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# Prospective, randomised, placebo-controlled, phase 2 clinical trial assessing the efficacy and safety of oral vancomycin in patients with primary sclerosing cholangitis with/out inflammatory bowel disease in Italy: study protocol of VanC-IT trial

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## ABSTRACT

**Background** Primary sclerosing cholangitis (PSC) is the classical hepatobiliary manifestation of inflammatory bowel disease (IBD). No therapy currently halts disease progression. The strong gut–liver axis implicated in PSC pathogenesis supports the investigation of microbiome-targeted treatments. Oral vancomycin (OV), an antibiotic with potential immunomodulatory properties, has shown encouraging results in improving clinical symptoms and liver biochemistry in PSC. However, prospective data on its safety and efficacy remain limited.

**Methods and analysis** Oral Vancomycin for primary sclerosing Cholangitis in ITaly (VanC-IT) is a phase II, dose-finding, randomised, placebo-controlled, trial designed to evaluate the efficacy and safety of OV in patients with PSC, with or without underlying IBD. Adults and adolescents aged 15–75 years will be enrolled following a 10-week screening and run-in period and randomised in a 1:1:1 ratio to receive either placebo, OV 750 mg/day or OV 1500 mg/day for 24 weeks. Randomisation will be stratified by baseline liver stiffness (< or ≥14.4 kPa). Participants will be followed at 4 and 12 weeks post-treatment. The primary efficacy outcome is the change in serum alkaline phosphatase at 24 weeks. Key secondary outcomes will assess the safety, the impact of OV on liver biochemistry, PSC risk scores, circulating and imaging markers of liver disease, IBD activity, quality of life and incidence of PSC-related clinical events. Key translational aims include sequencing of the faecal microbiota, metabolomic profiling of serum and stool samples and immunological profiling of serum associated with OV treatment.

**Ethics and dissemination** The protocol has been approved by the Ethics Committee CE Brianza on 10 February 2023, number 4017. Trial registration number NCT05876182. Participants will be required to provide written informed consent. The results of this trial will be disseminated through national and international presentations and peer-reviewed publications.

**Trial registration number** NCT05876182.

## STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Randomised, double-blind, placebo-controlled trial design enables rigorous assessment of the efficacy and safety of two oral vancomycin dosing regimens in primary sclerosing cholangitis (PSC).
⇒ Broad inclusion criteria allow enrolment across a spectrum of PSC phenotypes, including patients with and without inflammatory bowel disease, varying stages of fibrosis and adolescents aged 15–17 years.
⇒ Integration of clinical, biochemical, imaging, patient-reported outcomes and serial mucosal and stool sampling supports multimodal evaluation, although interpretation of microbiome data may be limited by small sample size and heterogeneous baseline.
⇒ Single-centre conduct with multicentre recruitment ensures protocol consistency and data quality, while reliance on remote follow-up and self-collected laboratory tests may introduce variability in adherence and data reliability.
⇒ Short trial duration allows detection of early treatment effects but limits evaluation of long-term efficacy and safety.

## INTRODUCTION

### Background and rationale

Primary sclerosing cholangitis (PSC) is a chronic fibroinflammatory disease of the

liver characterised by chronic inflammation and sclerosis of the intrahepatic and/or extrahepatic bile ducts, and at risk for progression to liver failure and development of colorectal and hepatobiliary cancer. Median survival after diagnosis is approximately 17 years[1] and no pharmacological therapy has been shown to halt the progression of PSC or prevent serious complications. Liver transplantation remains the only treatment option, although disease recurrence occurs in up to 30–40% of cases within 5 years.[2]

The prevalence of PSC varies; the highest rates are found in Northern European countries and North America (USA and Canada), with figures ranging from 3.85 to 16.2 per 100 000 individuals. In contrast, Italy reports a significantly lower prevalence, estimated at approximately 0.8 per 100 000 individuals. However, it might be an underestimation due to underdiagnosis and under-reporting.[3]

Nearly 70–80% of patients with PSC are affected by inflammatory bowel disease (IBD), most commonly ulcerative colitis (UC),[4] suggesting a pathophysiological link between the gut and the liver that remains poorly understood.

