CLINICAL STUDY REPORT SYNOPSIS

An open-label, phase IIa study of the safety, tolerability, pharmacokinetics and pharmacodynamics of oral GB2064 (a LOXL2 inhibitor) in participants with myelofibrosis (The MYLOX-1 study)

Study code:	MYLOX-1
EudraCT number:	2020-003087-45
IND number	152073
Sponsor:	Galecto Biotech AB COBIS Science Park Ole Maaloes Vej 3 DK-2200 Copenhagen Denmark
CRO:	OPIS s.r.l Via Matteotti,10 20832 Desio (MB) Italy
Version and date:	V1.0, 20 May, 2024

This study was conducted in compliance with the standards of Good Clinical Practice as defined in the ICH E6 (R2) " Guideline for Good Clinical practice", including the archiving of essential documents.

Name of Sponsor: Galecto Biotech AB
Name of Active Ingredient: GB2064
Study Code: MYLOX-1
Title of Study: An open-label, phase IIa study of the safety, tolerability, pharmacokinetics and pharmacodynamics of oral GB2064 (a LOXL2 inhibitor) in participants with myelofibrosis (The MYLOX-1 study)
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Study Centers: The study was conducted at 10 investigational sites (see above).
Publication (reference): NA

Studied period (years): Date of first enrolment (first subject first visit): June 9th, 2021 Date study finalized (last subject last visit): August 1st, 2024	**Phase of development:** IIa

Objectives:

Primary Objective:

To assess the safety and tolerability of 1000 mg BID GB2064 dosed to participants with primary myelofibrosis (PMF) or secondary myelofibrosis (SMF)

Secondary Objectives:

- To assess the pharmacokinetics (PK) in blood of GB2064 in participants with PMF or SMF
- To assess the effect of GB2064 on clinical parameters of disease activity in participants with PMF or SMF
- To assess the effect of GB2064 on myelofibrosis-related symptoms and quality of life (QoL)
- To assess the effect of GB2064 on the bone marrow

Exploratory Objective:

- To assess the pharmacodynamics (PD) in blood of GB2064 in participants with PMF or SMF
- To assess the relationship between exposure and effects on PD, disease activity in participants with PMF or SMF
- To assess the effect of GB2064 on blood biomarkers of disease activity in participants with PMF or SMF
- To assess the effect of GB2064 on the bone marrow

Methodology:

The MYLOX-1 study is an open-label, Phase IIa study of oral GB2064 (an inhibitor of LOXL2) in participants with primary (PMF) or secondary myelofibrosis (SMF). It consists of two Phases, a Core Phase and an Extension Phase. The study results of the Core Phase are reported here. Upon the last participant completing the last visit in the Extension Phase, the findings of the Extension Phase will be reported as an addendum to this CSR.
After signing the consent form, participants were screened and, if eligible, enrolled in the study. The period between screening and Day 1 was up to 28 days. Participants visited the clinic on 10 occasions during the trial: Screening visit, Days 1, 7 and 15, Months 1, 3, 4, 6, 9 and one follow-up visit. Safety, tolerability, PK, PD and appropriate myelofibrosis (MF)-specific assessments took place at all visits, except Day 7, Day 15 and Month 4, when only safety and tolerability were assessed. A bone marrow biopsy was taken at an appropriate time between screening and Day 1, at Month 3, Month 6 and Month 9. A follow-up visit occurred after the last dose (1 month post last dose).
During the Core Phase of the study, GB2064 was administered orally at a daily dose of 1000 mg BID (4 x 250 mg tablets BID) for nine months.

