meeting other eligibility criteria will be included for the study.

Run in period

Eligible patients will be subjected to a run-in period of 2 weeks prior to randomisation. During this period baseline data on the subjects' glycaemic control, sleep, and physical activity will be obtained. This period will also ensure standardization of treatment prior to randomisation. Subsequently patients will be randomized into either of the 2 groups

Group A (Intervention group): Will receive Twin Precision treatment along with standard of care treatment for 2 years
Group B (Control group): Will receive standard of care treatment for NAFLD/NASH for 2 years

6.2. Randomisation: Patients will be randomised into either control group or intervention group in 1;1 ratio using central randomisation. Randomisation code will be generated using online software using block randomisation. Patients will be stratified based on presence or absence of diabetes. Specific treatment will be assigned using IWRS
Blinding: The study will be open label

6.3. Details of intervention

6.3.1 Standard of care treatment (Control group):

There is currently no approved pharmacological treatment which can reverse NAFLD or control disease progression. Hence current treatment strategies are focused on lifestyle modifications. All patients in the standard care arm will receive interventions based on the recommendations of NICE and Asia pacific guidelines.
Lifestyle change will be focused on weight loss achieved by physical activity (aerobic activities and resistance training) and healthy diet. Participants will be encouraged to achieve approximately 7% -10% weight loss.
Energy restriction will include a low calorie (1200-1600 kcal/d), low fat (less than 10% of saturated fatty acid), low carbohydrate diet (< 50% of total kcal)
Subjects will be advised physical activity (including aerobic activity and resistance training) for approximately 150 to 200 min/ week over 3 to 5 sessions.

Patient coach: Each participant will be assigned a coach who will guide them on the diet and physical activity. All participants will be given a study diary where they will enter the details related to their diet and physical activity.

Standard care medications: Since there are no approved medications for NAFLD/NASH no drug will be given as part of standard care for treating NAFLD/NASH. However, management of comorbid conditions such as diabetes, hypertension will be as per routine hospital practice based on guidelines.

6.3.2 Twin Precision treatment (Intervention group)

Patients will receive lifestyle modifications in the form of dietary interventions and physical activity based on patient precision approaches supported by artificial intelligence. Dietary intervention will include calorie restriction, micro and macronutrient supplementation

Patients will be asked to record their food intake on the Twin app each day. Data from BP, CGM, Body composition, and Fitbit sensors enabled with Bluetooth will be transmitted securely through a cellular network to the software each day. This information and software access were made available to the patients as biometric feedback via the TPN app.
Machine learning algorithms will analyze the macronutrients, micronutrients, and microbiome from the database to determine the drivers of glucose response to specific foods for each participant. Factors found to be associated with glycemic response will be analyzed for each participant. Participants will then be provided with a set of specific food recommendations each day with the aim to avoid glucose spikes.
Using CGM data, future blood glucose values of the participants will be predicted using machine learning algorithms and data fusion techniques.

The aim of the TPN Program is to provide the optimal combination of macronutrients, micronutrients, and microbiome, while simultaneously guiding individual patients to avoid foods that cause blood glucose spikes and to replace them with foods that do not produce glucose spikes. There will be no cap on calorie consumption, and patients will be allowed to consume food ad-libitum to satiety.
Meal syntaxes will be followed and Intermittent fasting will be employed.
Nutritional counselling will be provided by trained coaches through the app and via telephone.
Patients will be asked to cover 10000 steps per day (Fitbit sensor) and resistance exercises and breathing exercises will be added to the routine.
Sleep will be monitored (Fitbit sensor) and patients will be counselled to get at least 7 hours of sleep.

Management of diabetes and hypertension of patients on Twin Precision treatment will be done as mentioned below.

Treatment protocol for diabetes (table-2)

1.	Drug naive Type 2 Diabetes patients will be onboarded with Twin precision treatment without adding any anti-diabetic medications

2.	Patients who are on insulin at baseline their bolus insulin will be stopped and basal will be reduced to half at the beginning of the Twin program and further reduced and stopped based on one day average glucose obtained from CGM.
3.	GLP-1 analogues, Sulfonylureas, Thiazolidinediones, SGLT2 inhibitors and Alpha glucosidase inhibitors will be stopped at the beginning.
4.	Metformin will be given to all the patients except those satisfying rule-1, will be started on Metformin 1000mg B.D and down-titrated based on the five day average CGM glucose reading.
5.	Sitagliptin will be started in patients on dual therapy with metformin and sulfonylureas (or) AGIs (or) Glitazones (or) DPP4 inhibitors (or) SGLT2 inhibitors and down-titrated based on the five day average CGM glucose reading.