Emerging evidence shows that alteration in gut microbiota may contribute to PSC pathogenesis. One prominent theory, often referred to as the 'leaky gut' hypothesis, suggests that bacteria or their toxic metabolites may translocate from the inflamed intestinal mucosa into the portal circulation, ultimately reaching the liver. This process is thought to contribute to liver and biliary injury. Studies have shown that the gut microbiota of patients with PSC, when compared with individuals with IBD and healthy controls, exhibits a notable decrease in microbial diversity. Additionally, there is an over-representation of intestinal pathobionts—organisms that, under normal conditions, exist as harmless symbionts but may become pathogenic under certain circumstances.

Preclinical studies on murine models have demonstrated that antibiotic therapy can reduce the concentration of pathobionts and influence hepatic and biliary inflammation. These effects might be mediated by microbiota bile acid metabolism. Specific taxa, such as *Enterococcus gallinarum,* a vancomycin-sensitive strain of bacteria, can evade immune clearance and induce hepatic inflammation. This discovery[5] underscores the potential for manipulating the gut microbiota as a means of influencing both bile acid metabolism and hepatic inflammation, offering a promising pathway for therapeutic development aimed at modulating the composition and function of the microbiota.[1 6 7]

Several antibiotics, including oral vancomycin (OV) and metronidazole, have been explored for their immunomodulatory potential in PSC. The OV, a non-absorbable glycopeptide antibiotic, has been associated with improvements in clinical symptoms, liver biochemistry and PSC-associated IBD symptoms.[8–16]

Despite growing interest in OV use among patients with PSC, particularly in countries like Italy where off-label administration is increasingly common, prospective data from randomised trials in European populations are lacking. This underscores the need for rigorous evaluation of OV's efficacy and safety through a placebo-controlled design.[6 7]

### Hypothesis

We hypothesise that OV administration can reduce PSC disease activity by selectively modulating the gut microbiome, leading to sustained improvements in liver function and inflammation early in the disease course.

## METHODS AND ANALYSIS

This is an investigator-initiated phase 2 randomised, double-blind, placebo-controlled, single-centre with multicentre recruitment study to evaluate the safety and efficacy of two doses of OV, 750 mg and 1500 mg/day in subjects between 15 and 70 years old with PSC with or without IBD. The doses are chosen based on the literature data highlighting the efficacy of OV in PSC with a range of treatment dose between 750 mg to 1500 mg, with no evidence of superiority of one dose to the other. The study will consist of a 10-week screening period (including a run-in phase), 24 weeks of treatment and follow-up visits at 4 and 12 weeks after completion of treatment to evaluate what happens after treatment stops. The run-in period is postrecruitment and prerandomisation period where participants will be assessed for alkaline phosphatase (ALP) fluctuation, which, if greater than 30%, is one of the exclusion criteria (see below). The enrolment period will cover 48 months to include the required number of patients. After inclusion of the last patient, a 24-week period of randomised treatment and 12-week follow-up will take place. 3 months will be necessary for data analysis and reporting. Total duration is therefore 60 months (figure 1).

The trial started recruitment on 15 June 2023 and will end in December 2028.

### Study population

84 subjects meeting the study's entry criteria will be randomly assigned in a 1:1:1 ratio to three different treatment groups. Sample size calculations are based on an analysis of covariance (ANCOVA) model comparing mean ALP levels at 6 months across treatment groups, adjusted for baseline ALP. Assuming mean ALP values of 3×upper limit of normal range (ULN) (control), 2×ULN (OV 750 mg/day) and 1.5×ULN (OV 1500 mg/day), a sample size of 25 patients per arm (75 total) provides 80% power to detect a ≥1×ULN difference at a 5% significance level (with $R^2$=0.3 and common SD=2×ULN). Accounting for a 10% dropout rate, the final sample size is 28 patients per arm (84 total). Participant eligibility criteria are reported in table 1.