Number of participants (planned and analysed):

Planned: 16 evaluable participants

Screened: 26

Randomized: NA

Treated: 18

Study Population:

Inclusion criteria:

Participants had to satisfy all of the following criteria at the Screening visit:

1. Adult male or female participants ≥ 18 years of age at enrolment:
   a) Female participants could be of non-childbearing potential defined as permanently sterile or postmenopausal, or female participants considered to be of childbearing potential who agreed to use highly effective birth control methods (Appendix 4 of the protocol) until 90 days after the follow-up visit. Female participants were to refrain from ova donation from the date of Enrolment (Day -1) until 90 days after the follow-up visit.
   b) For German sites, female participants had to be of non-childbearing potential defined as permanently sterile or postmenopausal.

   c) Male participants had to agree to use contraception throughout the study and until 90-days after the Follow-up visit (Appendix 4 of the protocol). For German sites, male participants (even with a history of vasectomy) with partners of childbearing potential had to use a male barrier method of contraception (male condom with spermicide) in addition to a second method of acceptable contraception from the date of Enrolment until 90 days after the Follow-up visit (Appendix 4 of the protocol). Male participants had to agree to refrain from sperm donation from the date of Enrolment (Day -1) until 90 days after the follow-up visit.
2. Diagnosis of PMF or SMF (Barbui et al. 2018, Cruz et al. 2020) with intermediate-2 or high-risk disease according to the Dynamic International Prognostic Scoring System (DIPSS)-plus (Gangat et al. 2011) or if with low-risk disease or intermediate-1 then with symptomatic splenomegaly as defined by sonographic assessment as spleen length of > 12 cm or by physical examination as ≥ 5 cm below left costal margin.
3. Participants who were not currently taking a Janus kinase (JAK) inhibitor (e.g. ruxolitinib or fedratinib) and were therefore refractory, intolerant or ineligible for a JAK inhibitor according to appropriate guidelines (including local guidelines).
4. Eastern Cooperative Oncology Group (ECOG) performance status 0–2 (Azam et al. 2019).
5. Required baseline laboratory status:
   a) Absolute platelet count (APC) ≥ 50 x 10^9/L
   b) Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L (1500/mm^3)
   c) Serum direct bilirubin ≤ 2.0 x ULN (upper limit of normal)
   d) AST (SGOT) or ALT (SGPT) [if both measured, then this applied to both measurements] ≤ 2.5 x ULN, except for participants with MF involvement of the liver who had to have levels ≤ 5 x ULN
   e) Estimated Glomerular Filtration Rate (eGFR) or creatinine clearance (CrCl) (CrCl calculated by the Cockroft and Gault method) ≥ 30 ml/min/1.73 m^2.
   f) Peripheral blood blasts < 10%
6. Treatment-related toxicities from prior therapies had to be resolved to Common Terminology Criteria for Adverse Events (CTCAE) Grade ≤ 1.
7. Participants had to have transfusion records (if there had been any such transfusions) of the preceding 12 weeks to Day 1.

Exclusion criteria

1. Current treatment with a JAK inhibitor (e.g. ruxolitinib or fedratinib) or a history of treatment with a JAK inhibitor within two weeks of enrolment.
2. Positive hepatitis panel (Appendix 2 of the protocol) and/or positive HIV test.