Treatment protocol for hypertension (Table-3)

Tier number	**Medicines**	**Initial condition on enrolment**	**Reduction condition**	**Increase conditions**
Tier 0	No medicine	Initial no medicine & BP < 160/100 mm Hg		Move to Tier 1, if (5d-BP > 140/90) & (after staying in this tier for 5 days)
Tier 1	Monotherapy (A)	Monotherapy	Move to Tier 0, if ((1d-BP < 120/80 for 7 consecutive days) II (1d-BP < 90/60 for 3 consecutive days) II (Symptoms of Hypotension like Postural Giddiness)) && (EOD-)	Move to Tier 2, if (5d-BP > 140/90) && (after staying in this tier for 5 days)

Tier 2	Dual low dose combination (A+C)	Dual low dose combination	Move to Tier 1, if ((1d-BP < 120/80 for 7 consecutive days) II (1d-BP < 90/60 for 3 consecutive days) II (Symptoms of Hypotension like Postural Giddiness))	Move to Tier 3, if (5d-BP > 140/90) && (after staying in this tier for 5 days)
Tier 3	Dual full dose combination (A+C)	Dual full dose combination/ Triple combination/ Triple combination + spironolactone or Alpha blocker	Move to Tier 2, if ((1d-BP < 120/80 for 7 consecutive days) II (1d-BP < 90/60 for 3 consecutive days) II (Symptoms of Hypotension like Postural Giddiness))	Move to Tier 4, if (5d-BP > 140/90) && (after staying in this tier for 5 days)
Tier 4	Triple combination (A+C+B/ D/ Alpha blocker)	Triple combination/ Triple combination + spironolactone or Alpha blocker	Move to Tier 3, if ((1d-BP < 120/80 for 7 consecutive days) II (1d-BP < 90/60 for 3 consecutive days) II (Symptoms of Hypotension like Postural Giddiness))	Consider manual change of medication or withdraw

7. Study endpoints and assessment

7.1 Primary endpoints

1. In patients with NAFLD/NASH change in fibrosis score and liver fat content measured using MR elastography between the 2 groups at the end of 1 year.

   MR elastography will be used for assessing liver stiffness (kPa) and liver fat content. Relation between fibrosis stage and liver stiffness will be assessed as mentioned below

	F0	F1	F2	F3	F4
NAFLD (KPa)	< 2.5	2.5 - 3.4	3.5 - 3.9	4.0 - 4.9	> 5

Table-4 Contraindications for MR elastography

Absolute contraindications	Relative contraindications
1. Cardiac implantable electronic device (CIED) such as pacemakers, implantable cardioverter defibrillators (ICDs) and cardiac resynchronization therapy (CRT) devices 2. Metallic intraocular foreign bodies 3. Implantable neurostimulation systems 4. Cochlear implants/ear implant 5. Drug infusion pumps 6. Catheters with metallic components 7. Metallic fragments such as bullets, shotgun pellets, and metal shrapnel 8. Cerebral artery aneurysm clips 9. Magnetic dental implants 10. Tissue expander 11. Artificial limb 12. Hearing aid 13. Piercing	1. Patients who are unable to be still or obey breathing instructions 2. Patients with high body mass index (BMI) might have face difficulty to fit into the narrow bore of the MRI machine 3. Patient presenting with any of the following objects require an evaluation with caution before MRI a. Coronary and peripheral artery stents b. Programmable shunts c. Airway stents or tracheostomy d. Intrauterine device (IUD) e. Ocular prosthesis f. Stapes implants g. Surgical clips or wire sutures h. Penile prosthesis i. Joint replacement or prosthesis j. Inferior vena cava (IVC) filter k. Harrington rods l. Medication patch: The patches require removal before the procedure. m. Tattoos n. Colonoscopy procedure in the last eight weeks

2. **ELF score**

Enhanced Liver fibrosis test is a non invasive blood test based on 3 components involved in liver matrix metabolism namely hyaluronic acid (HA), procollagen III amino- terminal peptide (PIIINP), and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) A higher ELF score is associated with increased risk of fibrosis

