# CLINICAL STUDY PROTOCOL

## TITLE PAGE

| | |
|---|---|
| **Title** | A Randomized, Double-Blind, Placebo-Controlled, Multiple Ascending Dose Study to Evaluate the Safety, Tolerability and Pharmacokinetic Properties of BIO89-100 Administered Subcutaneously in Subjects with Nonalcoholic Steatohepatitis (NASH) or with Nonalcoholic Fatty Liver Disease (NAFLD)and at High Risk of (NASH) |
| **Protocol Number:** | BIO89-100-002 |
| **Compound Number:** | BIO89-100 |
| **Study Phase:** | Phase 1b/2a |
| **Sponsor Name:** | 89bio LTD. |
| **Legal Registered Address:** | 6 Hamada Street, Herzliya 4676672 Israel |
| **Regulatory Agency Identifier Number:** | IND: 131934 |
| **Sponsor Representative:** | [redacted] |
| **Approval Date:** | 11 March 2021 |
| **Version:** | 5.0 |
| **Amendment:** | 5.0 |

This clinical study will be conducted in accordance with current Good Clinical Practice (GCP) as directed by the provisions of the International Council for Harmonisation (ICH); United States (US) Code of Federal Regulations (CFR), and European Union (EU) Directives and Regulations (as applicable in the region of the study); national country legislation; and the Sponsor's Standard Operating Procedures (SOPs).

**CONFIDENTIALITY STATEMENT**

# SIGNATURE PAGE

**Sponsor Signatory:**

[redacted] 89bio, Ltd.

**Date**

**Medical Monitor Name and Contact Information will be provided separately.**

# PRINCIPAL INVESTIGATOR SIGNATURE PAGE

| | |
|---|---|
| **Protocol Title** | A Randomized, Double-Blind, Placebo-Controlled, Multiple Ascending Dose Study to Evaluate the Safety, Tolerability and Pharmacokinetic Properties of BIO89-100 Administered Subcutaneously in Subjects with Nonalcoholic Steatohepatitis (NASH) or with Nonalcoholic Fatty Liver Disease (NAFLD)and at High Risk of (NASH) |
| **Protocol Number** | BIO89-100-002 |
| **Version and Date** | 5.0, 11 March 2021 |
| **Amendment No.** | 5.0 |
| **IND Number** | IND: 131934 |

I, the undersigned, have read this protocol and agree to personally supervise conduct of this protocol in accordance with ethical principles as outlined in the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice, any applicable laws and requirements (including Part 54: Financial Disclosure by Clinical Investigators) and any additional conditions mandated by a regulatory authority and/or Institutional Review Board/Independent Ethics Committee (IRB/IEC).

I acknowledge that I am responsible for the overall study conduct; I approve of and will comply with all conditions, instructions and restrictions described in this protocol. I am aware that my adherence to the above protocol is mandatory and that any changes in the protocol or consent form, except those necessary to eliminate apparent immediate hazards to human subjects, must first be approved in writing by 89bio, Ltd. And the respective IRB/IEC.

I also agree that all information provided to me by the Sponsor, including this document, Investigator's Brochure, case report form, and verbal and written information, will be kept strictly confidential and confined to the clinical personnel involved in conducting the study. It is recognized that this information may be related in confidence to the IRB/IEC. I also understand that reports of information about the study or its progress will not be provided to anyone not involved in the study other than to the Principal Investigator, or in confidence to the IRB/IEC or to the FDA or other legally constituted authority.

_____ _____

Principal Investigator Signature Date

_____ _____ _____

Printed Name Institution City, Country

# PROTOCOL AMENDMENT SUMMARY OF CHANGES

| **DOCUMENT HISTORY** | |
|---|---|
| **Document** | **Date** |
| Version 5.0, Amendment 5.0 | 11 March 2021 |
| Version 4.1, Amendment 4.0 | 22 October 2020 |
| Version 4.0, Amendment 4.0 | 06 October 2020 |
| Version 3.0, Amendment 3.0 | 25 March 2020 |
| Version 2.0, Amendment 2.0 | 09 February 2020 |
| Version 1.1, Amendment 1.0 | 29 June 2019 |
| Original, Version 1.0 | 16 May 2019 |

**Amendment:** 5.0, 11 March 2021

**Overall Rationale for Changes implemented in Version 5.0:**

| **Section # and Name** | **Description of Change** | **Brief Rationale** |
|---|---|---|
| Section 4.3.1 Part 2 Inclusion Criteria | IC # 3b: Revised to specify fasting plasma glucose ≥126 mg/dL or previous diagnosis of T2DM<br>IC # 4b: Clarified the acceptable timeframe for historical liver biopsy<br>IC # 5a: Clarified that MRI-PDFF will be adequate to assess steatosis if a Fibroscan model with CAP technology is unavailable<br>IC # 7e and IC # 9: Clarified the definition of surgical sterilization and removed the requirement for medical documentation for eligibility consideration<br>IC # 14: Added that COVID-19 protocols may be excepted with Medical Monitor (or designee) approval<br>IC # 15: Removed the criterion | To clarify the inclusion criteria and to facilitate enrolment in Part 2 of the study |

| Section # and Name | Description of Change | Brief Rationale |
|---|---|---|
| Section 4.3.2 Part 2 Exclusion Criteria | Removed certain ECs: # 4 (cardiac arrhythmia), # 11 (major trauma or surgery), # 12 (acute illness), # 17 (cigarette use), # 31 (blood donation)<br>Clarified/revised the wording in the following ECs related to: (new #)<br>Medical conditions: # 1, 2, 4, 6, 7, 8, 9, 10, 12, and 13<br>Prior and concomitant therapy: #15, 18, and 19<br>Diagnostic assessments: # 21, 22, 23, 24, and 25<br>Other exclusions: 26, 27, and 31 | To clarify the exclusion criteria and to facilitate enrolment in Part 2 of the study |
| Section 5.6.1 Prohibited Medication | Included gastric banding and sleeve surgery as prohibited bariatric surgery<br>Removed insulin from the prohibited medication list<br>Revised the wording on Vitamin E supplementation<br>Specified that short term oral steroid bursts with/without taper as well as topical and inhaled steroids are allowed | For consistency with the changes made in the exclusion criteria |
| Appendix 4 Contraceptives | Removed the requirement for documentation of surgical sterilization | For consistency with the changes made in IC # 7e and IC # 9 |
| Signature Page | Changed the Sponsor Signatory | Update in Sponsor Personnel |

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## LIST OF TABLES

# 1. PROTOCOL SUMMARY

## 1.1. Synopsis

**Protocol title:** A Randomized, Double-Blind, Placebo-Controlled, Multiple Ascending Dose Study to Evaluate the Safety, Tolerability and Pharmacokinetic Properties of BIO89-100 Administered Subcutaneously in Subjects with Nonalcoholic Steatohepatitis (NASH) or with Nonalcoholic Fatty Liver Disease (NAFLD) and at High Risk of NASH

**Rationale**

BIO89-100, developed by 89bio, is a glycoPEGylated analogue of fibroblast growth factor 21 (FGF21), a metabolic hormone secreted by the liver, adipose tissue, skeletal muscle, and the pancreas, that is regulated by nutritional status and affects energy expenditure, and glucose and lipid metabolism (Lee, 2014) (Arner, 2008) (Park, 2016). Administration of exogenous FGF21 is being explored as a method to treat obesity-associated insulin-resistance disorders, a category that includes Non-Alcoholic Fatty Liver Disease (NAFLD), the most common chronic liver disorder worldwide, and its higher-risk variant Non-Alcoholic Steatohepatitis (NASH). There are currently no approved pharmaceutical treatments for NASH (Friedman, 2018). Several nonclinical and clinical studies have shown that administration of various FGF21 analogues had beneficial effects on serum lipid and insulin-resistance as well as on liver fat (Zhang 2014) (Gaich, 2013) (Sanyal, 2019).

This Phase 1b/2a study has 2 parts. Part 1 is designed to assess the safety and tolerability of repeated subcutaneous (SC) escalating doses of BIO89-100 in subjects with a diagnosis of NASH, or who have NAFLD with a high risk of NASH. Part 2 is open-label, designed to assess the effect of repeated SC doses of one dose level of BIO89-100, administered for 20 weeks, on liver histology in subjects with biopsy-proven NASH with NAFLD Activity Score (NAS) ≥4, fibrosis stage F1 with high risk, F2 or F3.

The study includes safety, pharmacokinetic (PK), pharmacodynamic (PD), and immunogenicity characterizations to allow a preliminary assessment of a safe and efficacious dose range to be studied in further clinical studies.

**Rationale for Part 2 (Cohort 7)**

To obtain an initial understanding of the potential histological benefit of BIO89-100, an open-label cohort (Cohort 7) has been added under Part 2 for evaluation of the effect of 20 weeks of dosing with BIO89-100 at a dose of 27 mg/week (QW) on liver histological endpoints. Assessments for this cohort will include safety, tolerability, PK, and PD parameters (with some modifications in comparison to Cohorts 1-6, see Cohort 7 Schedule of Activities [SoA], Table 6). This cohort will enroll 20 subjects with biopsy-proven NASH (NAS ≥4, fibrosis stage F1 with high risk, F2 or F3) as determined by a baseline biopsy (to be done during screening; subjects with historical biopsies performed within 24 weeks of Day 1, that are available for central read and determined to meet inclusion criteria by the central reader, will also be eligible). Subjects will undergo a second liver biopsy within [redacted] after the last dose of study intervention. Histological assessment will include ≥2-point improvement in NAS

with at least a 1-point improvement in ballooning or lobular inflammation, and no worsening of fibrosis (primary endpoint); improvement of fibrosis ≥1 stage without worsening of NAS (secondary endpoint); and NASH resolution without worsening of fibrosis[1] (secondary endpoint). Additional exploratory histological endpoints (e.g., other endpoints related to NAS, fibrosis, or both; histological assessments in subgroups; e.g., subjects with ≥30% relative reduction in hepatic fat as assessed by magnetic resonance imaging [MRI]-proton density fat fraction [PDFF] on Week 12 compared to baseline) will be evaluated. Biopsies will be assessed by a central reader; in addition, biopsies will be assessed with artificial intelligence (AI)-based technology.

**Objectives and Endpoints**

**Part 1 (Cohorts 1-6)**

| Objectives | Endpoints |
|---|---|
| Primary | |
| • To evaluate the safety and tolerability of ascending multiple SC injections of BIO89-100 in subjects with NASH or who have NAFLD and at a high risk of NASH | • Frequency and severity of adverse events (AEs) and serious adverse events (SAEs)<br>• Number of subjects who discontinued due to AEs and due to related AEs |
| • To characterize BIO89-100 PK | • Maximal observed serum concentrations ($C_{max}$) within a dosing interval<br>• Area under the serum drug concentration by time curve within a dosing interval ($AUC_{0\text{-}tau}$)<br>• Time to achieve $C_{max}$ ($t_{max}$)<br>• Terminal elimination half-life ($t_{½}$)<br>Additional PK parameters may be calculated if deemed appropriate.<br>The serum concentration-time data may also be used for population PK modeling with the results reported separately from the clinical study report. |
| Secondary | |

[1] Resolution of NASH includes the total absence of ballooning (score=0) and absent or mild inflammation (score 0 to 1) associated with at least a 2 point reduction in NAS, and no worsening if fibrosis (progression ≥ 1 stage).

| Objectives | Endpoints |
|---|---|
| • To evaluate the immunogenicity of BIO89-100 as measured by presence of anti-drug antibodies (ADA) | • Assessment of the incidence and characteristics of ADA after dosing (e.g., titer and/or binding specificity, to the FGF21, PEG part of BIO89-100, and neutralizing immunogenicity)<br>• Impact of the presence of ADAs on serum BIO89-100 concentrations and clinical safety<br>Additional ADA assessments will be performed if a severe hypersensitivity reaction (e.g., anaphylaxis) is observed. |
| • To characterize biomarkers, PD profile, and biological activity of BIO89-100 administered at ascending doses and with both QW and Q2W dosing intervals<br>• To evaluate the time, dose, and exposure relationship of BIO89-100 on biological activity, as assessed by biomarkers and PD | Change and percentage change from baseline in the following biomarkers/PD parameters:<br>• Anthropomorphic measurements:<br>– Body weight<br>• Laboratory parameters:<br>– Triglycerides<br>– Non-high density lipoprotein (non-HDL) cholesterol<br>– High density lipoprotein (HDL-c)<br>– Low density lipoprotein (LDL-c)<br>– Hemoglobin A1c (HbA1c)<br>– Homeostatic model assessment of insulin resistance (HOMA-IR)<br>– Liver function tests: alanine transaminase (ALT), aspartate transaminase (AST)<br>– Adiponectin<br>– N-terminal propeptide of type III collagen (Pro-C3)<br>– Free fatty acids and Adipo-IR (fasting free fatty acids × fasting insulin)<br>• Imaging measures: |

| Objectives | Endpoints |
|---|---|
| | – Magnetic resonance imaging – Proton density fat fraction (MRI-PDFF) |
| Other Safety Endpoints | |
| [redacted] | |