### Randomisation

Randomisation will be performed using a computer-generated permuted-block sequence and stratified by

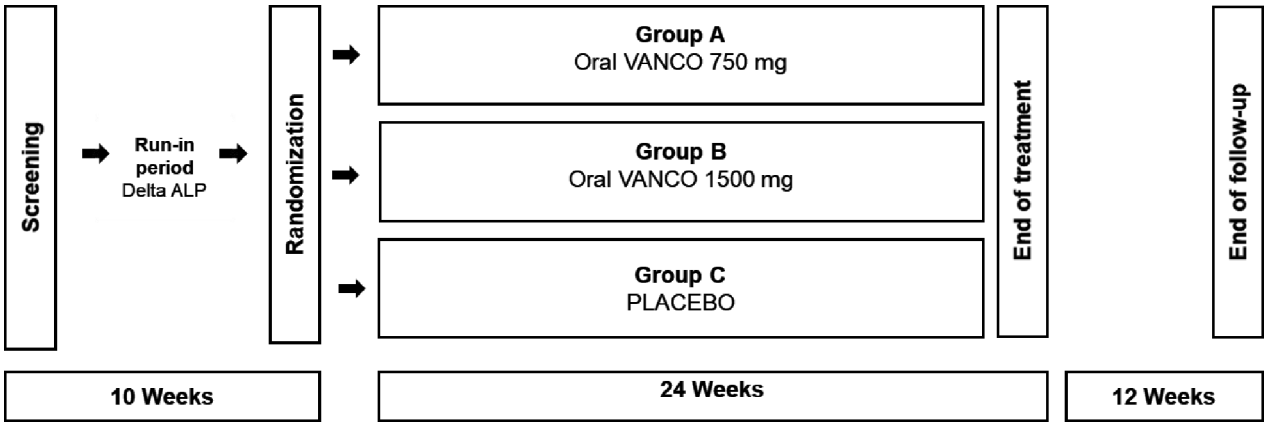


**Figure 1** Study design. Flow of participants through each stage of the VanC-IT trial (enrolment, randomisation, follow-up and analysis). ALP, alkaline phosphatase; VanC-IT, oral Vancomycin for primary sclerosing Cholangitis in ITaly; VANCO, vancomycin.

**Table 1** Eligibility criteria

| Inclusion criteria | Exclusion criteria |
|---|---|
| * Male and non-pregnant, non-lactating female subjects, including women of childbearing potential, between 15 and 70 years of age willing and able to give informed consent prior to any study specific procedure. | * Receiving an antibiotic or probiotic within 3 months prior to the study; or expected to receive antibiotics within the weeks leading up to enrolment (such as patients with recurrent cholangitis, ongoing infectious illnesses). |
| * Diagnosis of large-duct PSC based on cholangiogram (at MRCP, ERCP, PTC) according to the most recent published guidelines (EASL). | * Allergy to vancomycin or teicoplanin; history or active hearing problems. |
| * Baseline ALP ≥1.5 times upper limit normal at screening. | * Biliary intervention within 3 months prior to study enrolment or planned. |
| * Absence of biliary obstruction and/or malignancy within 6–12 months of entry into the study. | * Pregnancy and lactation. |
| * If a patient is on UDCA or 5-aminosalicylic acid, he or she is expected to remain on the same daily dose during the study period. | * Advanced renal disease (GFR <70); or advanced liver disease (history of variceal bleeding, ascites, hepatic encephalopathy and/or bilirubin >4 mg/dL); or on active transplantation list. |
| * Washout period of at least 3 months prior to study entry for antibiotics or probiotics, or obeticholic acid or other experimental therapies (eg, cilofexor and norUDCA) if prescribed for PSC, or rifampicin. Dose changes before the last 3 months prior to baseline of concomitant treatment with vitamin D or fibrates. | * Active hepatitis B and/or C infection; or other chronic or cholestatic liver diseases such as PBC, autoimmune hepatitis, non-alcoholic steatohepatitis, alcoholic liver disease, Wilson's disease, haemochromatosis, α–1 antitrypsin deficiency, IgG4-related sclerosing cholangitis and liver cancer. |
| * Male subjects with female partners of childbearing potential must use condoms during treatment and until the end of relevant systemic exposure. | * History of CCA or any active malignant disease; any known relevant infectious disease (eg, active tuberculosis, AIDS defining disease). |
| * PSC with or without IBD. IBD diagnosis should be documented and with a minimum disease duration of 6 months, as determined by endoscopic and histopathology assessment. IBD should be in clinical remission or mildly active according to CDAI and pMayo score for CD and UC, respectively (ie, patients with CDAI score <220 and pMayo score <5). Patients without documented IBD need a colonoscopy with segmental biopsies within 12 months prior to baseline visit. | * IBD with uncontrolled moderate-to-severe activity; or active treatment or within the previous 4 weeks (washout period) with any immunosuppressive medication for controlling IBD (ie, azathioprine, 6-mercaptopurine, tacrolimus, methotrexate, infliximab, adalimumab, golimumab, vedolizumab, ustekinumab, tofacitinib, ozanimod). Treatment with corticosteroids (including budesonide, budesonide MMX and beclomethasone) in the previous 4 weeks. |