7.2 Secondary endpoints

1. In patients with NAFLD/NASH change in fibrosis score and liver fat content measured using MR elastography between the 2 groups at the end of 2 years.
   (MRE will be performed every 6 months from randomisation)
2. Change in fibrosis score and liver fat content measured using Transient Elastography from baseline till 2 years (every 3 months)
3. **Metabolic parameters measured from baseline every 3 months till 2 years**
   Fasting plasma glucose and insulin, insulin resistance, hemoglobin A1c, lipid profile, adiponectin, and ALT and AST concentrations;
   Insulin resistance will be calculated using HOMA-IR as follows:
   HOMA-IR: [fasting glucose (mmol/L) × fasting insulin (μIU/mL)] ÷ 22.5

4. **Change in various non invasive markers for Fat and fibrosis measured from baseline every 3 months till end of study (2 years)**
   Fat scores will include Framingham steatosis index (FSI), Fatty Liver index (FLI), NAFLD liver fat score
   Fibrosis scores will include AST to platelet ratio index (APRI), Fibrosis-4 score, NAFLD fibrosis score

   Framingham steatosis index (FSI) will be calculated using the following variables based on the formula

   FSI = −7.981 + 0.011 × age (years) − 0.146 × sex (female = 1, male = 0) + 0.173 × BMI (kg/m2) + 0.007 × triglycerides (mg/dl) + 0.593 × hypertension (yes = 1, no = 0) + 0.789 × diabetes (yes = 1, no = 0) + 1.1 × ALT/AST ratio ≥ 1.33 (yes = 1, no = 0).

   Fatty Liver Index (FLI) = ey / (1 + ey) × 100
   Where y = 0.953 × ln(triglycerides, mg/dL) + 0.139 × BMI, kg/m2 + 0.718 × ln (GGT, U/L) + 0.053 × waist circumference, cm – 15.745

   The **NAFLD Liver Fat Score** is calculated using the presence of the metabolic syndrome, type 2 diabetes, fasting serum insulin, fasting serum AST and the AST/ALT ratio (AAR)

   NAFLD-LFS = - 2.89 + 1.18 x Metabolic Syndrome (Yes: 1, No: 0) + 0.45 x Type 2 Diabetes (Yes: 2, No: 0) + 0.15 x Insulin in mU/L + 0.04 x AST in U/L – 0.94 x AST/ALT

   AST to platelet ratio index (APRI) is calculated as follows:

   APRI = (AST in IU/L) / (AST Upper Limit of Normal in IU/L) / (Platelets in 10^9/L)

FIB-4 score- is a simple, and non-invasive method for assessing liver fibrosis based on the following components: age, platelet count, AST, and ALT.
It is calculated as follows: [age (years) × AST (U/L)]/[number of platelets (10^9 /L) × ALT (U/L) (1/2)]

A FIB-4 score <1.45 has a negative predictive value of 90% for advanced fibrosis (Ishak fibrosis score 4-6 which includes early bridging fibrosis to cirrhosis).

NAFLD fibrosis score

NAFLD Score = -1.675 + (0.037*age [years]) + (0.094*BMI [kg/m2]) + (1.13*IFG/diabetes [yes = 1, no = 0]) + (0.99*AST/ALT ratio) – (0.013*platelet count [×109/L]) – (0.66*albumin [g/dl])

5. **Change in quality of life from baseline measured every 3 months till end of study (2 years) as assessed using**
   - Chronic Liver Disease Questionnaire (CLDQ)-NASH
   - EuroQol Five Dimension (EQ-5D)

CLDQ is a validated tool for patients with chronic liver disease. It contains 29 items contained within six domains including abdominal symptoms (items 1, 5, 17), fatigue (items 2, 4, 8, 11, 13), systemic symptoms (items 3, 6, 21, 23, 27), activity (items 3, 6, 21, 23, 27), emotional function (items 10, 12, 15, 16, 19, 20, 24, 26) and worry (items 18, 22, 25, 28, 29). A Likert scale response format is given for all items ranging from 1 (most impairment) to 7 (least impairment).

EQ-5D comprises five dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems and extreme problems. The patient is asked to indicate his/her health state by ticking the box next to the most appropriate statement in each of the five dimensions.