**Part 2 (Cohort 7)**

| Objectives | Endpoints |
|---|---|
| Primary | |
| • To evaluate the safety and tolerability of SC injections of 27 mg BIO89-100, administered weekly for 20 weeks, in subjects with biopsy-proven NASH (NAS ≥4, fibrosis stage F1 with high risk, F2 or F3) | • Frequency of AEs and SAEs |
| • To characterize effect of BIO89-100 on liver histology | • At least a 2-point improvement in NAFLD Activity Score (NAS) with at least a 1-point improvement in ballooning or lobular inflammation, and no worsening of fibrosis |
| Secondary | |
| • To characterize biomarkers, PD profile, and biological activity of BIO89-100 | Change and percentage change from baseline in the following biomarkers/PD parameters:<br>• Anthropomorphic measurements:<br>– Body weight<br>• Laboratory parameters:<br>– Triglycerides |

| Objectives | Endpoints |
|---|---|
| | – non-HDL cholesterol<br>– HDL-c<br>– LDL-c<br>– HbA1c<br>– Liver function tests: ALT, AST<br>– Pro-C3<br>• Imaging measures:<br>– MRI-PDFF |
| • To characterize effect of BIO89-100 on liver histology | • Improvement of fibrosis ≥1 stage without worsening of NASH<br>• NASH resolution without worsening of fibrosis[2] |
| • To characterize BIO89-100 PK | • Trough concentration of BIO89-100 |
| Other Safety Endpoints | |
| [redacted] | |

[2] Resolution of NASH includes the total absence of ballooning (score=0) and absent or mild inflammation (score 0 to 1) associated with at least a 2-point reduction in NAS, and no worsening if fibrosis (progression ≥ 1 stage).

| Objectives | Endpoints |
|---|---|
| | [redacted] |

**Overall Design**

This study has 2 parts:

- Part 1 is a randomized, double-blind, placebo-controlled, multiple ascending dose (MAD) study to evaluate the safety, tolerability, PK and PD profiles, and immunogenicity of BIO89-100 administered SC in approximately 83 subjects with NASH, or with NAFLD who are at a high risk of NASH. This multi-site study will consist of 6 cohorts and will evaluate 2 dosing schedules, weekly (QW; Cohorts 1 to 4) and every 2 weeks (Q2W; Cohorts 5 and 6) (Table 1).
- Part 2 is an open-label cohort (Cohort 7) in which BIO89-100 will be administered SC weekly at a single dose level (27 mg QW) to 20 subjects with biopsy-proven NASH (NAS ≥4, fibrosis stage F1 with high risk, F2 or F3), to evaluate effect on histological endpoints.

The study will include a Screening period, a Treatment period, and a Follow-up period. After signing informed consent, subjects will undergo screening assessments to determine eligibility over a period of up to 60 days.

**Part 1 (Cohorts 1-6)**

Eligible subjects for Cohorts 1-6 will be randomized for each cohort as described in Section 5.4. Randomization to cohorts 3 and 5 will be stratified by biopsy-confirmed NASH with fibrosis status F1, F2, or F3 as described in Section 5.4.The decision to randomize into the next cohort (dose level) will be approved by a Safety Monitoring Committee (SMC), comprised of Principal Investigator participating in the study, the Clinical Research Organization (CRO) Medical Monitor, and the Sponsor Medical Monitor. The SMC will review blinded safety data (adverse events, clinical laboratory data, vital signs, and electrocardiograms [ECG]), summary of PK data, if available, and other blinded relevant data. There will be 2 dose escalation decisions. After Cohort 1 completes the Day 36 visit, the SMC will decide whether subjects can be randomized into Cohorts 2 and 5 (both cohorts to start concurrently). After at least 8 subjects from both Cohort 2 and Cohort 5, including at least 1 subject on placebo in each cohort, complete the Day 36 visit, the SMC will decide whether subjects can be randomized into Cohorts 3, 4, and 6 (all three cohorts to start concurrently).

For all subjects, treatment with study intervention may be continued for 8 additional weeks (total of 12 weeks) following review of individual safety data (AEs, ECG, physical examination, and safety laboratory) through Day 30 (4 weeks) by the site Investigator, CRO Medical Monitor and Sponsor Medical Monitor. Subjects who experience clinically significant treatment-emergent AEs that are assessed as a potential risk to subject safety will be discontinued from study intervention. The decision to discontinue study intervention will be made by the site Investigator following discussion with the Sponsor Medical Monitor.

Cohorts 1 to 4 (weekly regimen): On Day -1, eligible subjects will be randomized (as described above) and be treated with weekly (QW) SC injection of study intervention starting on Day 1 and continuing through Day 85; subjects will be domiciled at the Phase 1 unit from Day -1 (day before 1st dose) to Day 2 and from Day 28 (day before the 5th dose) to Day 30. Subjects will attend ambulatory clinic visits for dosing and evaluations per the Schedule of Activities (SoA; Section 1.2.1, Table 2). On the Day 8, Day 15, and Day 22 ambulatory dosing visits, subjects will remain at the study site for observation for at least 2 hours post dose.

Cohorts 5 and 6 (every 2 weeks regimen): On Day -1, eligible subjects will be randomized (as described above) and be treated with SC injection of study intervention every 2 weeks (Q2W) starting on Day 1 and continuing through Day 85; subjects will be domiciled at the Phase 1 unit from Day -1 (day before 1st dose) to Day 2 and from Day 28 (day before the 3rd dose) to Day 30. Subjects will attend ambulatory clinic visits for dosing and evaluations per the SOA (Section 1.2.1, Table 4). On the Day 15 ambulatory dosing visit, subjects will remain at the study site for observation for at least 2 hours post dose. On Days 64 and 78, subjects will be contacted by phone to inquire about AEs and concomitant medications.

Subjects in all cohorts will be followed up on Day 92 (1 week post last dose of study intervention) and Day 113, 4 weeks post last dose of study intervention (End of Study visit). Subjects who are found to be neutralizing anti-drug antibodies (NAb)-positive at the End of Study visit will be followed for 3-5 months after this visit or until stable or declining as deemed by the Medical Monitor.

**Part 2 (Cohort 7)**

Part 2 is an open-label cohort that will enroll 20 subjects with biopsy-proven NASH and fibrosis (NAS ≥4, fibrosis stage F1 with high risk, F2 or F3). Subjects will either undergo a liver biopsy during screening or have a recent liver biopsy (within ≤24 weeks before Day 1, available to be evaluated for eligibility by the central reader) that meets study inclusion criteria. Eligible subjects will be treated with weekly (QW) SC injection of study intervention starting on Day 1 and continuing through Day 134 (20 weeks of treatment). Subjects will attend ambulatory clinic visits for dosing and evaluations and home visits for dosing as per the Schedule of Activities[3] (SoA; Section 1.2.2, Table 6). Subjects will undergo a second liver biopsy within 14 days after the last dose of study intervention. Subjects will be followed up on Day 141 (1 week post last dose of study intervention) and Day 162 (4 weeks post last dose of study intervention, End of Study visit). [redacted]

[3] If home administration is not feasible, not desired by the subject, or deemed inappropriate option by the investigator for any reason, in-clinic study intervention administration may take place at some or all timepoints.

[redacted]. More frequent testing (e.g., every month) or testing for a longer period of time may be requested in the event of safety-related concerns.

### Number of Subjects and Intervention Groups

A total of approximately 103 subjects, consisting of: 6 subjects to receive active and 2 subjects to receive placebo in Cohort 1; 12 subjects to receive active and 3 subjects to receive placebo in Cohort 2; 9 subjects to receive active and 3 subjects to receive placebo in Cohorts 4 and 6; 14 subjects to receive active and 4 subjects to receive placebo in Cohorts 3 and 5; and 20 subjects to receive active (open-label) in Cohort 7. Dose escalation cohorts are shown in Table 1. Based on emerging data and SMC recommendation, the Sponsor can decide to modify the dose of any subsequent cohort.

**Table 1 Study Cohorts**

| Cohort | Dose Level[a] | Frequency and Route of Administration | Number of Subjects | |
|---|---|---|---|---|
| | | | BIO89-100 | Placebo |
| 1 | 3 mg | Weekly (QW), SC to abdomen (1 injection)<br>Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, and 85<br>Total doses (initial 4 weeks + 8-week extension): 5 + 8 | 6 | 2 |
| 2 | 9 mg | QW, SC to abdomen (1 injection)<br>Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, and 85<br>Total doses (initial 4 weeks + 8-week extension): 5 + 8 | 12 | 3 |
| 3 | 18 mg | QW, SC to abdomen (1 injection)<br>Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, and 85<br>Total doses (initial 4 weeks + 8-week extension): 5 + 8 | 14 | 4 |
| 4 | 27mg | QW, SC to abdomen (2 injections)<br>Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, and 85<br>Total doses (initial 4 weeks + 8-week extension): 5 + 8 | 9 | 3 |
| 5 | 18 mg | Every 2 weeks (Q2W), SC to abdomen (1 injection)<br>Days 1, 15, 29, 43, 57, 71, and 85.<br>Total doses (initial 4 weeks + 8-week extension): 3 + 4 | 14 | 4 |

| Cohort | Dose Level[a] | Frequency and Route of Administration | Number of Subjects | |
|---|---|---|---|---|
| | | | BIO89-100 | Placebo |
| 6 | 36 mg | Q2W, SC to abdomen (2 injections)<br>Days 1, 15, 29, 43, 57, 71, and 85.<br>Total doses (initial 4 weeks + 8-week extension): 3 + 4 | 9 | 3 |
| 7 (open-label) | 27 mg | QW, SC to abdomen (2 injections)<br>Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 92, 99, 106, 113, 120, 127, and 134<br>Total doses: 20 | 20 | NA |

[a] The actual doses will be ±5% the mg dose due to technical considerations related to drug withdrawal from the vials into the syringes for injection. This difference is considered negligible for subject exposure.

**Study Duration**

**Part 1 (Cohorts 1-6)**

For each subject in Part 1, the total duration of study participation will be up to 172 days (24.5 weeks), excluding the potential study interruption due to the COVID-19 pandemic:

Screening period: ≤ 60 days (8.5 weeks)

Treatment period: 84 days (12 weeks)

Follow-up period 28 days (4 weeks)

**Part 2 (Cohort 7)**

For each subject in Part 2 (Cohort 7), the total duration of study participation will be up to 221 days (31.5 weeks), excluding the potential study interruption due to the COVID-19 pandemic:

Screening period: ≤ 60 days (8.5 weeks)

Treatment period: 134 days (20 doses in 19 weeks)

Follow-up period 28 days (4 weeks [4])

**Safety Monitoring Committee**: Yes (Cohorts 1-6)

[4] Subjects who are found to be NAb-positive at the End of Study visit will be followed every 3 months starting from when the site has been notified of the positive result, until: (1) neutralizing antibodies are no longer detectable or (2) the subject has been followed for a period of at least 1 year (± 4 weeks). More frequent testing (e.g., every month) or testing for a longer period of time may be requested in the event of safety-related concerns.