ALP, alkaline phosphatase; CCA, cholangiocarcinoma; CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; EASL, European Association for the Study of the Liver; ERCP, endoscopic retrograde cholangiopancreatography; GFR, glomerular filtration rate; IBD, inflammatory bowel disease; IgG4, immunoglobulin G4; MMX, MultiMatrix System; MRCP, magnetic resonance cholangiopancreatography; PBC, primary biliary cholangitis; pMayo, Partial Mayo Score; PSC, primary sclerosing cholangitis; PTC, percutaneous transhepatic cholangiography; UC, ulcerative colitis; UDCA, ursodeoxycholic acid.

the presence or absence of fibrosis by Fibroscan value at baseline (< or ≥14.4 kPa corresponding to F4 fibrosis), as this parameter could affect the likelihood of reaching the primary outcome measure. Randomisation lists will be generated by R software package RandomizeR for centralised randomisation and treatment assignment. Investigative site personnel will obtain the subject's study drug assignment from the lists. The study drug will be dispensed by the study pharmacist or designee. The drug and the placebo pills are provided for free by *Genetic spa*.

## Blinding

During the randomisation phase, subjects and all personnel directly involved in the conduct of the study will be blinded to the treatment assignment.

Unblinding will occur in the case of participant emergencies and at the conclusion of the study.

## Recruitment and trial schedule

The trial is conducted at the hospital *IRCCS San Gerardo dei Tintori* in Monza, Italy.

*Screening visit:* Potential participants will be identified based on the inclusion criteria. Blood, urine and stool tests will be conducted, and all patients will undergo a magnetic resonance cholangiopancreatography (MRCP) and Fibroscan. Bowel ultrasound and colonoscopy will be performed for patients with known IBD (if a complete, well-conducted and well-documented ileocolonoscopy with multiple random biopsies has not been done in the previous 3 months), as well as for patients without IBD (if an ileocolonoscopy has not been performed within the last 12 months). 2 weeks after the screening visit, patients will repeat the blood test for ALP to assess any fluctuation, with a threshold of 30% accepted for inclusion in the study.

*Baseline visit:* Blood, urine and stool tests will be performed, as well as a rectal swab for vancomycin resistant enterococci (VRE). Study participants will be randomly assigned to either the OV or placebo group, with the investigational drug and placebo dispensed accordingly. Quality of life will be evaluated using the following questionnaires: Visual Analogue Scale (VAS) score for itch, Chronic Liver Disease Questionnaire (CLDQ), EuroQoL-5 Dimension-5 Levels (EQ-5D-5L) questionnaire, PSC patient reported outcome (PSC-PRO) and Inflammatory Bowel Disease Questionnaire (IBDQ).

*Week 4:* A virtual visit will be scheduled for screening adverse events (AEs), with blood tests performed by the patient at a certified local laboratory of their choice and results will be shared with the study team for review.

*Week 12–24 treatment assessments:* Blood, urine and stool tests will be conducted. Serious AEs (SAEs) and all AEs will be recorded. The study drug will be dispensed at week 12, and drug compliance will be reviewed and reconciled using pill counts at weeks 12 and 24. Quality of life will be assessed through the questionnaires. At week 24, Fibroscan and MRCP will be performed on all patients, while colonoscopy and bowel ultrasound will be conducted only for patients with concomitant IBD. Rectal swabs for VRE will also be collected from all patients.