3. Any concurrent severe and/or uncontrolled medical conditions that could have increased the participant's risk for toxicity while in the study or that could have confounded discrimination between disease- and study treatment-related toxicities. Any planned major surgery during the study period.
4. Impaired cardiac function or clinically significant cardiac diseases, including any of the following:
    a. History or presence of ventricular tachyarrhythmia.
    b. Presence of unstable atrial fibrillation (ventricular response > 100 bpm). Participants with stable atrial fibrillation were eligible provided they did not meet any of the other cardiac exclusion criteria.
    c. Clinically significant resting bradycardia (< 50 bpm) and use of a cardiac pacemaker or implantable cardioverter defibrillator.
    d. Angina pectoris or acute myocardial infarction ≤ 90 days prior to starting study drug.
    e. Other clinically significant heart disease (e.g., symptomatic congestive heart failure; uncontrolled arrhythmia or hypertension; history of labile hypertension or poor compliance with an antihypertensive regimen).
5. Immunosuppressive treatment other than the allowed dose of corticosteroids had to be discontinued prior to starting study drug.
6. Participants with impairment of gastrointestinal (GI) function or GI disease that could significantly alter the absorption of GB2064 as per physician's opinion.
7. Participants who received radiotherapy within the last month prior to screening procedures, or patients who received splenectomy in the previous three months or were scheduled for the procedure in the next three months.
8. Participants who had a history of malignancy in the past 3 years, except for treated early stage squamous, basal cell carcinoma or treated, localized prostate cancer.
9. Presence of clinically meaningful active bacterial, fungal, parasitic or viral infection which required therapy.
10. Previous history of Progressive Multifocal Leuko-encephalopathy (PML).
11. Pregnant or breast feeding (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive β- HCG laboratory test.
12. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, unless they were using highly effective methods of contraception during dosing and for 90 days after study treatment. Highly effective contraception methods had to be used (Appendix 4 of the protocol). For German sites, women of childbearing potential were fully excluded from the trial.
13. Sexually active males (including vasectomized) had to use a condom during intercourse while taking the drug and for 90 days after stopping study drug and were not to father a child in this period. A condom was required to be used also by vasectomized men in order to prevent delivery of the drug via seminal fluid.
14. Hypersensitivity to GB2064 and/or its excipients.
15. Participants unable or unwilling to comply with protocol requirements.
16. Participants related to PI/site staff.
17. Participants who had had a hematopoietic stem cell transplantation.
18. Participants who were eligible, had a donor and were willing to undergo a hematopoietic stem cell transplantation within the clinical study period.

Study duration:

The duration of the whole study per participant in this MYLOX-1 Core phase was ten months (nine months of treatment and one month follow-up) plus a screening period of up to 28 days. Participants who continued in the Extension study are expected to undergo up to 45 months of treatment plus one month follow-up.

Test and reference products, dose and mode of administration, batch number

GB2064 administered daily at 1000 mg BID (4 x 250 mg tablets BID) by oral route.

Test product batch: P221220-01, P221220-02, P221220-03, P221220-04, P221220-05, P221220-06, and P221220-07

Criteria for evaluation

Primary endpoints

- Adverse events (AE), serious adverse events (SAE), clinical laboratory assessments, vital signs, ECG, physical examination, body weight

Secondary endpoints

- Pre-dose and post-dose plasma levels of GB2064
- Clinical parameters including, but not limited to changes in anemia, platelets and transfusion dependence, spleen volume
- Clinical Benefit Rate (CBR) and Overall Survival (OS)
- Patient-reported assessments of symptoms and quality of life, as measured by Myeloproliferative Neoplasm score (MPN10) and European Quality of Life 5 Dimensions 5 Level Version (EQ-5D-5L)
- Histopathological examination of the extent of collagen and reticulin in fibrosis in bone marrow biopsies

Exploratory endpoints

- LOXL2 binding assay in the circulation
- Relationships between PK plasma exposures, PD markers, PK plasma exposures and markers of clinical activity
- Biomarkers of fibrosis and inflammation including, but not limited to: YKL-40, PAI-1, PDGF, CCN2, collagen formation and degradation neoepitopes
- PK, PD and biomarkers in bone marrow biopsy tissue

Statistical methods:

Sample Size:

The study was not formally powered. The primary objective of the study was to assess the safety and tolerability of GB2064 in participants with myelofibrosis. A sample size of 16 participants was selected in order to provide adequate safety and tolerability data to address this objective, whilst also limiting the number of participants exposed to experimental treatment and procedures.

A secondary objective of the study was to provide preliminary evidence of the clinical activity of GB2064 in participants with myelofibrosis. Based on the IWG-MRT and ELN criteria (Tefferi et al. 2013), a participant would be considered to have shown clinical benefit if the criteria for Clinical Improvement (Anemia Response, Spleen Response or Symptoms Response) were met at least once post baseline. If the true CBR in this population were 25%, there would be an 80% probability of observing at least 3 participants with clinical benefit out of 16 (an observed CBR of 18.75%). A true CBR of 10% or lower would not be considered favorable in this indication. If the true CBR were 10% or lower, there would be only a 21% chance of achieving 3 or more participants with clinical benefit out of 16.

Methods of Analysis:

No formal statistical analyses were planned.