## 1.2. Schedule of Activities (SoA)

### 1.2.1. Part 1

**Table 2 Part 1 Schedule of Activities for Cohorts 1-4**

| Cohort 1-4 Assessments | Screening Period | | | Treatment Period | | | | | | | | | | | | | | | FU Period | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Visit | | | | | | | | | | | | | | | | | | | | |
| Study Day | | | | | | | | | | | | | | | | | | | | |
| Informed consent | X | | | | | | | | | | | | | | | | | | | |
| Medical history/demographics | X | | | | | | | | | | | | | | | | | | | |
| Percutaneous liver biopsy (optional)[b] | X | | | | | | | | | | | | | | | | | | | |
| Prior medications | X | | | | | | | | | | | | | | | | | | | |
| Inclusion and exclusion criteria | X | | X | | | | | | | | | | | | | | | | | |
| Complete physical exam[c] | X | | | | | | | | | | | | X | | | | | | X | X |
| Symptom-directed physical exam[d] | | | X | B | P | | | | B | B | B | | B | | | | | B | | |
| Body weight[e] | X | | | B | | | | | B | B | B | | B | | | | | B | | |
| Waist and hip measurements | X | | | B | | | | | | | | | B | | | | | | X | X |
| Fibroscan[f] | X | | | | | | | | | | | | | | | | | | X | |
| 12-lead ECG (single)[g] | X | | | B | X | | | | B | B | B | | B | X | | | | B | X | X |
| Urine drug screen and, alcohol breath test | X | | X[h] | | | | | | | | | | | | | | | | | |
| Clinical laboratory tests[i] | X | X | | B | X | | | | B | B | B | | B | X | | | | B | X | X |
| Cortisol (24 hour urine collection)[j] | X | | | | | | | | | | | | | | | | | | X | |
| Urinalysis | X | | X | | | | | | | | | | | X | | | | | X | X |
| HbA1C | X | | X | | | | | | | | | | | | | | | | X | X |
| Serology[k] | X | | | | | | | | | | | | | | | | | | | |
| TSH | X | | | | | | | | | | | | | | | | | | | |

| **Cohort 1-4 Assessments** | **Screening Period** | | | **Treatment Period** | | | | | | | | | | | | | | | **FU Period** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Visit** | [redacted] | | | | | | | | | | | | | | | | | | | |
| **Study Day** | | | | | | | | | | | | | | | | | | | | |
| Pregnancy test in WOCBP only[l] | X(S) | | X | | | | | B | B | B | | B | | | | | B | B | X | X |
| Vital signs[m] | X | | X | X | P | X | X | X | X | X | X | X | P | X | X | X | X | X | X | X |
| Columbia-Suicide Severity Rating Scale (C-SSRS) | | | X | | | | | | | | | | | | | | D50 | | X | |
| Randomization | | | X | | | | | | | | | | | | | | | | | |
| Study Intervention administration[n] | | | | X | | | | X | X | X | | X | | | | | X | X | | |
| PK blood collection[o] | | | | X | X | X | X | B | B | B | | X | X | X | X | X | [redacted] | B | X | |
| PD and biomarker blood sampling: | | | | | | | | | | | | | | | | | | | | |
| IGF-1, total | | | | B | | | | B | B | | | B | | | | | | | X | |
| Adiponectin | | | | B | | | | B | | | | B | | | | | | | X | X |
| CK-18, ELF panel and Pro-C3 | | | | B | | | | | | | | B | | | | | | | X | [redacted] |
| Free fatty acid | | | | B | | | | B | | | | B | | | | | | | X | X |
| Oral glucose tolerance test (OGTT)[p] | | | X | | | | | | | | | | | | | | | | X | |
| Insulin (not part of OGTT) | | | | B | | | | B | | | | B | | | | | | | | X |
| HOMA-IR calculation | | | | X | | | | | | | | | | | | | | | X | X |
| MRI-PDFF; visceral fat; and subcutaneous fat[q] | X | | | | | | | | | | | | | | | | | | X | |
| Plasma sample for potential future bone biomarkers analysis[r] | | | X | | | | | | | | | | | | | | | | X | X |
| Pharmacogenomic (DNA) blood sampling | | | X | | | | | | | | | | | | | | | | | |
| Exploratory biomarker analysis[s] | | | X | | | | | | | | | | | | | | | | X | X |
| Immunogenicity [t] | | | | B | | | | | B | | | B | | | | | | | X[u] | X |
| Endogenous FGF21[v] | | | | B | | | | | | | | | | | | | | | | |

| Cohort 1-4 Assessments | Screening Period | | | Treatment Period | | | | | | | | | | | | | | FU Period | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Visit** | | | | | | | | | | | | | | | | | | | |
| **Study Day** | | | | | | | | | | | | | | | | | | | |
| Adverse event monitoring[w] | X | X | X | ←-==========→ | | | | | | | | | | | | | | X | X |
| Concomitant medication | X | X | X | ←-==========→ | | | | | | | | | | | | | | X | X |
| Domiciled[x] | | | X | X | X | | | | | | | X | X | X | | | | | |

Abbreviations: B = predose; D = Day; CK = cytokeratin; DNA = deoxyribonucleic acid; ECG=electrocardiogram; ELF = enhanced liver fibrosis; EOS = end of study; ET= early termination; FGF21 = fibroblast growth factor 21; FU = Follow-Up; HbA1c=hemoglobin A1c; IGF-1 = insulin-like growth factor; MRI-PDFF = magnetic resonance imaging based proton density fat fraction; P = Pre-discharge; PK=pharmacokinetic; Pro-C3 = N-terminal propeptide of type III collagen; S = serum; TSH = thyroid stimulating hormone; WOCBP=women of child-bearing potential.

## Table 2 footnotes

All outpatient visits will have a study window of ±1 day.

a. For any subject who withdraws before completion of the study, an Early Termination (ET) visit will be conducted, if possible; with the same assessments as the Day 92 visit.
b. Optional liver biopsy may be performed for subjects who do not have a medical contraindication to undergoing a liver biopsy, and did not have a liver biopsy in the 24 months prior to screening.|For subjects who underwent a previous liver biopsy >24 months prior to screening, that would not have qualified for the study based on results of the initial biopsy, there should be a sound clinical basis to expect different findings in a repeat biopsy based on medical judgment. Any biopsy will need to be approved in advance by Sponsor and only after the subject has met all other inclusion and exclusion criteria,
c. Complete physical exam done at screening to include recording height, weight, and calculating BMI.
d. Symptom-directed physical exam will be done pre-dose and prior to discharge from the Phase 1 unit on domiciled dosing visits (1st and 5th dose), and pre-dose on ambulatory dosing visits. Additional physical examinations will be performed if clinically indicated.
e. On domiciled and ambulatory visits, body weight will be measured pre-dose.
f. Fibroscan should be performed during screening, prior to magnetic resonance imaging (MRI) for all subjects.
g. 12-lead safety ECGs will be recorded as single bedside measurements; on domiciled dosing visits (1st and 5th dose), ECG will be measured pre-dose and 24 hours post-dose. On ambulatory dosing visits, ECG will be measured pre-dose. Additional ECG may be conducted if clinically indicated.
h. On Day -1, urine drug screen can be done at a local laboratory, however, a sample should also be collected for central laboratory evaluation.
i. Clinical laboratory tests will include biochemistry, hematology, and coagulation, and FSH for determination of post-menopausal status ; on domiciled dosing visits (1st and 5th dose), clinical laboratory will be collected pre-dose and 24 hours post dose; on ambulatory visits, clinical laboratory will be collected pre-dose. For all subjects, alanine transaminase (ALT) and aspartate transaminase (AST) will be collected twice during the Screening period (at least 2 weeks apart), with the 2nd assessment to be collected at Day -7 to Day -3. A 3rd assessment, if required, will be collected via unscheduled visit (refer to Exclusion criterion 3).
j. Ambulatory 24-hour urine collection for cortisol to be done within 14 days from baseline, on Day 50 and Day 92/ET. It is recommended that subjects collect urine for the 24 hours prior to D-7 to-3 visit and bring the container with them to this visit. Alternatively, urine can be collected for the 24 hours

prior to the randomization visit (D-1) and brought to the site on D-1 (the result is not required for eligibility confirmation). For Day 50 and D92/ET – subject will start collection 24 hours before coming into the clinic and bring the sample to the visit.

k. Serology tests will include Hepatitis B surface antigen, Hepatitis C Virus, and Human Immunodeficiency Virus (HIV) 1 and 2 antibodies.

l. Serum urine pregnancy test will be conducted at screening; at all other timepoints urine pregnancy test will be done to guide clinical decisions to dose on dosing days. Prior to the 1st dosing, the baseline urine pregnancy test will be performed on D-1 to allow for randomization on that day. If urine test is positive, a confirmatory serum pregnancy test will be conducted.

m. [k] Vital signs include supine blood pressure, pulse, body temperature, and respiratory rate; vital signs will be measured pre-dose (prior to scheduled blood draws and study intervention administration); 1, 12 and 24 hours post-dose and prior to discharge on domiciled dosing visits (1st and 5th doses); on dosing ambulatory visits, vital signs will be measured pre-dose (prior to scheduled blood draws and study intervention administration) and prior to discharge; on non-dosing visits, vital signs will be measured prior to scheduled blood draws. Starting from randomization, blood pressure and pulse will be measured in duplicate, the first measurement will be taken up to 15 minutes before the indicated timepoint. Additional vital signs measurement may be done if clinically indicated. Subjects must be in a supine or semi-erect/seated position and resting for at least 5 minutes prior to measurements.

n. Study intervention will be administered SC to the abdomen region by qualified study personnel.

o. PK blood samples will be collected as shown in Table 3. *Additional blood samples for PK analysis may be collected if clinically indicated (e.g., in case of SAE)*. For PK sample collection instruction/procedures, refer to the relevant manual.

p. Blood samples for OGTT test will be collected under fasting conditions (10 hours) at the following timepoints: 0 minutes (just prior to ingesting glucose), 30 minutes, 60 minutes, 90 minutes 120 minutes and 180 minutes. On Day 92/ET the insulin will be captured from the OGTT.

q. At Screening, MRI-PDFF to be done within 35 days of baseline (Day 1). On Days 50 and 92, MRI-PDFF to be done within ± 2 days of the planned visit date. If out of the window, MRI-PDFF should still be performed as close to the target day as possible, but protocol deviation should be recorded.

r. Samples for carboxy-terminal collagen crosslinks (CTX) and N-terminal propeptide of type 1 collagen (P1NP) will be obtained at the designated timepoints for storage and potential future analysis. The D42 sample will be obtained pre-dose.

s. Samples for RNA as well as plasma and serum samples will be collected for potential future exploratory assessments, to increase understanding of BIO89-100 biological activity and to identify potential existing and/or emerging biomarkers.

t. [redacted]

u. Immunogenicity sample on Day 92/ET will only be collected for subjects who early terminate from the study.

v. Baseline samples of endogenous FGF21 will be analyzed: [redacted]

w. The sites may take non-personally identifying photographs of potential injection site reactions (optional)

x. Subjects in Cohorts 1 to 4 will be domiciled at the clinic from 1 day prior to dosing until 24 hours post the 1st dose and the 5th dose. Other study visits will be ambulatory. On Day 8, Day 15 and Day 22 (ambulatory dosing visits), subjects will remain at the site for observation for at least 2 hours post dosing.

**Table 3 Part 1 PK Sample Collection for Cohorts 1-4 – QW Dosing Interval**

| Study Day | Dosing Day | Ambulatory visit | Hours relative to 1st dose (Day 1) |
|---|---|---|---|
| [redacted] | X | | [redacted] |
| | | | |
| | | X | |
| | | X | |
| | | X | |
| | X | X | |
| | X | X | |
| | X | X | |
| | X | | |
| | | | |
| | | X | |
| | | X | |
| | | X | |
| | X | X | |
| | X | X | |
| | X | X | |
| | X | X | |
| | X | X | |
| | | X | |

**Table 4 Part 1 Schedule of Activities for Cohorts 5 and 6**

| Cohorts 5-6 Assessments | Screening Period | | | Treatment Period | | | | | | | | | | | | | | | | FU Period | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Visit | | | | | | | | | | | | | | | | | | | | | |
| Study Day | | | | | | | | | | | | | | | | | | | | | |
| Informed consent | X | | | | | | | | | | | | | | | | | | | | |
| Medical history/demographics | X | | | | | | | | | | | | | | | | | | | | |
| Percutaneous liver biopsy (optional)[b] | X | | | | | | | | | | | | | | | | | | | | |
| Prior medications | X | | | | | | | | | | | | | | | | | | | | |
| Inclusion and exclusion criteria | X | | X | | | | | | | | | | | | | | | | | | |
| Complete physical exam[c] | X | | | | | | | | | | | | X | | | | | | | X | X |
| Symptom-directed physical exam[d] | | | X | B | P | | | | X | B | X | | B | | | | | B | | B | | |
| Body weight[e] | X | | | B | | | | | X | B | X | | B | | | | | B | | B | | |
| Waist and hip measurements | X | | | B | | | | | | | | | B | | | | | | X | | X | X |
| Fibroscan[f] | X | | | | | | | | | | | | | | | | | | X | | X | |
| 12-lead ECG (single)[g] | X | | | B | X | | | | X | B | | | B | X | | | | | | | X | X |
| Urine drug screen and alcohol breath test | X | | X[h] | | | | | | | | | | | | | | | | | | | |
| Clinical laboratory tests[i] | X | X | | B | X | | | | X | B | X | | B | X | | | | | X | | X | X |
| Cortisol (24 hour urine collection)[j] | X | | | | | | | | | | | | | | | | | | X | | X | |
| Urinalysis | X | | X | | | | | | | | | | | X | | | | | X | | X | X |
| HbA1C | X | | X | | | | | | | | | | | | | | | | X | | X | X |

| Cohorts 5-6 Assessments | Screening Period | | | Treatment Period | | | | | | | | | | | | | | | | FU Period | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Visit** | [redacted] | | | | | | | | | | | | | | | | | | | | |
| **Study Day** | | | | | | | | | | | | | | | | | | | | | |
| Serology[k] | X | | | | | | | | | | | | | | | | | | | | |
| TSH | X | | | | | | | | | | | | | | | | | | | | |
| Pregnancy test in WOCBP only[l] | X (S) | | X | | | | | | B | | | B | | | | | B | | B | X | X |
| Vital signs[m] | X | | X | X | P | X | X | X | X | X | X | X | P | X | X | X | X | X | X | X | X |
| Columbia-Suicide Severity Rating Scale (C-SSRS) | | | X | | | | | | | | | | | | | | | X | | X | |
| Randomization | | | X | | | | | | | | | | | | | | | | | | |
| Study Intervention Administration[n] | | | | X | | | | | | X | | | X | | | | | X | | X | | |
| PK blood collection[o] | | | | X | X | X | X | X | X | B | X | | X | X | X | X | X | | X | [redacted] | X | |
| PD and biomarker blood sampling: | | | | | | | | | | | | | | | | | | | | | |
| IGF-1, total | | | | B | | | | | X | B | | | B | | | | | | | X | |
| Adiponectin | | | | B | | | | | X | | | | B | | | | | | X | X | X |
| CK-18, ELF panel and Pro-C3 | | | | B | | | | | | | | | B | | | | | | X | X | [redacted] |
| Free fatty acid | | | | B | | | | | X | | | | B | | | | | | X | X | X |
| Oral glucose tolerance test (OGTT)[p] | | | X | | | | | | | | | | | | | | | | | X | |
| Insulin (not part of OGTT) | | | | B | | | | | X | | | | B | | | | | | X | | X |
| HOMA-IR calculation | | | | X | | | | | | | | | | | | | | | X | X | X |