*Early termination (EOT) visit:* Patients who discontinue treatment prematurely will complete an EOT visit within 30 days of their last dose. Blood, urine and stool tests will be performed, and quality of life will be evaluated using the questionnaires. Fibroscan will be conducted if the EOT visit occurs after 12 weeks of treatment.

*Follow-up week 4–12 after treatment discontinuation:* Patients will be contacted by phone for a follow-up visit at 4 weeks after treatment discontinuation, with blood tests performed by the patient at a certified local laboratory and results will be shared with the study team for review. A face-to-face follow-up visit will take place at 12 weeks after the Week 24 or EOT visit, where blood, urine and stool tests will be conducted, quality of life will be assessed using the questionnaires and any AEs or SAEs since the last visit will be recorded.

## Primary endpoints

The primary objective is to compare the effect of two different doses of OV versus placebo evaluating ALP levels at 6 months. The choice of this primary endpoint is based on the evidence from different independent studies that showed patients with ALP <1.5 per ULN have a significantly reduced risk of death, liver transplantation, cholangiocarcinoma and liver decompensation during a 10-year follow-up (OR 0.094, 95% CI 0.33 to 0.027).[17]

## Secondary endpoints

Secondary objective is to determine the safety and tolerability of OV in each treatment arm, and the effect of OV, evaluating biochemical biomarkers of inflammation and cholestasis (reduction of ALP levels ≤40%, reduction of gamma-glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, total and direct bilirubin).

The evolution of the available prognostic score (ie, Amsterdam Oxford score, revised Mayo risk score), the progression of liver fibrosis assessed by liver stiffness measurement using transient elastography; the progression of bile duct strictures at MRCP, evaluated with traditional semiquantitative scoring (Anali criteria) and continuous scoring using *MRCP+* and *Liver multiscan* technology (Perspectum Diagnostics Ltd); the change of non-invasive biomarkers of liver fibrosis (ie, ELF, PRO-C3, PRO-C5; C3M, C4M and BGM), cell apoptosis and necrosis (ie, CK18 M30 and M65), cytokines (TGF-β, interleukin (IL)-4, IL-13, IL-10), peripheral blood mononuclear cells (T helper cell (Th)1 and Th17 subsets) and biomarkers of farnesoid X receptor activity will also be measured (FGF-19, C4 and bile acids). IBD activity and any subsequent changes will be evaluated using the Crohn's Disease Activity Index (CDAI) score and partial Mayo Score (pMCS) for Crohn's disease (CD) and UC, respectively. Additional assessments will include C-reactive protein and faecal calprotectin levels, as well as changes in Simple Endoscopic Score for CD (SES-CD) and the endoscopic Mayo score for CD and UC, respectively, along with

variations in the Nancy Histological Index for UC and Global Histologic Disease Activity Score (GHAS) for CD. Clinical remission will be defined as a CDAI score of <150 for CD or a pMCS of <2 for UC, with evaluations occurring at baseline, weeks 4, 12, 24 and week 12 of follow-up. Endoscopic remission, defined as an SES-CD ≤2 for CD or an endoscopic Mayo score <1 for UC, will be assessed at baseline and week 24. Histological healing, defined as a GHAS≤4 for CD or a Nancy Histological Index <1 for UC, will be measured at baseline and week 24. Changes in ultrasound activity indices, including lesion length, bowel wall thickness, colour Doppler signals, bowel wall stratification, inflammatory mesenteric fat and intestinal complications, will also be assessed at week 24. Finally, quality of life will be investigated by the VAS score for itch, CLDQ, EQ-5D-5L questionnaire, PSC-PRO and IBDQ.

### Exploratory endpoints

Exploratory objectives are to investigate whether OV impacts on hard endpoints such as liver decompensation, ascending cholangitis, dominant strictures, cholangiocarcinoma, hepatocellular carcinoma, liver transplantation or model for end-stage liver disease ≥15 and mortality.