The data has been listed and summarized using descriptive statistics. Continuous variables have been summarized using the number of observations, mean, standard deviation, median, and range as appropriate. Categorical values

have been summarized using the number of observations and percentages as appropriate. Time-to-event endpoints have been estimated using Kaplan-Meier methodology. Graphical displays were also produced.

SUMMARY – CONCLUSIONS

Baseline and disease characteristics

- A total of 26 participants were screened, and 19 (73.08%) were enrolled in the Core Phase of the study. Out of the 19 participants who were enrolled, 18 (94.74%) initiated GB2064 treatment, 7 (36.84%) completed the study while 12 (63.16%) discontinued from study, with reasons including adverse events, progressive disease and withdrawal by the participant.
- Recruitment was stopped because the trial exceeded the pre-defined target of a ≥ 1 grade reduction in collagen fibrosis in at least three out of sixteen evaluable patients (intermediate assessment showed collagen reduction in 4 out of 5 evaluable patients).
- The mean age of participants was 66.2±8.43, 10 were male and 8 female. All participants had ECOG performance status of 0 or 1, indicating that the population were ambulatory and active with little restriction in physical activity; and most participants fell into the category of Intermediate-2 in terms of DIPSS-plus score. Nine participants (50%) had primary MF while the other 9 (50%) had secondary MF.
- In terms of previous use of JAKi inhibitor such as ruxolitinib, 6 participants (33.33%) were JAKi naïve, 8 (44.44%) refractory, 3 (16.67%) intolerant, and 1 (5.56%) ineligible. All participants harbored "driver mutations" including CALR, JAK2, and MPL. About 40% of the enrolled participants exhibited unfavorable mutations.

Efficacy results

- To assess the systemic pharmacokinetics of GB2064 in participants with MF, GB2064 plasma concentrations were measured prior to and 2 hours after administration of the study drug on Day 1, Month 1, Month 3, Month 6, and Month 9. Post-dose concentrations at all timepoints were in the range of 4698 to 6106 ng/mL, indicating stable exposure of GB2064 over time without additional accumulation once steady-state has been reached.
- Clinical parameters were evaluated to assess the effect of GB2064 on disease activity in MF participants. One participant (5.56%) showed an anemia response at Month 6 during the study. Only 1 participant developed new transfusion requirement while on the study.
- Hemoglobin and platelet counts generally did not change during the study for most participants. Platelet count shifts were observed in a small number of participants who showed improvement from "low" to "normal", or "high" to "normal" at certain timepoints. Similarly, shifts in hemoglobin levels were observed in a small number of participants who showed hemoglobin levels reaching normal range, but there was also a small number of participants who exhibited reduction in hemoglobin levels (from "normal" to "low").
- For MRI spleen assessments, two participants showed a ≥ 35% spleen volume reduction (SVR35) at Month 6. Of these, only 1 participant was deemed to have achieved a spleen volume response by the investigator. *(The MRI results of certain parameters of another participant was ambigous which lead to SVR35 not being achieved)*
- Seven (38.89%) out of 18 participants achieved a reduction in DIPSS Plus Score relative to baseline, and 2 participants (11.11%) out of 18 achieved a reduction in ECOG Score relative to baseline.
- The CBR is defined as the proportion of participants deriving clinical benefit during the study. No clinical benefit event was seen at Month 1, and the CBR was 0.06 at Months 3, 6, and 9 (One participant showed benefit at Months 3 and 6; whereas another participant showed benefit at Month 9. When splitting by time period, only 1 appeared at Months 3, 6, and 9). Overall survival could not be estimated as only 1 death occurred.
- Two participants had a TSS50 at Month 6 indicating that they expressed a reduction in symptom burden associated with MF during the study with a number of participants showing a relatively stable or improved EQ-5D-5L score.