| Cohorts 5-6 Assessments | Screening Period | | | Treatment Period | | | | | | | | | | | | | | | | FU Period | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Visit | | | | | | | | | | | | | | | | | | | | | |
| Study Day | | | | | | | | | | | | | | | | | | | | | |
| MRI-PDFF; visceral fat; and subcutaneous fat [q] | X | | | | | | | | | | | | | | | | | X | | X | |
| Plasma sample for potential future bone biomarkers analysis[r] | | | X | | | | | | | | | | | | | | | X | | X | X |
| Pharmacogenomic (DNA) blood sampling | | | X | | | | | | | | | | | | | | | | | | |
| Exploratory biomarker analysis [s] | | | X | | | | | | | | | | | | | | | X | | X | X |
| Immunogenicity[t] | | | | B | | | | | | B | | | B | | | | | X | | X[u] | X |
| Endogenous FGF21[v] | | | | B | | | | | | | | | | | | | | | | | |
| Adverse event monitoring[w] | X | X | X | ←-═══════════════════════════════════→ | | | | | | | | | | | | | | | | X | X |
| Concomitant medication | X | X | X | ←-═══════════════════════════════════→ | | | | | | | | | | | | | | | | X | X |
| Domiciled[x] | | | X | X | X | | | | | | | X | X | X | | | | | | | |

Abbreviations: B = predose; D = Day; CK = cytokeratin; DNA = deoxyribonucleic acid; ECG=electrocardiogram; ELF = enhanced liver fibrosis; EOS = end of study; ET= early termination; FGF21 = fibroblast growth factor 21; FU = Follow-Up; HbA1c=hemoglobin A1c; IGF-1 = insulin-like growth factor; MRI-PDFF = magnetic resonance imaging based proton density fat fraction; P = Pre-discharge; PK=pharmacokinetic; Pro-C3 = N-terminal propeptide of type III collagen; S = serum; TSH = thyroid stimulating hormone; WOCBP=women of child-bearing potential.

## Table 4 footnotes

All outpatient visits will have a study window of ±1 day

* Between Visits 17 to 19, on Days 64 and 78 subjects will be contacted by phone to inquire about AEs and concomitant medications

a. For any subject who withdraws before completion of the study, an Early Termination (ET) visit will be conducted, if possible; with the same assessments as the Day 92 visit.
b. Optional liver biopsy may be performed for subjects who do not have a medical contraindication to undergoing a liver biopsy, and did not have a liver biopsy in the 24 months prior to screening.|For subjects who underwent a previous liver biopsy >24 months prior to screening, that would not have

qualified for the study based on results of the initial biopsy, there should be a sound clinical basis to expect different findings in a repeat biopsy based on medical judgment. Any biopsy will need to be approved in advance by Sponsor and only after the subject has met all other inclusion and exclusion criteria.

c. Complete physical exam done at screening to include recording height, weight, and calculating BMI.
d. Symptom-directed physical exam will be done pre-dose and prior to discharge from the Phase 1 unit on domiciled dosing visits (1st and 3rd dose), and pre-dose on ambulatory dosing visits. Additional physical examinations will also be performed if clinically indicated.
e. On domiciled and ambulatory visits, body weight will be measured pre-dose.
f. Fibroscan should be performed during screening, prior to magnetic resonance imaging (MRI) for all subjects.
g. 12-lead safety ECGs will be recorded as single bedside measurements. On domiciled dosing visits (1$^{st}$ and 3$^{rd}$ dose), ECG will be measured pre-dose and 24 hours post-dose; on ambulatory dosing visits, ECG will be measured pre-dose. Additional ECG may be conducted if clinically indicated.
h. On Day -1, urine drug screen can be done at a local laboratory, however, a sample should also be collected for central laboratory evaluation
i. Clinical laboratory tests will include biochemistry, hematology, and coagulation, and FSH for determination of post-menopausal status ; on domiciled dosing visits (1$^{st}$ and 3$^{rd}$ dose), clinical laboratory will be collected pre-dose and 24 hours post dose; on ambulatory visits, clinical laboratory will be collected pre-dose. For all subjects, alanine transaminase (ALT) and aspartate transaminase (AST) will be collected twice during the Screening period (at least 2 weeks apart), with the 2$^{nd}$ assessment to be collected at Day -7 to Day -3. A 3$^{rd}$ assessment, if required, will be collected via unscheduled visit (refer to Exclusion criterion 3).
j. Ambulatory 24 hour urine collection for cortisol to be done within 14 days from baseline, on Day 50 and Day 92/ET. It is recommended that subjects collect urine for the 24 hours prior to D-7 to-3 visit and bring the container with them to this visit. Alternatively, urine can be collected for the 24 hours prior to the randomization visit (D-1) and brought to the site on D-1 (the result is not required for eligibility confirmation). For Day 50 and D92/ET – subject will start collection 24 hours before coming into the clinic and bring the sample to the visit.
k. Serology tests will include Hepatitis B surface antigen, Hepatitis C Virus, and Human Immunodeficiency Virus (HIV) 1 and 2 antibodies
l. Serum urine pregnancy test will be conducted at screening; at all other timepoints urine pregnancy test will be done to guide clinical decisions to dose on dosing days. Prior to the 1$^{st}$ dosing, the baseline urine pregnancy test will be performed on D-1 to allow for randomization on that day. If urine test is positive, a confirmatory serum pregnancy test will be conducted.
m. Vital signs include supine blood pressure, pulse, body temperature, and respiratory rate; vital signs will be measured pre-dose (prior to scheduled blood draws and study intervention administration); 1, 12 and 24 hours post-dose and prior to discharge on domiciled dosing visits (1$^{st}$ and 3$^{rd}$ doses); on dosing ambulatory visits, vital signs will be measured pre-dose (prior to scheduled blood draws and study intervention administration) and prior to discharge; on non-dosing visits, vital signs will be measured prior to scheduled blood draws. Starting from randomization, blood pressure and pulse will be measured in duplicate, the first measurement will be taken up to 15 minutes before the indicated timepoint. Additional vital signs measurement may be done if clinically indicated. Subjects must be in a supine or semi-erect/seated position and resting for at least 5 minutes prior to measurements.
n. Study intervention will be administered SC to the abdomen region by qualified study personnel.
o. PK blood samples will be collected as shown in Table 5. *Additional blood samples for PK analysis may be collected if clinically indicated (e.g., in case of SAE).* For PK sample collection instruction/procedures, refer to the relevant manual.
p. Blood samples for OGTT test will be collected under fasting conditions (10 hours) at the following timepoints: 0 minutes (just prior to ingesting glucose), 30 minutes, 60 minutes, 90 minutes 120 minutes and 180 minutes. . On Day 92/ET the insulin will be captured from the OGTT.
q. At Screening, MRI-PDFF to be done within 35 days of baseline (Day 1). On Days 50 and 92, MRI-PDFF to be done within ± 2 days of the planned visit date. If out of the window, MRI-PDFF should still be performed as close to the target day as possible, but protocol deviation should be recorded.
r. Samples for carboxy-terminal collagen crosslinks (CTX) and N-terminal propeptide of type 1 collagen (P1NP) will be obtained at the designated timepoints for storage and potential future analysis. The D42 sample will be obtained pre-dose

s. Samples for RNA as well as plasma and serum samples will be collected for potential future exploratory assessments, to increase understanding of BIO89-100 biological activity and to identify potential existing and/or emerging biomarkers.
[redacted]
u. Immunogenicity sample on Day 92/ET will only be collected for subjects who early terminate from the study.
v. Baseline samples of endogenous FGF21 will be analyzed; [redacted]
w. The sites may take non-personally identifying photographs of potential injection site reactions (optional)
x. Subjects in Cohort 5 and 6 will be domiciled at the clinic from 1 day prior to dosing until 24 hours post the 1st dose and 24 hours post the 3rd dose; other study visits will be ambulatory. On the Day 15 ambulatory dosing visit, subjects will remain at the study site for observation for at least 2 hours post dosing.

**Table 5 Part 1 PK Sample Collection for Cohorts 5 and 6 – Q2W Dosing Interval**

| Study Day | Dosing Day | Ambulatory visit | Hours relative to 1st dose (Day 1) |
|---|---|---|---|
| [redacted] | X | | [redacted] |
| | | | |
| | | X | |
| | | X | |
| | | X | |
| | | X | |
| | X | X | |
| | | X | |
| | X | | |
| | | | |
| | | X | |
| | | X | |
| | | X | |
| | | X | |
| | X | X | |
| | X | X | |
| | X | X | |
| | X | X | |
| | | X | |

### 1.2.2. Part 2

**Table 6 Schedule of Activities for Part 2 (Cohort 7)**

| Cohort 7 Assessments | Screening Period | | Treatment Period | | | FU Period | |
|---|---|---|---|---|---|---|---|
| **Study Visit** | [redacted] | | | | | | |
| **Study Day (mandatory in clinic visits)** | | | | | | | |
| Informed consent | X | | | | | | |
| Medical history/demographics | X | | | | | | |
| Percutaneous liver biopsy[b] | X | | | | | X | |
| Histology machine read (PathAI) | X | | | | | X | |
| Prior medications | X | | | | | | |
| Inclusion and exclusion criteria | X | | B | | | | |
| Complete physical exam[c] | X | | | | | X | X |
| Symptom-directed physical exam[d] | | | B | | | | |
| Body weight | X | | B | | | X | X |
| Waist and hip circumference | X | | B | | | X | |
| Lifestyle counseling | X | X | X | | | | |
| Fibroscan[e] | X | | | | | X | |
| 12-lead ECG (single)[f] | X | | B | | | X | X |
| Urine drug screen and alcohol breath test | X | | B[g] | | | | |
| Clinical laboratory tests[h] | X | X | B | | | X | X |
| Urinalysis | X | | B | | | X | X |
| HbA1C | X | | B | | | X | X |
| Serology[i] | X | | | | | | |
| TSH | X | | | | | | |
| Pregnancy test in WOCBP only[j] | X(S) | | B | | | X | X |
| Vital signs[k] | X | | B | | | X | X |

| Cohort 7 Assessments | Screening Period | | Treatment Period | | FU Period | |
|---|---|---|---|---|---|---|
| **Study Visit** | [redacted] | | | | | |
| **Study Day (mandatory in clinic visits)** | | | | | | |
| Columbia-Suicide Severity Rating Scale (C-SSRS) | X | | | | | |
| Study intervention in-clinic (mandatory) administration[l] | | | X | X | | |
| Home or in-clinic study intervention administration[m] | | | | [redacted] | | |
| PK blood collection[n] | | | B | | X | |
| Pro-C3 | | | B | | X | |
| Adiponectin | | | B | | X | |
| MRI-PDFF[o] | X | | | | X | |
| Bone biomarkers analysis[p] | | | B | | X | X |
| Immunogenicity [q] | | | B | | X | X |
| Endogenous FGF21[r] | | | B | | | |
| Adverse event monitoring[s] | X | X | X | ←==========X==========→ | X | X |
| Concomitant medication | X | X | X | ←==========X==========→ | X | X |

Abbreviations: B = predose; D = Day; AI = artificial intelligence; ECG=electrocardiogram; EOS = end of study; ET= early termination; FGF21 = fibroblast growth factor 21; FU = Follow-Up; HbA1c=hemoglobin A1c; MRI-PDFF = magnetic resonance imaging based proton density fat fraction; PK=pharmacokinetic; Pro-C3 = N-terminal propeptide of type III collagen; S = serum; TSH = thyroid stimulating hormone; WOCBP=women of child-bearing potential.

## Table 6 Footnotes

All ambulatory clinic visits will have a study window of ±1 day. Window for MRI-PDFF will be ±7 days. The first liver biopsy will be done during the screening period. The second liver biopsy will be performed within 14 days after the last dose of study intervention. Liver biopsies (first and second) will be done after MRI-PDFF.

a. For any subject who withdraws before completion of the study, an Early Termination (ET) visit will be conducted, if possible; with the same assessments as the Day 141 visit.
b. A liver biopsy with NASH that meets study inclusion criteria, performed within 24 weeks from baseline visit with the sample deemed interpretable by the central reader is acceptable instead of the baseline liver biopsy. A second liver biopsy will be done within 14 days of the last dose of study intervention.