The need for treatment of dominant strictures by endoscopic retrograde cholangiopancreatography or percutaneous transhepatic cholangiography and the lack of progression in bile duct strictures and dilatation (evaluated at *MCRP+*, Perspectum Diagnostics Ltd) will be assessed. Soft endpoints such as changes of the biliary tree and the liver by using advanced imaging protocols (*MRCP+* and *LiverMultiScan*, Perspectum Diagnostics Ltd) and Δ of cT1, an objective mpMR measurement of fibroinflammation (*LiverMultiScan*, Perspectum Diagnostics Ltd) will be explored. Finally, microbiota changes will be assessed at 3 months, 6 months and at 3 months post-treatment (ie, 9 months from baseline).

### Safety

Investigators will record AEs using patients' verbatim description and assign codes and toxicity grades based on Common Terminology Criteria for Adverse Events V.5.0 while ensuring patient safety. AEs will be assessed for their potential relationship to the study treatment or procedures, considering underlying disease, concomitant treatments and other conditions. A 'reasonable possibility' implies a causal link between the study intervention and the AE.

SAEs include death, hospitalisation, life-threatening experiences, significant disability/incapacity, congenital anomalies or other medically significant conditions. Suspected unexpected serious adverse reactions are SAEs not listed in the Investigator Brochure but deemed treatment-related by the investigator will also be reported as per national regulatory requirements.

### Side effects and adverse reactions

The main side effect of OV may be the development of VRE, which is a rare but potentially serious infection. Individuals exposed to vancomycin over prolonged periods may be at risk of VRE colonisation and potentially VRE infections.[18–20] While it has been rarely reported in patients with PSC using OV, we will collect stool samples from study participants and perform rectal swabs to screen for VRE.

Gastrointestinal absorption of orally administered vancomycin is negligible and trivial amounts of orally administered vancomycin reach the blood circulation[21]; however, serum vancomycin trough levels will be obtained in participants who develop any type of colitis, IBD flare and/or kidney injury (creatinine clearance 60–75 mL/min) during treatment.

Rarely, patients taking OV experience nausea, bloating, drug rash and leucopenia. These side effects have rarely been reported in patients with PSC taking OV.

On very rare occasions, the use of OV has been associated with drug-induced liver injury characterised by marked elevated liver aminotransferases. If the study subject meets the criteria for drug-induced liver injury, his/her study anticipation shall be terminated and the patient carefully monitored.

### Statistical analysis

The primary endpoint will be analysed using ANCOVA to assess the association of treatment groups with ALP at 6 months adjusting for ALP baseline values and for the randomisation stratification factor (ie, fibrosis stage).

Comparisons between the mean of ALP in the control group versus both experimental treatment groups are planned to show a trend such that the higher dose tends to have a better response. To this aim and to account for the issue of multiple testing, we plan to use the Bonferroni-Holm method to control for the family-wise error rate at 5% level.

Secondary/exploratory continuous or binary endpoints will be analysed by means of multivariable linear or logistic regression models, adjusting for the stratification factor and the baseline values, where appropriate.

### Patients and public involvement

Patients were involved in the development of the study through consultation with the patient association group Associazione Malattie Autoimmuni del Fegato (AMAF). Their input helped inform aspects of the study design and ensured that the trial addresses outcomes relevant to patients. AMAF will also be engaged in the dissemination of the study findings.

## ETHICS AND DISSEMINATION

The protocol has been approved by the Ethics Committee CE Brianza on 10 February 2023, number 4017. Trial registration number: NCT05876182. The trial was registered at ClinicalTrials.gov on 3 July 2023. The study will comply with the Declaration of Helsinki, Good Clinical Practice (GCP) (Ministerial Decree 15 July 1997), and applicable regulatory requirements. This clinical trial

is conducted in accordance with Regulation (EU) No 536/2014, as implemented in Italy through Legislative Decree No. 52/2019 and follows the operational procedures and ethical guidelines issued by the Italian Medicines Agency (AIFA) and the Italian Ministry of Health. The findings will be reported at national and international gastroenterology meetings and published in peer-reviewed journals.