6. Microbiome assessment
Change in gut microbiome pattern from baseline to end of treatment (2 years) For this stool sample will be obtained at baseline, 1month, 3 months (when introduction of OFY (orange for you) is done 1 year, 2 years and during follow up. Composition, abundance and diversity of gut microbiome will be compared between the 2 groups

Procedure for sample collection

- Stool samples will be collected from all participants who gets accepted for the study.
- Each participant will be given a single stool collection kit, with detailed instructions about sample collection.
- The stool collection tube contains 1ml of DNA stabilizing solution and an integrated spoon that can be used to scoop out stool into the tube.

Steps for DNA extraction and sequencing

- Once the sample is received at the processing site, a unique working ID will be assigned.
- Extraction of DNA, will be done with a predefined validated protocol and the quantity and quality will be assessed to meet the sequencing requirements.
- Whole metagenome sequencing will be performed using long read sequencing technology from Oxford Nanopore. Basecalling and demultiplexing of sequence reads will be performed and raw sequencing reads will be generated in FastQ format for further computational analysis.
- The composition [all species], abundance [% estimates], and diversity of the microbiome in each sample will be analysed

7. Safety assessment

The investigator will monitor the patients throughout the study period for any adverse event. This will include clinical examinations, vital signs and lab investigations.

7.3. Exploratory endpoints

1. **Resolution of NASH**

In patients with NASH, resolution of definite non-alcoholic steatohepatitis with no worsening in fibrosis [Time frame baseline to 2 years]. Liver biopsy will not be done as part of the study. Instead data will be collected from patients with available follow up liver biopsy Resolution of NASH (based on histopathological examination from liver biopsy) is defined as the absence of ballooning (0) and inflammation (0–1) after 1 year of therapy in patients with a diagnosis of definite NASH at baseline.

Adjudication of histopathology findings: Each biopsy will be assessed centrally by two independent expert pathologists to determine the activity score for nonalcoholic fatty liver disease and the fibrosis stage. They will be blinded to the treatment group.

2. **Improvement of fibrosis stage**

Improvement of at least one fibrosis stage and no worsening of NASH (with worsening defined as an increase of ≥1 point in either the lobular inflammation score or the hepatocyte ballooning score according to the NASH Clinical Research Network criteria) [Time Frame baseline to 2 years]

The NASH ranges from 0 to 8 (highest activity) and is calculated as the sum of scores of the three components of the histologic scoring system

NAS = steatosis (0–3) + lobular inflammation (0–3) + hepatocyte ballooning (0–2).

The histologic scoring system was developed and validated by the NASH Clinical Research Network (NASH-CRN) pathology committee and currently recommended for NASH-related clinical trials

Table-5 Description of NAFLD score

Item	Definition	Score
Steatosis	< 5%	0
	5%-33%	1
	> 33%-66%	2
	> 66%	3
Lobular inflammation	No foci	0
	< 2 foci per 200 × field	1
	2-4 foci per 200 × field	2
	> 4 foci per 200 × field	3
Ballooning	None	0
	Few balloon cells	1
	Many cells/prominent ballooning	2

Table-6 Staging of fibrosis

Stage 1	Zone 3 perisinusoidal/pericellular fibrosis, focal or extensive
Stage 2	Zone 3 perisinusoidal/pericellular fibrosis + focal or extensive periportal fibrosis
Stage 3	Zone 3 perisinusoidal/pericellular fibrosis + portal fibrosis + bridging fibrosis
Stage 4	Cirrhosis

3. Progression of liver disease

To compare the proportion of patients showing progression or worsening of liver disease between the 2 groups at the end of 2 years Progression or worsening of liver disease includes progression in fibrosis stage or cirrhosis, episodes of hepatic decompensation.

4. Major Adverse Cardiovascular events

To study the incidence of major adverse cardiovascular events (MACE) such as MI, stroke, cardiovascular death between the 2 groups at the end of 2 years

Criteria for diagnosis of MI will be symptoms of MI with either increase in cardiac enzymes or ECG changes (development of Q wave). Criteria for diagnosis will be focal neurological deficit lasting more than 24 hours associated with ischaemic or hemorrhagic changes in the brain (confirmed by radiological imaging whenever possible).