Liver biopsies (first and second) will be done after MRI-PDFF. If out of a window, a liver biopsy should still be performed as close to the target date as possible, but a protocol deviation should be recorded.

c. Complete physical exam done at screening to include recording height, weight, and calculating BMI.
d. Physical examinations will be performed if clinically indicated.
e. Fibroscan should be performed during screening, prior to magnetic resonance imaging (MRI) for all subjects. Fibroscan will be repeated on D141/ET.
f. 12-lead safety ECGs will be recorded as single bedside measurements. Additional ECG may be conducted if clinically indicated.
g. On Day 1, urine drug screen can be done at a local laboratory.
h. Clinical laboratory tests (performed under fasting conditions, ≥10 hours) will include biochemistry, hematology, and coagulation, and FSH for determination of post-menopausal status. For all subjects, alanine transaminase (ALT) and aspartate transaminase (AST) will be collected twice during the Screening period (at least 2 weeks apart), with the 2nd assessment to be collected at Day -7 to Day -3. A 3rd assessment, if required, will be collected via unscheduled visit (refer to Exclusion criterion 3).
i. Serology tests will include Hepatitis B surface antigen, Hepatitis C Virus, and Human Immunodeficiency Virus (HIV) 1 and 2 antibodies.
j. Serum urine pregnancy test will be conducted at screening; at all other timepoints urine pregnancy test will be done to guide clinical decisions to dose on dosing days. On dosing days in the clinic, a urine pregnancy test will be obtained locally; in addition, a urine sample will be sent to the central lab. If urine test is positive, a confirmatory serum pregnancy test will be conducted.
k. Vital signs include blood pressure, pulse, body temperature, and respiratory rate; vital signs will be measured pre-dose (prior to scheduled blood draws and study intervention administration. Blood pressure and pulse will be measured in duplicate, the first measurement will be taken up to 15 minutes before the indicated timepoint. Additional vital signs measurement may be done if clinically indicated. Subjects must be in a supine or semi-erect/seated position and resting for at least 5 minutes prior to measurements.
l. Study intervention will be administered SC to the abdomen region by qualified study personnel or by the subject (under supervision) at ambulatory clinic visit and will be administered at home by the subject at the rest of the planned dosing days. Subjects will be observed for 15 minutes after each dose.
m. At the designated timepoints, study intervention will be administered at the subject's home. If home administration is not feasible, not desired by the subject, or deemed inappropriate option by the investigator for any reason, in-clinic study intervention administration may take place at some or all of these timepoints.
n. PK blood samples will be collected at the specified timepoints. *Additional blood samples for PK analysis may be collected if clinically indicated (e.g., in case of SAE)*. For PK sample collection instruction/procedures, refer to the relevant manual.
o. At Screening, MRI-PDFF to be done within 35 days of baseline (Day 1). On Days 85 and 141, MRI-PDFF to be done within ± 7 days of the planned visit date. If out of the window, MRI-PDFF should still be performed as close to the target day as possible, but protocol deviation should be recorded.
p. Samples for carboxy-terminal collagen crosslinks (CTX), N-terminal propeptide of type 1 collagen (P1NP) and osteocalcin will be obtained at the designated timepoints.
q. [redacted]

r. Baseline samples of endogenous FGF21 will be obtained in all subjects; [redacted]

s. The sites may take non-personally identifying photographs of potential injection site reactions (optional).

# 2. INTRODUCTION

## 2.1. Study Rationale

BIO89-100, developed by 89bio, is a glycoPEGylated analog of FGF21, a metabolic hormone secreted by the liver, adipose tissue, skeletal muscle, and the pancreas. FGF21 is regulated by nutritional status, affects energy expenditure, and glucose and lipid metabolism (Lee, 2014) (Arner, 2008) (Park, 2016), and therefore may play a role in metabolic disease, e.g., NAFLD, a common chronic liver disease affecting 30% of adult population worldwide (Chalasani, 2012). Several nonclinical and clinical studies have shown that administration of similar FGF21 analogs had beneficial effects on serum lipid and insulin-resistance as well as on liver fat (Zhang 2014) (Gaich, 2013) (Sanyal, 2019). There are currently no approved pharmaceutical treatments for NASH (Friedman, 2018).

This Phase 1b/2a study has 2 parts. Part 1 is designed to assess the safety and tolerability of repeated SC escalating doses of BIO89-100 in subjects with a diagnosis of NASH, or who have NAFLD with a high risk of NASH. Part 2 is open-label, designed to assess the effect of repeated SC doses of one dose level of BIO89-100 (27 mg QW), administered for 20 weeks, on liver histology in subjects with biopsy-proven NASH with NAFLD Activity Score (NAS) ≥4, fibrosis stage F1 with high risk, F2 or F3.

The study includes safety, PK, PD, and immunogenicity characterizations to allow a preliminary assessment of a safe and efficacious dose range to be studied in further clinical studies.

## 2.2. Background

### 2.2.1. Non-alcoholic Steatohepatitis

NASH is a chronic liver disease, characterized histologically by hepatic steatosis in ≥5% of hepatocytes, injury (ballooning)(Chalasani, 2012). It is part of the spectrum of NAFLD that includes NASH, and cirrhosis resultant from fatty liver (Estes, 2018). In North America, the prevalence of NAFLD is estimated at ~24%. NAFLD patients tend to be obese, with insulin resistance and/or type 2 diabetes mellitus (T2DM), dyslipidemia, hypertriglyceridemia, and hypertension, and NAFLD is increasingly recognized as the liver disease component of the metabolic syndrome (MetS) (Chalasani, 2018).

The pathophysiologic mechanisms that lead to NAFLD are thought to be similar to those in the development of T2DM, as insulin resistance is a hallmark in both disease states. Insulin resistance in adipocytes leads to dysfunction in the normal regulation of lipolysis, contributing to elevated circulating free fatty acids, accumulation of triglycerides in the liver (Saponaro, 2015) and lipotoxicity, manifested as inflammation and cellular injury (Byrne, 2015), and ultimately – fibrosis.

NAFLD is usually asymptomatic, unless progression to cirrhosis has occurred. It is often diagnosed by demonstration of hepatic steatosis on liver imaging (e.g., ultrasound or magnetic resonance imaging (MRI)) in subjects, commonly with features of the metabolic syndrome, in whom no alternative etiology for liver fat accumulation can be identified (e.g., alcoholic liver disease, medications). Validated noninvasive tests for diagnosis of steatohepatitis are not

currently available, and a liver biopsy is still needed to diagnose the inflammation and cellular ballooning features of NASH (Torres, 2012).

There is a high unmet need in NASH (Friedman, 2018). The disease progresses to fibrosis and cirrhosis in approximately 20% of patients (Vernon, 2011; Khan, 2015), and 45% of patients with cirrhosis will progress to decompensated cirrhosis within 10 years (Rinella, 2015). Eight percent of patients with advanced fibrosis will develop hepatocellular carcinoma (HCC) within 5 years (Hashimoto, 2009), and there is increasing evidence that non-cirrhotic subjects with NAFLD may also be at an increased risk of HCC (Mittal, 2016). Pre-cirrhotic NASH has a relative risk of death of 1.7 over 15 years compared to the general population, and approximately 20% of NAFLD patients die of liver-related causes (Soderberg, 2010). A variety of therapeutic agents are being developed for NASH, targeting metabolic pathways, inflammatory pathways or fibrosis. Positive results from the REGENERATE study, the first and largest successful pivotal Phase 3 study in patients with liver fibrosis due to NASH have been reported (Younossi, 2019). However, as of this time, there are no approved therapies for treatment of patients with NASH.

### 2.2.2. FGF21

FGF21 is a metabolic hormone, regulated by nutritional status, that affects energy expenditure and glucose and lipid metabolism. It is secreted mainly by the liver, but also by white adipose tissue, skeletal muscle, and the pancreas. The activation of fibroblast growth factor receptors (FGFRs) by FGF21 requires the transmembrane protein cofactor β-Klotho, expressed predominantly in metabolic organs, including the liver, white adipose tissue, and the pancreas, conferring organ specificity to FGF21 (Ogawa, 2007; Li, 2015).

In patients with insulin resistance and NASH, circulating and tissue levels of FGF21 are increased and correlate with disease severity, indicating the presence of FGF21 resistance, which can be overcome by administration of pharmacological doses of FGF21. On this basis, administration of exogenous FGF21 has been explored as a method to treat obesity-associated insulin-resistance disorders, including NASH.

Native FGF21 has a short life (~2 hours), limiting the potential to use it as a therapeutic agent. Consequently, various methods have been developed to extend the half-life of FGF21, including PEGylation. PEGylated FGF21 was shown to provide comparable efficacy to wild-type FGF21 despite lower dosing frequency and total cumulative dose, a likely result of greatly increased in vivo half-life (Mu, 2012). In humans, the effects of pegbelfermin (BMS-986036; a PEGylated analog of FGF21, which differs from BIO89-100 in [redacted]) on biopsy confirmed NASH patients (both genders; fibrosis stage F1-F3) with hepatic fat fraction ≥10% (per MRI-PDFF) were recently assessed in a 16-week randomized placebo-controlled study (n=74) (Sanyal, 2019). The study demonstrated a statistically significant reduction in liver fat versus placebo at week 16 (primary endpoint; measured by MRI-PDFF), with up to 6.8% (p=0.0004) and 5.2% (p=0.008) absolute reduction for a 10 mg daily dose and 20 mg weekly dose, respectively. The study also showed improvements in Pro-C3, serum biomarker of fibrosis, magnetic resonance elastography (measure of liver stiffness), adiponectin, and liver enzymes (ALT, and AST). Improvements in triglycerides, LDL-c, and HDL-c were also noted primarily with the 10 mg daily dose. Finally, safety was considered favorable, with no deaths, treatment-related SAEs, or discontinuations due to AEs reported. The most frequently reported AEs were diarrhea, nausea and frequent bowel movement, most of these were mild, and none were considered as severe in intensity.

### 2.2.3. BIO89-100

BIO89-100 is a [redacted] PEGylated analog of FGF21 that has an [redacted] It was evaluated in nonclinical pharmacology, PK, and toxicology studies and in a recently completed, first-in-human, single ascending dose study (TV47948-SAD-10122).

In nonclinical pharmacology studies, BIO89-100 was found to be effective in two NASH models in mice and in spontaneously diabetic monkeys, with liver-related and metabolic benefits, including improved transaminase levels and liver histology, decreased body weight, improved glycemic parameters and lipid profile, and increased adiponectin levels. Nonclinical safety studies with BIO89-100 have shown good tolerability to treatment at doses that were time-folds higher than the effective doses in animal pharmacological models and over systemic human exposure to BIO89-100 following single administration of clinical doses (TV47948-SAD-10122 study). Data from repeated nonclinical toxicology GLP studies in mice suggest that at high doses, BIO89-100 may have an adverse effect on bone formation and linear growth, and on the female reproductive system (refer to Section 2.3.1). The determined no observed adverse effect level (NOAEL) in males and female mice is much higher than the suggested clinical doses in BIO89-100-002.

TV47948-SAD-10122, a Phase 1, first-in-human, randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, and PK properties of BIO89-100 following SC administration of single ascending doses (SAD), has recently been completed. The study randomized and treated 58 subjects, 43 of whom were dosed with BIO89-100 at 7 dose levels (0.45 mg; 1.2 mg; 3 mg; 9.1 mg; 18.2 mg; 39 mg; and 78 mg). No significant safety issues have been identified. [redacted]

A detailed description of the chemistry, pharmacology, efficacy, and safety of BIO89-100 is provided in the Investigator's Brochure (IB).

## 2.3. Benefit / Risk Assessment

Information about the expected benefits and potential risks (adverse event) of BIO89-100 can be found in the IB.

### 2.3.1. Risk Assessment

BIO89-100 has not been administered previously to patients with NASH or NAFLD who are at high risk for NASH. Mild injection site reactions and sporadic GI effects have been reported in a recently completed SAD study (Study TV47948-SAD-10122) with BIO89-100. Potential risks based on nonclinical data with BIO89-100 and published class effects include immunogenicity, bone effects and female reproductive effects. It is important to note that none of these potential risks have been reported as significant safety concerns in the recently published Phase 2 proof-of-concept study in which subjects with biopsy-proven NASH were treated for 16 weeks with

pegbelfermin, a PEGylated FGF21 analogue. Additional information regarding risks to subjects with BIO89-100 may be found in the IB.

#### 2.3.1.1. Potential Risk: Immunogenicity

As with any biologic drug, exposure to BIO89-100 may potentially provoke an immune response, with formation of ADAs, that may lead to loss of efficacy or adverse effects (e.g., hypersensitivity, loss of endogenous FGF21 activity). After single administrations to healthy subjects in study TV47948-SAD-10122, [redacted] of BIO89-100-treated subjects had positive ADA, all with [redacted] ADAs to BIO89-100 have [redacted], without a discernible effect on PK or PD parameters that were evaluated in these studies. No severe hypersensitivity event has been reported in Cohorts 1-6 (completed). Of note, formation of ADAs in nonclinical species is expected, and is generally not considered predictive for immunogenicity in humans.