- Histopathological examination of bone marrow biopsy tissue including an evaluation of the extent of fibrosis was performed. GB2064 is hypothesized to inhibit crosslinking of extracellular matrix collagen and elastin. Two out of 10 participants belonging to the Full Analysis Set (FAS))(with available Baseline and Month-6 bone marrow biopsy)) showed a ≥ 1 grade reduction in reticulin fibrosis, and 6 out of 10 FAS participants showed a ≥ 1 grade reduction in collagen fibrosis. As bone marrow fibrosis is a central pathological feature of myelofibrosis, the observed reduction of bone marrow fibrosis in this study may indicate a disease-modifying potential of GB2064 in the treatment of myelofibrosis.
- All participants had high levels of serum lactate dehydrogenase (LDH) throughout the study. This is typically seen in patients with PMF and has been associated with increased cell turnover from clonal myeloproliferation, including leukocytosis, hemolysis and extra-medullary hematopoiesis in the liver. LDH assessments in this study were performed locally and were not part of the central laboratory assessments in the protocol, however from an observatory perspective, a reduction in LDH values was seen in 3 participants out of results received from 12 participants, which may be of clinical significance.
- Clinical efficacy signals such as bone-marrow collagen fibrosis reduction were seen in 6 participants, TSS50 in 2 participants, spleen response (SVR35) in one participant and anemia response in one participant.
- Out of the 19 participants enrolled and 18 treated in the study, 5 participants were enrolled into the Extension Phase as the Investigator deemed they would benefit from longer treatment.

Safety results

- Seventeen participants (94.44%) reported at least one treatment-emergent adverse event (TEAE), 7 (38.89%) reported at least one serious TEAE, 12 (66.67%) at least one IMP-related TEAE, and 9 (50.00%) at least one Grade 3 or above TEAE. The most common TEAEs by System Organ Class (SOC) were gastrointestinal disorders (13 patients, 72.22%; 29 GI-related TEAEs) including nausea and vomiting.
- GI-related AEs were mostly mild or moderate (Grade 1 or 2) and were self-limiting or responded to standard anti-emetic therapy, however there were four GI-related AEs that led to study discontinuation, including one G3 diarrhea,, one G1-G2 nausea, one G2 nausea and one G1 vomiting.
- The most frequently observed IMP-related AEs by SOC were also “gastrointestinal disorders” (9 patients, 50.00%). There was only 1 participant (5.56%) who reported an IMP-related serious adverse event, which was a classified as a “fall” by Preferred Term (PT).
- One (5.56%) participant died during the study, and the cause of death was attributed to sepsis with multiple organ failure.
- No major liver function derangements were observed across the study. One participant demonstrated a case of mild to moderate (G1-G2) elevated levels of GGT and ALP after one month of dosing. The participant remained on GB2064 for the entire duration of the study, and the liver function test values slowly declined. The participant wasn’t referred to a hepatologist, and the root cause of the event remains unknown.
- No significant worsening of anemia and thrombocytopenia was observed in the study.

Conclusion

- GB2064 showed stable plasma concentrations in MF patients throughout the study and no accumulation effect was observed upon reaching the steady-state.
- Six out of ten participants evaluable for BM fibrosis were responders to GB2064 therapy and demonstrated a ≥ 1-grade reduction in bone marrow collagen fibrosis over the course of treatment. This unique reduction in collagen bone marrow fibrosis may be a notable contributor to overall clinical benefit. In addition, two out of ten evaluable participants for BM fibrosis also showed a ≥ 1-grade reduction in reticulin fibrosis.
- The participants that experienced a ≥1-grade reduction in bone marrow collagen fibrosis in MYLOX-1 all showed disease stabilization when progression would typically have been expected.

- Two participants achieved a TSS50 response, one had an Anemia Response, and one had a spleen response in the MYLOX-1 Core phase. These 4 participants also had a corresponding bone marrow fibrosis reduction.
- Five participants demonstrated clinical benefit at Month 6 and 9, as assessed by the investigator, and continued into the extension phase of the study, allowing for up to three additional years of GB2064 therapy.
- AEs were generally mild and tolerable. GI effects that initially led to study discontinuations were less of a concern as the study progressed through the effective use of anti-emetics.
- GB2064 appears to have an acceptable safety and tolerability profile and may have a disease-modifying effect in patients with MF.

Date of report: 20 May 2024