8. Patient follow up and study period:

The duration of study will be for 2 years (intervention period) and 6 months (follow up)

All patients will be followed monthly for the first 3 months followed by every 3 months until the end of the study period.

9. Schedule of events and assessments (table-7)

	Pre Screening (-3 weeks)	Run in period	Baseline Day0	1 M (±1 wk)	3 M (±1 wk)	6 M (±1 wk)	9 M (±1 wk)	12 M (±1 wk)	15 M (±1 wk)	18 M (±1 wk)	21 M (±1 wk)	24M EOT (±1 wk)	30 M Post intervention 6M EOS (±1wk)
	V0		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
Eligibility assessment & Informed consent	X												
Medical history, medications, clinical examination & anthropometric	X		X		X	X	X	X	X	X	X	X	X

assessment													
MR Elastography			X			X		X		X		X	X
FibroScan (Transient Elastography)			X		X	X	X	X	X	X	X	X	X
Dexa			X			X		X				X	X
ECHO			X					X				X	X
CIMT			X					X				X	X
ECG			X					X				X	X
USG Abdomen			X										
Fundus examination by ophtamologist			X					X				X	X
Fundus examination Remedio			X			X		X		X		X	X
Liver biopsy (if available)*													
Blood investigations# ● Metabolic parameters ● Liver function test ● Complete blood count ● Non-invasive biomarkers for fat and fibrosis ● Exploratory biomarkers			X	X	X	X	X	X	X	X	X	X	X
CGM*		X	X		X	X	X	X	X	X	X	X	X
Questionnaire ● CLDQ-NAS			X		X	X	X	X	X	X	X	X	X

H ● EQ-5D ● AUDIT													
Stool sample for microbiome			X	X	X			X				X	X
Adverse events				X	X	X	X	X	X	X	X	X	X

*Apart from this all subjects will be monitored daily using a fitbit sensor for monitoring physical activity, duration of sleep.
Stool samples for microbiome analysis will be done at baseline, 1 month, 3 months and 1 year in a small sub group.
CGM- Complete glucose monitoring will be done continuously for first 6 months followed by once (14 days) every quarterly
Complete list of blood investigation is included in annexure IV

Parameters collected from sensors

- Continuous glucose monitor: Abbott Libre Pro CGM Diabetes Sensor. CGM data will be collected continuously during the first 6 months followed by once (14 days data) every quarterly.
- Systolic and Diastolic BP: Blood Pressure Monitor TAIDOC TD-3140
- Body composition parameters like weight, visceral fat, muscle mass, bone mass: Powermax BCA-130 Bluetooth Smart Scale
- Resting heart rate (RHR), step count: Fitbit charge 2 wristband
- Sleep parameters: Fitbit charge 2 wristband

*Resting heart rate, sleep parameters only for patients in the TPT group

Assessment of alcohol consumption

Frequency of alcohol consumption will be assessed monthly using AUDIT questionnaire in all patients. The Alcohol Use Disorders Identification Test (AUDIT) is a 10-item screening tool developed by the World Health Organization (WHO) to assess alcohol consumption, drinking behaviors, and alcohol-related problems.

Interpretation of AUDIT score

The score ranges from 0 to 40 where 0 indicates an abstainer who has never had any problems from alcohol. A score of 1 to 7 suggests low-risk consumption according to World Health Organization (WHO) guidelines. Scores from 8 to 14 suggest hazardous or harmful alcohol consumption and a score of 15 or more indicates the likelihood of alcohol dependence (moderate-severe alcohol use disorder).

Liver biopsy for NASH subgroup

Liver biopsy will not be done as part of the study. Only data will be collected from patients who have a follow up liver biopsy.

10. Criteria for stopping the trial or discontinuation of study intervention

1. Increase in serum AST and/or ALT > 3 times the baseline value OR > 500 IU/L (on one reading unless the sample is hemolysed)
2. New onset jaundice (total bilirubin ≥ 3 mg/dl) irrespective of other liver biochemistries
3. Increase in serum AST and/or ALT < 3 times the baseline value but associated with symptoms (fatigue, nausea, vomiting) where the investigator finds it unsafe to continue
4. International normalized ratio (INR) greater than or equal to 1.7
5. Alkaline phosphatase more than 2 ULN (less than 250–300 271 U/L
6. Patients with hepatic decompensation events (e.g., hepatic encephalopathy, variceal bleeding, ascites)
7. Patients with AUDIT score > 8
8. If participants are not following the process of the trial (food log ,nutrition)with in 30 days in the project will be withdraw from the trail

   For patients who discontinue study intervention, attempts will be made to follow the patients for primary and secondary endpoints until end of study.