In study BIO89-100-102, the following mitigation measures will be implemented to address the potential risk of severe hypersensitivity reactions, including anaphylaxis:

a. Subjects with any prior exposure to an FGF21 analogue, if known, will be excluded from participating in the study.
b. In Part 1, subjects will be under observation and intensive monitoring (including multiple vital signs measurements) for 48 hours in 2 domiciled visits (1st and 5th dose for Cohorts 1-4, 1st and 3rd dose for Cohorts 5 and 6), and for 2 hours post dose during ambulatory dosing visits in the first month. In Part 2, subjects will be observed for at least 15 minutes after each dose of study intervention.
c. All study sites will have appropriate measures (personnel and equipment) to diagnose and address severe hypersensitivity reactions, including proximity to a hospital to which a subject may be evacuated, if needed. Details on identification and management of severe hypersensitivity reactions after home administration will be provided in the Home Administration Manual.
d. Severe hypersensitivity reactions (including anaphylaxis) will be an event to monitor.
e. Stopping rules include serious hypersensitivity reactions to BIO89-100.
f. Hypersensitivity-related AEs will be captured as part of the general safety assessment in all subjects.
g. Immunogenicity testing will be done frequently, at baseline and multiple timepoints during study treatment. In addition, an immunogenicity sample will be obtained in the event of a significant hypersensitivity reaction. Subjects who test positive for neutralizing antibodies to BIO89-100 at the EOS/ET visit will be asked to return for additional follow-up testing. This testing should occur approximately every [redacted]. More frequent testing (e.g., every month) or testing for a longer period of time may be requested in the event of safety-related concerns. Follow-up testing will not be required where it is established that the subject did not receive BIO89-100 (Part 1). All follow-up results, both positive and negative, will be communicated to the sites. A blood sample for ADA assessment will also be collected upon observation of any severe hypersensitivity reaction (e.g., anaphylaxis). Subjects who test positive for binding, non-neutralizing antibodies and

have clinical sequelae that are considered potentially related to an anti-BIO89-100 antibody response may also be asked to return for additional follow-up testing.

#### 2.3.1.2. Potential Risk: Bone Effects

The literature suggests that FGF21 may be involved in bone turnover (Wei, 2012; Owen, 2013). In nonclinical studies with BIO89-100 in mice, non-adverse reductions in bone formation and resorption markers were observed in young, healthy mice, as well as decreases in tibia size and bone density. The relevance of these findings to humans is not known. Interestingly, in a study in 40 healthy volunteers, fasting plasma FGF21 levels correlated positively with total bone mineral density (BMD) ($R^2$=0.69, p=0.003) and spine BMD ($R^2$=0.76, p=0.001) in women, a correlation that remained significant after adjusting for age, ethnicity, and body composition; there was no association between FGF21 concentrations and body composition in men (Lee, 2013).

Bone-related assessments have been conducted in studies of 2 long-acting FGF analogues in humans: PF-05231023, a long-acting synthetic FGF21 analogue generated by covalent linking of 2 recombinant human FGF21 molecules to the Fab portion of a proprietary scaffold antibody, and pegbelfermin, a PEGylated FGF21 analogue. Modest changes in bone turnover markers were observed with PF-05231023 over 4 weeks of treatment (Kim, 2017), especially in the context of weight loss, although the clinical significance of these findings is not established. With pegbelfermin no bone findings were observed relative to placebo treatment over 16 weeks of treatment.

Bone-related AEs were not reported in a single ascending dose study with BIO89-100 (study TV47948-SAD-10122) however, this short-term study may not be informative for this type of risk. The determined NOAEL of BIO89-100 in males and female mice (up to 13 weeks) and monkeys (up to 28 days) is much higher than the suggested clinical doses in BIO89-100-002. It is notable that in addition to the presence of an adequate safety margin relative to the highest dose to be administered in study BIO89-100-102, the risk of a clinically significant impact on bone is considered to be low in the setting of a 12-week study, as significant changes to bone typically require longer exposures. Nevertheless, as a precautionary measure, in study BIO89-100-102 subjects younger than 21 year of age and subjects with a history of bone trauma, bone fracture, or bone surgery within 2 months of screening will be excluded. In Part 2, exclusion of subjects with bone disorders, including as osteoporosis, osteomalacia, and known, untreated severe vitamin D deficiency (serum 25-hydroxy-vitamin D ≤5 ng/mL) has been added as an additional precautionary measure, given the longer exposure.

Bone-related AEs will be captured as part of the general safety assessment in all subjects. Plasma samples will be collected for potential future analysis of bone turnover biomarkers, if deemed necessary. In Part 2, samples of bone turnover biomarkers will be analyzed.

#### 2.3.1.3. Potential Risk: Female Reproductive Effects

In nonclinical studies in mice exposed to high doses of BIO89-100, [redacted]. These are known effects of FGF21 in mice that have previously been described in the literature (Wei, 2012;, Owen, 2013). Importantly, the established NOAEL of 15 mg/kg in female mice provides an adequate safety margin (16.2- and 52.4-fold margin for $AUC_{168,\,ss}$ and $C_{max}$ for the 27 mg QW clinical dose) for the female reproductive findings that were observed in nonclinical studies with

BIO89-100. Consequently, women of childbearing potential (WOCBP) will be allowed to enroll in study BIO89-100-102. It is worth noting that WOCBP were enrolled in the recent Phase 2a study with pegbelfermin (BMS-986036); no reproduction-related AEs were reported (Sanyal, 2019). Female reproductive AEs were not reported in a single ascending dose study with BIO89-100 (study TV47948-SAD-10122). Specific assessments of the female reproductive system were not performed in this short-term study, which enrolled only females who were not of childbearing potential, and the study is not considered to be informative for this type of risk. WOCBP and their partners will be required to use highly effective contraception, as per current standards for investigational products with an unknown potential for embryotoxicity. Female reproductive system-related AEs will be captured as part of the general safety assessment in all female subjects.

#### 2.3.1.4. Potential Risk: Injection Site Reactions

As with other protein drugs that are administered subcutaneously, there is a potential for injection site reactions (erythema, induration, ecchymosis, tenderness, warmth, swelling, and - at times – more severe reactions, such as lipodystrophy, ulceration, or necrosis).

Injection site reactions following subcutaneous BIO89-100 injections in nonclinical species were non- adverse and reversible after a treatment-free period. Some mild (CTCAE Grade 1) injection site reactions were reported in a single ascending dose study with BIO89-100 (study TV47948-SAD-10122) that completely resolved and did not require treatment; few injection site reactions were also reported in the placebo group. [redacted]

#### 2.3.1.5. Potential Risk: Gastrointestinal Effects

Gastrointestinal (GI) effects have been reported in clinical studies with other FGF21 analogues. In a recent Phase 2a study with pegbelfermin (BMS-986036), in which NASH patients were administered 10 mg daily or 20 mg weekly regimens of pegbelfermin (N=49) or placebo (N=26), the most frequently reported AEs were diarrhoea and nausea, which were more common in pegbelfermin-treated than in placebo-treated patients. There were 28 GI-related AEs, including diarrhoea, nausea, frequent bowel movements, upper abdominal pain, and vomiting, 11 of which were considered to be treatment-related. The frequency of GI AEs was higher in patients treated with pegbelfermin than in patients who received placebo but there was no clear association between frequency of AEs and pegbelfermin dose. All GI events were considered mild or moderate and only 1/11 treatment-related GI AEs required treatment for resolution (probiotics). No GI-related effects were observed in nonclinical studies with BIO89-100 in either mice or monkeys.

A few mild, sporadic GI effects (nausea, dyspepsia, abdominal pain, constipation, diarrhea, flatulence, frequent bowel movements), most of which were considered not related to study drug and all of which resolved without treatment, were reported in a single ascending dose study with BIO89-100 (study TV47948-SAD-10122).

#### 2.3.1.6. Potential Risk of Liver Biopsy

Following liver biopsy, the overall rate of serious complications has been reported to be approximately 1% (Seeff, 2010; Boyum, 2016) and the overall mortality risk has been estimated to be 0.2% (West, 2010). Most complications (83% to 96%) occur within 24 hours of the procedure; 60% occur within 2 hours (Piccinino, 1986; Boyum, 2016). Approximately 2% to 3% percent of patients require hospital admission for management of complications; pain or hypotension are the predominant causes (Garcia-Tsao, 1993; Janes, 1993).

Pain is the most common complication following percutaneous liver biopsy with approximately 25% of patients experiencing pain in the right upper quadrant or right shoulder (Rockey, 2009). The pain usually resolves completely within a few hours.

The most common serious complication of liver biopsy is intraperitoneal hemorrhage. Severe bleeding that results in hemodynamic compromise or requires intervention has been reported following 0.01% to 0.5% of liver biopsies (Boyum, 2016). Bleeding usually becomes clinically apparent within 3 to 4 hours after the biopsy. Hypotension and/or tachycardia following a biopsy, particularly when associated with abdominal pain, are usually related to hemorrhage.

### 2.3.2. Benefit Assessment

There are currently no approved products for NASH in the US. As discussed in previous sections, the FGF21 receptor is a promising mode of action for the treatment of NASH, and several FGF21 analogues are being evaluated for this indication. Recently, encouraging clinical data has been reported for a Phase 2 proof-of-concept study with pegbelfermin, a PEGylated FGF21 analogue, that was administered to patients with biopsy-proven NASH for 16 weeks (Sanyal, 2019). In this study, a significant reduction of hepatic fat (as assessed by MRI-PDFF) was evident, as well as improvement in fibrosis-related and metabolic parameters. While no clinical data for BIO89-100 are yet available, nonclinical data in mice and monkeys are promising, with significant pharmacological effects on liver-related and metabolic parameters in disease animal models. Taken together, it is estimated that 12 weeks of treatment with BIO89-100 may have a positive impact on liver-related (e.g., reduction of hepatic fat) and metabolic parameters in patients with NASH or with NAFLD at a high risk for NASH (Part 1), and 20 weeks of treatment with BIO89-100 may have a positive impact on liver histology in patients with biopsy-proven NASH and fibrosis (Part 2). These patients may thus derive benefit from this treatment.

### 2.3.3. Update: Preliminary Data from Part 1

At the time of finalization of this amendment, preliminary top-line data from Part 1 of this study (Cohorts 1-6) have become available. Analyses of the full dataset are ongoing.

At Week 13, all BIO89-100 dose groups (Cohorts 1-6) showed significant absolute and relative reductions in MRI-PDFF. Up to 88% of BIO89-100 subjects achieved ≥30% MRI-PDFF reduction vs. baseline ($p<0.001$), a threshold of MRI-PDFF relative reduction that has been shown to be associated with histological benefit (Loomba, 2015; Loomba, 2017; Harrison, 2019). Significant decreases in ALT vs. placebo were observed with BIO89-100, which were maximal with 27 mg QW (30 U/L decrease from baseline, $p<0.001$) and also prominent in the subgroup of subjects (n=17) with baseline ALT >45 U/L (35 U/L decrease from baseline, $p<0.05$). Reductions in Pro-C3, a fibrosis marker, were also noted. Metabolic benefits of

BIO89-100 included a favorable effect on lipids, with significant reductions in triglycerides (up to 28% in overall population, up to 49% in the subgroup [n=15] with baseline TG ≥200 mg/mL); non-HDL cholesterol and LDL-C (up to 15% and 16%, respectively), and increased adiponectin (up to +61%).

BIO89-100 had a favorable tolerability profile. There were no deaths or related SAEs. One BIO89-100-treated subject discontinued due to a related AE (localized skin rash). Mild increased appetite (15.9% in pooled BIO89-100-treated subjects) was the most common AE. The frequency of gastrointestinal AEs compared favorably to placebo; diarrhea (BIO89-100 12.7%; placebo 22.2%) and nausea (BIO89-100 7.9%; placebo 16.7%) were the only GI AEs in ≥5% of BIO89-100-treated subjects. Injection site reactions, all mild, were reported in 6.3% of pooled BIO89-100-treated subjects. No hypersensitivity reactions were reported.

### 2.3.4. Overall Benefit Risk Assessment

As discussed, patients with NASH or with NAFLD at high risk for NASH (Part 1) or biopsy-proven NASH (NAS ≥4 and fibrosis stage F1 with high risk, F2 or F3; Part 2) may derive benefit from treatment with BIO89-100. The preliminary data from Part 1 of this study (Cohorts 1-6) demonstrating robust, clinically meaningful reductions in liver fat assessed by MRI-PDFF and ALT at thresholds that have been associated with histological benefit in patients with NASH, combined with additional metabolic benefits, provide further support for a potential benefit in patients with NASH.