### Informed consent

A subject is considered enrolled once written informed consent is voluntarily provided. No study procedures will begin before consent is signed. Prior to this, the investigator must clearly explain the study's purpose, procedures, potential benefits, expected and unforeseen risks, using language the subject can understand and allowing ample time for questions. The consent form is given at the start of the screening visit, and the subject may choose to sign immediately or take it home for further consideration.

### Enrolment procedures

Study participants will be recruited from the University of Milan-Bicocca main site: IRCCS San Gerardo dei Tintori, European Reference Network (ERN) RARE-LIVER Center, Monza, Italy

### Treatment of personal data

Personal data must be processed in accordance with the General Data Protection Regulation (GDPR), Legislative Decree 196/2003, and all applicable Italian data protection laws. The institute and the sponsor are joint data controllers for pseudoanonymised data, each within their area of responsibility and must both comply with the relevant regulations. They are required to adopt appropriate technical and organisational measures to ensure GDPR compliance. The institute is responsible for informing investigators and research staff about how their personal data may be processed. In the event of a data breach, the party becoming aware must notify the others within 24 hours and cooperate fully to address the breach as defined by Articles 33 and 34 of the GDPR.

### Insurance

The sponsor will obtain appropriate clinical insurance to cover any injury, death or loss related to drug administration or study procedures, in compliance with the protocol and legal requirements. All participants will be covered by a civil liability policy in accordance with Ministerial Decree 14 July 2009.

### Collection, management and storage of data: CRF

The participating centre must maintain medical records and research data in accordance with Internationa Conference of Harmonisation (ICH)-GCP E6 (Section 4.9), ensuring patient confidentiality. Authorised personnel from the sponsor and regulatory agencies may access medical records for monitoring, auditing and safety assessments. Source documents include medical charts, lab records, patient diaries, drug logs and any original data necessary to reconstruct the study. Data will be collected by site staff under the supervision of the principal investigator using electronic case report forms (eCRF) via the REDCap Cloud platform. Data must be accurate, complete and traceable to source documents. Any discrepancies or corrections must be explained and documented. Essential study documents must be retained for at least 15 years poststudy unless otherwise instructed by the sponsor. Data will be pseudoanonymised using a unique alphanumeric code (eg, HEITMB-001). Each centre will maintain a separate, encrypted, password-protected file linking the code to patient identifiers. This file will not be shared with the sponsor or data analysts. eCRF access is protected by individual login credentials. Passwords must be changed after the first login and will expire every 3 months. Only the study admin is authorised to extract data from the system.

### Quality assurance and control

The trial will follow the approved protocol, ICH-GCP guidelines, applicable regulations and Standard Operating Procedures (SOPs). Site monitoring will ensure subject safety, data accuracy and compliance with ethical standards based on the Declaration of Helsinki and regulatory requirements.

### Clinical monitoring

Monitoring will be conducted by BiCRO (Bicocca Clinical Research Office) using a centralised monitoring approach to ensure patient safety, protocol adherence and data quality. Trained monitors will review eCRFs for completeness and accuracy, resolve queries with site staff and verify source documentation. Interim visits include site initiation, periodic monitoring and close-out visits, with additional visits as needed. Monitoring may occur on-site or remotely. Certified monitors will ensure compliance with the protocol, ICH-GCP and local regulations, documenting all activities and addressing any deviations accordingly.

## TRIAL STATUS

The trial opened for recruitment on 15 June 2023. At the time of protocol submission, 43 patients have been screened for the study, with 24 patients who completed the treatment period of 6 months.

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**Contributors** LC and MC conceived and designed the study; MC secured funding. LC, DD, CM, DPB, MED, SMIM, FF, MMF, AG, ER, FM, PT, MEC, RC, DI, SG, PI and MC were all involved in developing the clinical protocol and authoring the manuscript, with LC being the lead author and investigator, DD the subinvestigator and MC the chief investigator. DPB, SG and ER are the senior statisticians of the project, involved in the trial design, the development of the statistical analysis plan and contributing the bioinformatics expertise. PT is the study coordinator. We used the SPIRIT checklist when writing this protocol. MC is the guarantor. ChatGPT for text editing.

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**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

**Patient consent for publication** Not applicable.

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