11. Medications allowed and contraindicated

- Drugs known to cause hepatic steatosis (e.g., amiodarone, valproate, tamoxifen, methotrexate, steroids) saroglitazar, obeticholic acid are not allowed during the study.
- Antidiabetic drugs that influence liver fat, including thiazolidinediones and glucagon-like peptide 1 receptor agonists, or recent initiation of any SGLT-2 inhibitor. However patients on stable doses of pioglitazone and vitamin E for 3 months or more can be included.
- Patients who are on stable medications for diabetes, hypertension and dyslipidemia for 3 months other than what is mentioned in the above list will be allowed in the study.

12. Statistical considerations

12.1 Sample size calculation for NAFLD

In a randomized controlled trial conducted by Chehregosha H et al (2021), the efficacy of empagliflozin (24 weeks therapy) was compared with pioglitazone and placebo in patients with NAFLD. Liver stiffness measured using fibroscan (KPa) showed a reduction from 7.49 ± 2.65 (at baseline) to 7.17 ± 2.67 (week 24)

with a P value of 0.27 in the placebo group. In the empagliflozin group the liver stiffness reduced from 6.83 ± 2.44 (baseline) to 6.01 ± 1.65 (week 24) with a P value of 0.05 the empagliflozin group.

Expecting a similar difference in liver stiffness measurement between 2 two groups at the end of 1 year, with 90% power and 5% alpha error the minimum required sample size is 154 patients (77 in each group). Adding for 20% anticipated drop out rate the final sample size is 194 (97 in each arm). The final sample size will be rounded to 200 patients

12.2 Statistical analysis plan

Data will be analyzed using SPSS software version 21. Baseline patient characteristics will be described using descriptive statistics. Continuous variables will be expressed using mean and standard deviation or median with range depending on the distribution of data. Categorical variables will be expressed using frequency and percentage. Primary analysis will be done using a modified intention to treat (mITT) analysis where only subjects with at least one follow up assessment done will be included in the analysis.

Mean change in fibrosis score and liver fat fraction from baseline to 1 year will be compared between the 2 groups using Independent T test or Mann Whitney U test depending on the distribution of data. For the secondary endpoints continuous variables will be compared using Independent T test or Mann Whitney U test and categorical variables will be compared using Chi square test. Repeated measures ANOVA will be used for analysis of change in variables from baseline till end of study. P value less than 0.05 will be considered statistically significant. Safety outcomes will be described using descriptive statistics. For patients who were lost to follow up, the last available value will be used for analysis. For patients who discontinue study intervention, attempts will be made to follow the patients for primary and secondary endpoints until end of study and the nearest available value will be used. Similarly for patients who missed assessment at 1 year and 2 years, the nearest available value will be considered

Change in composition, abundance, and diversity of the microbiome will be compared between 2 groups from baseline till end of study. The alpha diversity and beta diversity metrics along with other relevant indices would be generated and compared across the samples.

Categorical data(Sex,MRE,Fibroscan scores etc) will be represented by Percentages. Continuous or quantitative variables(BMI,Age,weight, waist circumference etc. will be assessed for the normality using Shapiro-Wilk's test. If the quantitative variables follow normal distribution, then they will be expressed as mean + SD. Variables which are not following Gaussian distribution after attempting logarithmic transformation or other transformation, will be expressed by median (Interquartile range). The primary analysis will compare the change in fibrosis score and liver fat fraction measured using MRE between the intervention and standard care arm between baseline and 2 years using an independent sample 't' test. Non normally distributed continuous variables of the same will be done by Mann-Whitney U test. ELF scores between baseline and 2 years will also be compared by independent sample 't' test between the groups.Categorical variables will be compared either by Chi square test or Fisher's Exact test based on