Available data from clinical studies with other FGF21 analogues, including a recent Phase 2 study with pegbelfermin in a patient population that is very similar to the intended target population for study BIO89-100-102, do not suggest clinically significant safety concerns. Data from the Phase 1 SAD study TV47948-SAD-10122 in healthy subjects suggest a good safety and tolerability profile with single SC doses of BIO89-100 up to 78 mg; mild injection site reactions and mild, sporadic GI effects were reported in BIO89-100-treated subjects. Preliminary data from Part 1 (Cohorts 1-6) of study BIO89-100-002 (the current study) also suggest a good safety and tolerability profile.

As with any biologic drug, there is a potential risk of immunogenicity; measures have been implemented in this protocol to characterize this risk and appropriately identify and address any clinically significant events related to immunogenicity, including the potential for severe hypersensitivity reactions (including anaphylaxis). Bone and female reproductive findings have been observed at high doses in toxicology studies in mice; these risks are mitigated by the presence of adequate safety margins and additional precautionary measures, as detailed in the section on potential risks.

Taken together, considering the benefit, potential risks, and risk mitigation measures that have been implemented, the sponsor considers the benefit-risk profile for administering BIO89-100 to patients with NASH or NAFLD at high risk for NASH and patients with biopsy-proven NASH (NAS ≥4 and fibrosis stage F1 with high risk, F2 or F3) in study BIO89-100-102 to be favorable. The overall benefit-risk associated with administration of BIO89-100 will be continually re-assessed with the emergence of additional data.

# 3. PART 1

## 3.1. Part 1 Objectives and Endpoints

| Objectives | Endpoints |
|---|---|
| Primary | |
| • To evaluate the safety and tolerability of ascending multiple SC injections of BIO89-100 in subjects with NASH or who have NAFLD and at a high risk of NASH | • Frequency and severity of AEs and SAEs<br>• Number of subjects who discontinued due to AEs and due to related AEs |
| • To characterize BIO89-100 PK | • $C_{max}$ within a dosing interval<br>• $AUC_{0\text{-}tau}$ within a dosing interval<br>• $t_{max}$<br>• $t_{½}$<br>Additional PK parameters may be calculated if deemed appropriate.<br>The serum concentration-time data may also be used for population PK modeling with the results reported separately from the clinical study report. |
| Secondary | |
| • To evaluate the immunogenicity of BIO89-100 as measured by presence of ADA | • Assessment of the incidence and characteristics of ADA after dosing (e.g., titer and/or binding specificity, to the FGF21 and PEG part of BIO89-100).<br>• Impact of the presence of ADAs on serum BIO89-100 concentrations and clinical safety.<br>Additional ADA assessments will be performed if a severe hypersensitivity reaction (e.g., anaphylaxis) is observed. |
| • To characterize biomarkers, PD profile and biological activity of BIO89-100 administered at ascending doses and with both QW and Q2W dosing intervals | Change and percentage change from baseline in the following biomarkers/PD parameters:<br>• Anthropomorphic measurements:<br>– Body weight<br>• Laboratory parameters: |

| Objectives | Endpoints |
|---|---|
| • To evaluate the time, dose, and exposure relationship of BIO89-100 on biological activity, as assessed by biomarkers and PD | – Triglycerides<br>– Non-HDL cholesterol<br>– HDL-c<br>– LDL-c<br>– HbA1c<br>– HOMA-IR<br>– Liver function tests: ALT, AST<br>– Adiponectin<br>– Pro-C3<br>– Free fatty acids and Adipo-IR (fasting free fatty acids × fasting insulin)<br>• Imaging measures:<br>– MRI-PDFF |
| Other Safety Endpoints | |
| [redacted] | |
| Exploratory Endpoints | |
| [redacted] | |

| Objectives | Endpoints |
|---|---|
| [redacted] | |

## 3.2. Part 1 Study Design

Part 1 is a randomized, double-blind, placebo-controlled, MAD study to evaluate the safety, tolerability, PK and PD profiles, and immunogenicity of BIO89-100 administered SC in approximately 83 subjects with NASH, or with NAFLD who are at a high risk of NASH. This multi-site study will consist of 6 cohorts and will evaluate 2 dosing schedules, QW (Cohorts 1 to 4) and Q2W (Cohorts 5 and 6) (Table 1). Based on emerging data and SMC recommendation, the Sponsor can decide to modify the dose of any subsequent cohort.

Part 1 will include a Screening period, a Treatment period, and a Follow-up period. After signing informed consent, subjects will undergo screening assessments to determine eligibility over a period of up to 60 days.

Eligible subjects will be randomized for each cohort as described in Section 5.4. Randomization to Cohorts 3 and 5 will be stratified as described in Section 5.4. The decision to randomize into the next cohort (dose level) will be approved by an SMC, comprised of at least a Principal Investigator participating in the study, the CRO Medical Monitor, and the Sponsor Medical Monitor. The SMC will review blinded safety data (adverse events, clinical laboratory data, vital signs, and ECGs), summary of PK data, if available, and other blinded relevant data. There will be 2 dose escalation decisions. After Cohort 1 completes the Day 36 visit, the SMC will decide whether subjects can be randomized into Cohorts 2 and 5 (both cohorts to start concurrently). After at least 8 subjects from both Cohort 2 and Cohort 5, including at least 1 subject on placebo

in each cohort, complete the Day 36 visit, the SMC will decide whether subjects can be randomized into Cohorts 3, 4, and 6 (all three cohorts to start concurrently).

For all subjects, treatment with study intervention may be continued for 8 additional weeks (total of 12 weeks) following review of individual safety data (AEs, ECGs, physical examination, and safety laboratory) through Day 30 (4 weeks) by the site Investigator, CRO Medical Monitor, and Sponsor Medical Monitor. Subjects who experience clinically significant TEAEs that are assessed as a potential risk to subject safety will be discontinued from study intervention and undergo an early termination visit. The decision to discontinue study intervention will be made by the site Investigator following discussion with the Sponsor Medical Monitor.

Cohorts 1 to 4 (weekly regimen): On Day -1, eligible subjects will be randomized (as described above) and be treated with SC injection of study intervention QW starting on Day 1 through Day 85; subjects will be domiciled at the Phase 1 unit from Day -1 (day before 1st dose) to Day 2 and from Day 28 (day before the 5th dose) to Day 30. Subjects will attend ambulatory clinic visits for dosing and evaluations per the SoA (Section 1.2.1, Table 2). On the Day 8, Day 15 and Day 22 ambulatory dosing visits, subjects will remain at the study site for observation for at least 2 hours post dose.

Cohorts 5 and 6 (every 2 weeks regimen): On Day -1, eligible subjects will be randomized (as described above) and be treated Q2W with SC injection of study intervention starting on Day 1 through Day 85; subjects will be domiciled at the Phase 1 unit from Day -1 (day before 1st dose) to Day 2 and from Day 28 (day before the 3rd dose) to Day 30. Subjects will attend ambulatory clinic visits for dosing and evaluations per the SOA (Section 1.2.1, Table 4). On the Day 15 ambulatory dosing visit, subjects will remain at the study site for observation for at least 2 hours post dose. On Days 64 and 78 subjects will be contacted by phone to inquire about AEs and concomitant medications.

Subjects in all cohorts will be followed up on Day 92 (1 week post last dose of study intervention) and Day 113, 4 weeks post last dose of study intervention (End of Study visit). Subjects who are found to be ADA positive at the end of study visit will be followed for 3-5 months after this visit or until stable or declining as deemed by the Medical Monitor.

Assessments during the study period will be done as specified in the Schedule of Activities (SoA; Table 2 for Cohorts 1-4 and Table 4 for Cohorts 5-6).

For each subject, the total duration of study participation will be up to 172 days (24.5 weeks) excluding the potential dose interruption due to the COVID-19 pandemic:

| | |
|---|---|
| Screening period: | ≤ 60 days (8.5 weeks) |
| Treatment period: | 84 days (12 weeks) |
| Follow-up period | 28 days (4 weeks) |

### 3.2.1. Scientific Rationale for Study Design

Part 1 is a randomized, double-blind, placebo-controlled, multiple ascending dose, 4-week study designed as a typical dose range finding study. The primary endpoints, safety and PK, are characteristic for this type of study. The study will be followed by an 8-week extension, and a number of secondary pharmacodynamic endpoints, including change in liver fat content as assessed by MRI-PDFF, will be evaluated. These will provide early proof-of-concept data to inform dose selection in future clinical studies.

### 3.2.2. Rationale for Study Population

In recent years, there has been a shift in regulatory expectations for early, Phase 1 clinical studies, whereby it is increasingly expected that the population enrolled into such studies would consist of patients who are similar to the intended target population for the drug. BIO89-100 is being developed for NASH with fibrosis; in line with this approach, study BIO89-100-002 will enroll subjects with NASH or with NAFLD and at a high risk for NASH. Subjects will be required to have evidence of ≥ 10% liver steatosis by MRI-PDFF, together with either biopsy-proven NASH (liver biopsy within 2 years before enrollment), or at least one of the following: (1) Obesity with T2DM or (2) Obesity with evidence of liver injury (latter defined as either increased ALT and/or Fibroscan vibration-controlled transient elastography (VCTE) score ≥7 KPa). It is notable that central obesity by itself increases the risk for NASH in patients with NAFLD, and that the additional requirement for presence of T2DM, a well characterized risk factor for NASH and advanced fibrosis, or evidence of liver injury, is considered appropriate to define a population of NAFLD patients who are at high risk for NASH. This population of patients with biopsy-proven NASH or NAFLD with a high risk for NASH also meets regulatory expectations for patients to be enrolled in early proof-of-concept studies for NASH, in which change in liver fat as measured by MRI-PDFF is a typical endpoint. Change in liver fat as determined by MRI-PDFF has emerged as the leading noninvasive quantitative biomarker for liver fat quantification as it is accurate, reproducible, precise, has excellent inter-rater and intra-rater reproducibility, and has a robust correlation with MRI spectroscopy-based quantification (the gold standard to quantify liver triglyceride content) for liver fat, with a correlation coefficient ranging from 0.98 to 0.99 (Tapper, 2018).The study population is thus also appropriate for proof-of-concept assessment of this and other pharmacodynamic endpoints.

### 3.2.3. Justification of Treatment Duration

The treatment duration will be 12 weeks, consisting of a 4-week study for initial safety assessment (primary endpoint) with an 8-week extension phase for efficacy and safety assessments. The study includes an individual safety checkpoint after 4 weeks of treatment, to ensure that only individuals with an acceptable safety profile will proceed to the 8-week extension phase. The study design will enable to obtain early proof-of-concept data, including assessment of change in liver fat (measured by MRI-PDFF) and additional biomarkers after 12 weeks of treatment with BIO89-100. For additional information, please also refer to the BIO89-100 IB.

Factors affecting the rate of liver de-fattening are not well characterized, and it is difficult to predict the dynamics of improvement in hepatic steatosis based on a drug's mechanism of action. Studies assessing change in liver fat by MRI-PDFF, including a recent study with another pegylated FGF21 product, pegbelfermin, typically had readouts at 12 weeks or later (Harrison, 2018a; Harrison, 2018b; Sanyal, 2019). Recently, ≥30% reduction in hepatic steatosis by MRI-PDFF at week 12 was found to be correlated with reduction in the ballooning and inflammation components of NAFLD Activity Score, and to be predictive of NASH resolution on liver biopsy at 36 weeks. In line with these considerations, the Sponsor plans to assess change in liver fat by MRI-PDFF and other pharmacodynamic biomarkers after 12 weeks of treatment.

### 3.2.4. Justification for Dose

Six dose cohorts are planned: 4 cohorts to be dosed QW and 2 cohorts to be dosed Q2W (Table 1). Based on emerging data and SMC recommendation, the Sponsor can decide to modify the dose of any subsequent cohort.

These dose regimens were selected based on nonclinical safety and pharmacology studies, interim review of PK and blinded safety data from the single ascending dose clinical study TV47948-SAD-10122 and PK modelling.

[redacted]

The exposure at the NOAEL for female and male mice as well as for monkey are compared to the observed $AUC_{(0\text{-}168)}$ from the highest dose in Study TV47948-SAD-10122. In determining the predicted safety margins for the highest dose planned in the Study BIO89-100-002 we used a partial fitting approach by employing PK data from Study TV47948-SAD-10122 and a previously developed model in monkeys. When using the predicted $AUC_{(0-168)}$ at steady state for the highest dose regimen, 27 mg QW, the safety margins ranged from [redacted] The predicted $C_{max}$ safety margins for the highest administered dose, 36 mg Q2W, [redacted]. The safety margins for both $AUC_{(0-168)}$ at steady state and for $C_{max}$ should provide more than adequate coverage for subjects in Study BIO89-100-002.