number of observations . Pearson correlation coefficient will be computed to evaluate the correlation between the continuous variables. Comparison of Pre and Post normally distributed continuous variables within the arm will be evaluated using Paired 't' test, if they are non normally distributed Wilcoxon Signed Rank test. Comparison of mean of normally distributed continuous variables more than two groups will be taken care of by ANOVA test, otherwise equivalent non parametric test viz, Kruskal Wallis H test will be used. Percentage changes of continuous variables to know the relative change of individuals will be computed from baseline to end of treatment that will also be compared between standard care arm and intervention arm using the above mentioned statistical tests.Exploring the possibilities of developing regression models with covariates based on the availability of univariate results. Data capturing will be automated in Tableau software and will be validated through Microsoft Excel. Data analysis will be carried out by IBM SPSS Statistics for Windows, Version 28.0,IBM Corp, Armonk; NY. Two tailed $p<0.05$ will be considered as statistically significant.

13. Ethical considerations

Study will be initiated after obtaining permission from the Institutional Review Board. Written informed consent will be obtained from all study participants. The patient will be explained that their participation is purely voluntary. Patient confidentiality will be maintained throughout the study period. All patients will be identified using an unique study ID. There will be no additional expense to the participant related to the study.
Treatment will be provided at free of cost for any study related injury as per local requirements..

13.1 Risk to the patients

Since the intervention is in the form of lifestyle modifications and dietary interventions based on micro and macronutrients, we do not foresee any serious adverse events. occasionally there can be a drop in blood sugar levels in diabetic patients due to improvement in glycemic control. However all these patients will be continuously monitored for any change in blood sugar level and drug dose will be titrated accordingly.

13.2 Potential benefits to the participants

The study may or may not benefit the patients directly. Based on ongoing diabetes studies we have found improvements in blood sugar, reduction in blood pressure, weight loss in patients on Twin Precision treatment. If the intervention works in this group of patients, there can be reduction in disease progression and its associated complications. This includes progression to cirrhosis, need for liver transplant and risk of hepatocellular cancer.

14. Reporting of adverse events

Events that are both serious and unexpected for the patient population under study and that occur in participants treated with study medication will be reported to the sponsor/ Institute ethics committee. Adverse events that are not serious, but which lead to permanent discontinuation of study medication will be recorded and included in the final study report. All serious adverse events will be notified to the ethics committee and sponsor as per the timelines of the local IRB/ regulations.

An Adverse Event is any untoward medical occurrence in a patient that may or may not have a causal relationship to the study medication. It may be an unfavorable and unintended sign; symptom or medical condition occurring after the informed consent form is signed. Medical conditions/diseases present before consent is signed are considered AEs if they worsen after consent is signed. Information about every AE will be collected and recorded on the CRF. Adverse events, whether reported by the subject, information received during general discussion by the Principal Investigator, or detected through physical examination, laboratory test or other means will be recorded on the CRF and followed carefully until they resolve.

Abnormal laboratory values or test results should not generally be considered AEs, unless they induce clinical signs or symptoms or require therapeutic intervention; then, they should be recorded on the CRF with an appropriate justification.

Each AE will also be described by:
1. its duration (start and end time and date)
2. the severity grade (mild, moderate, severe)
3. its relationship to the study intervention (none, possible, probable, definite)
4. The action(s) taken by the Principal Investigator.

The severity grade of an AE provides a qualitative assessment of the extent or intensity of an AE, as determined by the investigator or as reported by the subject. The severity grade does not reflect the clinical seriousness of the event, only the degree or extent of the affliction or occurrence (e.g. severe nausea, mild seizure), and does not reflect the relationship to study supplement**.**

15. Regulatory and ethical considerations

The study does not involve use of any investigational drug product and is considered as a non regulatory trial.
The study will be conducted in accordance with the protocol and with the following:

- Applicable ICH GCP guidelines
- Applicable laws and regulations (National ethical guidelines for biomedical and health research involving human participant)

The protocol, amendments, informed consent forms and other relevant documents must be reviewed and approved by the sponsor, submitted to an IRB/IEC by the investigator, and reviewed and approved by the IRB/IEC before the study is initiated.

15.2 Insurance of study participants

All study participants in the trial will be covered under the insurance which is in line with applicable laws and/or regulations.

16. Operational considerations

16.1 Data management

All data will be de-identified for any patient identifiers and confidentiality will be maintained throughout. Each patient will be identified by a unique enrollment number. Only enrolment number, subject initials will be recorded on the CRF. If the subject's name appears on any other document collected the name will be obliterated and stored. Subjects will be informed that all personal information made available for inspection will be handled in the strictest confidence.

Data will be captured in an electronic case report form which has access restriction, password protection and backup of data.

Source documents will be maintained for all data entered in the CRF and archived for a minimum period of three years from the time of completion of trial or as per local requirements.

16.2 Protocol deviation and violation

All study activities will be conducted in accordance with the protocol. No change in the protocol can be implemented without permission from the ethics committee except in scenarios to avoid an immediate hazard to trial subjects or for other medically compelling reasons and the same will be notified to the ethics committee as early as possible. All protocol deviations will be notified to the ethics committee along with measures taken for corrective and preventive actions

16.3 Publication policy

The results of the study will be published in a peer reviewed journal. Authorship of publications of the overall study results will be determined by mutual agreement as per the International Committee of Medical Journal Editors authorship requirements. The sponsor will comply with the requirements of publication of the overall study results covering all participating sites. No data or part of the study can be published without the permission of the sponsor. Any information published will not reveal the identity of the study patients and confidentiality will be maintained.

17. References

1. Sanyal AJ, Brunt EM, Kleiner DE, Kowdley K, Chalasani N, Lavine J et al. Endpoints and Clinical trial design for non alcoholic steatohepatitis. Hepatology. 2011 July ; 54(1): 344–353
2. Kobyliak N, Abenavoli L, Mykhalchyshyn G, Kononenko L, Boccuto L, Kyriienko D et al. A Multi-strain Probiotic Reduces the Fatty Liver Index, Cytokines and Aminotransferase levels in NAFLD Patients: Evidence from a Randomized Clinical Trial. J Gastrointestin Liver Dis, March 2018 Vol. 27 No 1: 41-4
3. Takahashi Y, Fukusato T. Histopathology of nonalcoholic fatty liver disease/ nonalcoholic steatohepatitis. World J Gastroenterol 2014; 20(42): 15539-15548

4. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M et al. The Diagnosis and Management of Nonalcoholic Fatty Liver Disease: Practice Guidance From the American Association for the Study of Liver Diseases. Practice Guidance. Hepatology. 2018
5. Safa Hoodeshenas, MD, Department of Radiology, Mayo Clinic, Rochester, MN, United States Meng Yin, PhD, and Department of Radiology, Mayo Clinic, Rochester, MN, United States Sudhakar Kundapur Venkatesh, MD Department of Radiology, Mayo Clinic, Rochester, MN, United States
6. Fatty liver index Available from https://www.mdcalc.com/fatty-liver-index#evidence
7. NAFLD Liver fat score Available from https://www.mdapp.co/non-alcoholic-fatty-liver-disease-liver-fat-score-nafld-lfs-calculator-358/
8. APRI Available from https://www.mdcalc.com/ast-platelet-ratio-index-apri#evidence
9. FIB-4 score Available from https://www.hepatitisc.uw.edu/page/clinical-calculators/fib-4
10. NAFLD fibrosis score Available from NAFLD (Non-Alcoholic Fatty Liver Disease) Fibrosis Score
11. Leoni S, Tivoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. *World J Gastroenterol*. 2018;24(30):3361-3373.
12. Adeniyi OV, Yogeswaran P, Longo-Mbenza B, Ter Goon D. Uncontrolled Hypertension and Its Determinants in Patients with Concomitant Type 2 Diabetes Mellitus (T2DM) in Rural South Africa. PLoS One. 2016;11(3):e0150033.
13. Ghadimi M, Sapra A. Magnetic Resonance Imaging Contraindications. [Updated 2021 May 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK551669/
14. Houttu V, Csader S, Nieuwdorp M, Holleboom AG and Schwab U. Dietary Interventions in Patients With Non-alcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. Front. Nutr. 2021; 8:716783.
15. Seeff LB, Everson GT, Morgan TR, et al. Complication rate of percutaneous liver biopsies among persons with advanced chronic liver disease in the HALT-C trial. *Clin Gastroenterol Hepatol*. 2010;8(10):877-883.

18. Annexures

1. Chronic Liver Disease Questionnaire (CLDQ)-NASH
2. EuroQol Five Dimension (EQ-5D)
3. AUDIT questionnaire for alcohol consumption
4. List of investigations