Clinical safety observations also support the dose regimens for Study BIO89-100-002. Clinical safety in healthy subjects is being established with single SC doses up to 78 mg in study TV47948-SAD-10122. The study has been recently completed; review of blinded safety data did not identify clinically significant safety concerns. Notably, the starting dose in BIO89-100-002, 3 mg QW for 13 doses, represents a total exposure of [redacted]

A PK modeling and simulation framework was undertaken to estimate the systemic exposure anticipated with the proposed dose regimens for Study BIO89-100-002. Based on these analyses, across the proposed dose range, plasma concentrations (median, 2.5$^{th}$ and 97.5$^{th}$ percentiles) are predicted to increase proportionally with dose up to and including 27 mg QW and 36 mg Q2W. The maximum proposed dose is anticipated to achieve maximal suppression of key PD responses associated with important clinical outcomes over the dosing interval, once steady-state is achieved. A 3 mg QW dose is anticipated to produce close to the half-maximal response whereas doses greater than 27 mg, administered QW, are not anticipated to produce substantially larger responses over a 27 mg dose. Therefore, the QW regimens are proposed to range from 3 mg to 27 mg, SC. Similar magnitude of maximal response is anticipated for a 36 mg dose administered Q2W.

Based on these data, the dose regimens are anticipated to be safe, of sufficient duration to reach steady state, to maintain PD responses over the dosing intervals, and to cover the dose range where key PD responses are predicted to be associated with important clinical outcomes to be

assessed in subsequent studies. Depending on emerging study findings, the range of doses in study BIO89-100-102 may be modified, and other intermediate doses may be added.

#### 3.2.4.1. Justification for Weekly (QW) and Once Every Two Weeks (Q2W) Dosing Regimens

Nonclinical data from 2 pharmacological studies in spontaneously diabetic cynomolgus monkeys, in which the effect of BIO89-100 (up to 2 mg/kg) was evaluated following either QW or Q2W dosing regimens, provides the rationale for the dosing regimens with BIO89-100 in the current study. In these nonclinical studies, statistically significant reductions were observed in body weight, food intake, glucose, LDL, triglycerides, HbA1c and ALT along with improvement in oral glucose test results and an increase in adiponectin levels in monkeys treated with QW or Q2W regimens of BIO89-100, compared to the vehicle group. The exposure profile of the once QW and Q2W regimens was similar, except for the expected higher trough levels on Day 14 and Day 28 with the QW dose. As the half-life of BIO89-100 in these monkeys was approximately 50 hours, the findings suggest the absence of a direct PK-PD correlation.

### 3.2.5. End of Study Definition

A subject is considered to have completed the study if he or she has completed all study periods including the End of Study (EOS)/Day 113 follow-up visit. The end of the study is defined as the date of the last visit of the last subject in the study.

## 3.3. Part 1 Eligibility Criteria

### 3.3.1. Part 1 Inclusion Criteria

Subjects are eligible to be included in the study only if all of the following criteria apply:

**Age and Gender**

1. Subjects must be 21 to 75 years of age inclusive, at the time of signing the informed consent form (ICF).
2. Male or female.

**Type of Subject and Disease Characteristics**

3. Evidence of steatosis by Fibroscan and MRI-PDFF:
    a. All subjects must complete a Fibroscan with Controlled Attenuation Parameter (CAP) measurement. Fibroscan must be done with a subject-appropriate probe during screening; CAP score should be ≥300 dB/m, unless a MRI-PDFF ≥10% within the last 12 months is available and the current CAP score is similar to CAP score when previous MRI-PDFF was done.
    b. MRI-PDFF ≥10% at screening (MRI must be completed no more than 35 days prior to randomization)

For the purpose of these inclusion criteria, use the following definitions:

**T2DM:** History of T2DM diagnosed at least 6 months prior to screening [diagnosis based on documentation of fasting plasma glucose (≥126 mg/dL), plasma glucose in the 75g OGTT (≥200 mg/dL) or HbA1c (≥ 6.5 %)]. Subjects must have a glycated hemoglobin (HbA1C) level

at <9.5%, without known macrovascular or clinically significant microvascular complications, and – if receiving antidiabetic medications – must be on a stable dose of antidiabetic medications before screening (6 months for Glucagon-Like Peptide 1 (GLP-1) analogs or Dipeptidyl Peptidase IV (DPP-IV) antagonists; 3 months for other oral or injectable medications; thiazolidinediones or insulin are NOT allowed in this study).

**Central Obesity:** Waist circumference of >102 cm for males, >88 cm for females, or body mass index (BMI) >30 kg/m$^2$. BMI should not exceed 45 kg/m$^2$.

**Increased ALT:** ALT ≥ 40 in males, ALT ≥ 30 in females. ALT level should not exceed 200 U/L.

4. NASH or high risk for NASH as reflected by **AT LEAST ONE** of the following:
    a. Diagnosis of NASH with fibrosis (stages F1, F2 or F3), without cirrhosis, by percutaneous liver biopsy meeting one of the following:
        a. Performed within 24 months prior to screening
        b. For subjects who did not undergo a liver biopsy in the 24 months prior to screening, a liver biopsy may be performed during screening if not contraindicated (optional, requires pre-approval by Sponsor). For subjects who underwent a previous liver biopsy >24 months prior to screening that would not have qualified for the study based on results of the initial biopsy, there should be a sound clinical basis to expect different findings in a repeat biopsy based on medical judgment.
    b. Central obesity WITH T2DM
    c. Central obesity WITH either increased ALT and/or Fibroscan VCTE score ≥7 KPa.

**Pregnancy and Contraception**

Contraceptive use by men or women should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

5. All subjects (male or female) who are of childbearing potential must agree to use highly effective, double contraception (both male and female partners) during the study. Double contraception is defined as use of a condom by the male partner, combined with use of one of the following forms of highly effective contraception by the female partner:
    a. a. Oral contraceptive pills,
    b. b. depot or injectable contraceptive,
    c. c. Intrauterine device (IUD),
    d. d. Contraceptive patch (e.g. Xulane®) or NuvaRing®, or
    e. e. documented evidence of surgical sterilization at least 6 months prior to screening visit (i.e. tubal ligation or hysterectomy).

Use of a condom in a male subject who underwent vasectomy is also acceptable as double contraception. Use of highly-effective, double contraception must continue for 30 days or 5 half-lives (whichever is longer) after the last dose of investigational product. Female subjects should not donate oocytes during this time. Male subjects must not donate sperm during this time. Rhythm methods are not considered as highly-effective methods of birth control. Subject abstinence for the duration of the study and 30 days or 5 half-lives (whichever is longer) after last dose of investigational product is acceptable if it is the subject's regular practice.

6. Females of childbearing potential must have a negative serum pregnancy test at Screening and a negative urine pregnancy test on Day -1. Female of childbearing potential must agree to undergo a pregnancy test prior to each administration of study intervention.
7. Females not of childbearing potential will be defined for this study as postmenopausal (defined as cessation of regular menstrual periods for at least 12 months) and confirmed by follicle stimulating hormone (FSH) level OR sterile.

   Medical documentation indicating that the subject had undergone a surgical sterilization procedure will be required from female study subjects to be considered not of childbearing potential in this study.

   Presumptive fertile male subjects who are partnered with women who are not of childbearing potential, either post-menopausal or through sterilization or other surgical procedures (e.g., hysterectomy) will confirm that their partners have been determined to be sterile or post-menopausal based on medical judgment; this can be confirmed either by statement or medical records, if presented by the subject.

   Men will not be required to use other contraception when sexually active with a sterile or post-menopausal partner.

   Men who are sexually active with fertile women need to confirm that they will use 2 forms of contraception as per protocol.

**Informed Consent**

8. Capable of giving signed informed consent which includes compliance with the requirements and restrictions listed in the ICF and in this protocol.

### 3.3.2. Part 1 Exclusion Criteria

Subjects are excluded from the study if any of the following criteria apply:

**Medical Conditions**

1. History of, or current symptoms of, cardiovascular disease or cerebrovascular disease including clinical congestive heart failure (CHF), symptomatic coronary artery disease (CAD), peripheral arterial disease, and/or abdominal aortic aneurysm (AAA), history of transient ischemic attack (TIA), peripheral vascular disease (PVD), or symptomatic carotid stenosis.
2. Clinically significant respiratory; hepatic (other than NAFLD or NASH); renal; gastrointestinal; neurological; immunological; hematologic, infectious or psychiatric disorder(s) (e.g. schizophrenia, generalized anxiety disorder, panic disorder, etc.); or a history of any illness that, in the opinion of the Investigator, might confound the results of the study, or pose additional risk to the subject by participation in the study. Individual cases which the Investigator deems the subject appropriate for inclusion despite a chronic medical condition should be discussed with and approved by the Medical Monitor. Subjects who are known to have tested positive for COVID-19 will be excluded, even they are asymptomatic.

   The following conditions are **NOT EXCLUDED**:

- **Hypertension**: Subjects with a history of hypertension, whose hypertension is controlled and are clinically stable, may be enrolled if they have been on a stable dose of no more than 2 antihypertensive medications at least 2 months before screening.
- **Dyslipidemia:** Subjects with dyslipidemia, who are clinically stable, may be enrolled, including subjects who have been on a stable statin dose at least 2 months before screening.
- **T2DM:** Subjects with T2DM may be enrolled if their HbA1c level does not exceed 9.5 %, there are no known macrovascular or clinically significant microvascular complications and they have been on a stable dose of antidiabetic medications in the 6 months before screening (for GLP-1 agonists or DPP-IV antagonists) or 3 months for other oral or injectable treatments. Subjects treated with thiazolidinediones or insulin are excluded from this study.
- **Depression**: Subjects with stable, controlled depression, who have not been hospitalized in the past for depression and have been on a stable dose of no more than one antidepressant in the 2 months before screening, may be enrolled.

3. Greater than 40% increase in ALT or AST between 2 screening assessments, to be done at least 2 weeks apart, as per the table below:

| **ALT/AST Screening Assessments** | | | **Eligibility status** |
|---|---|---|---|
| **Assessment 1** | **Assessment 2** | **Assessment 3 (if applicable)** | |
| Normal | Normal | Not applicable | Eligible |
| Normal | Abnormal and ≤40% increase from Assessment 1 | Not applicable | Eligible |
| Normal | Abnormal and >40% increase from Assessment 1 | Normal or ≤40% increase from Assessment 1 | Eligible |
| | | Abnormal and >40% increase from Assessment 1 | **Excluded** |
| Abnormal | ≤40% increase from Assessment 1 | Not applicable | Eligible |
| Abnormal | >40% increase from Assessment 1 | ≤40% increase from Assessment 1 | Eligible |
| | | >40% increase from Assessment 1 | **Excluded** |

Normal is defined as ≤ ULN; abnormal is defined as >ULN
Note: Clinical judgment should be used for subjects with isolated AST increases in whom there is suggestion of another cause of AST increase (e.g. muscle injury as evident by concurrent creatine phosphokinase elevation).

4. A personal or family history of arrhythmia, sudden unexplained death at a young age (before 40 years) in a first-degree relative, or long QT syndrome.
5. History of bariatric surgery or plan to have bariatric surgery during conduct of study.
6. History of type 1 diabetes.

7. Weight loss of more than 5% within 3 months prior to Day -1 or more than 10% within 6 months prior to Day -1 or planning to try to lose weight during conduct of study.
8. History of a liver disorder other than NASH or clinical suspicion of a liver disorder other than NASH, including but not limited to hepatitis B and hepatitis C, autoimmune hepatitis, hemochromatosis, alcoholic liver disease, primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), or Wilson's disease.
9. History of liver transplantation.
10. History of cirrhosis or evidence of cirrhosis (clinical, imaging-liver appearance or splenomegaly), advanced fibrosis (F4) on biopsy, VCTE-based Fibroscan >12.5 kPa or Fibrosis-4 (Fib-4) index[5] >3.25. In cases in which a patient is determined to have stage 4 fibrosis based on VCTE or FIB4, but has a liver biopsy within 12 months of screening and reported to show fibrosis stage F0, F1, F2 or F3, the subject will be considered to be eligible based on the liver biopsy result.
11. Major trauma or surgery in the 2 months before screening or at any time between screening and Day -1.
12. Recent clinically significant acute illness (within 4 weeks of screening) unless per the Investigator's clinical discretion a full recovery is apparent.
13. History of bone trauma, fracture or surgery within 2 months of screening.
14. Have any known malignancy or history of malignancy, except for basal cell skin cancer that has been treated with no evidence of recurrence for at least 3 months prior to Day -1. In specific circumstances in which there is basis to consider the subject as cured from cancer, exceptions may be considered. Any exception would need to be approved in advance by the Sponsor's Medical Monitor.
15. History of alcohol use disorder (per DSM-5) or risky drinking (defined as alcohol intake >14 standard drink units per week or 4 standard drinks on a single occasion in men; and alcohol intake >7 standard drink units per week or 3 standard drinks on a single occasion in women) within the 24 months before Day -1, or had a positive alcohol test at screening and/or check-in. A unit of alcohol is defined as 14 gram or as 355 mL of beer (5%), 1 glass of wine (150 mL; 12%), or 1 shot of hard liquor (45 mL; 40%). During the study, subjects will be encouraged to abstain from alcohol. Alcohol intake will be limited to 2 drinks per day for men and 1 drink per day for women.
16. History of drug abuse, or any other substance dependence (with the exception of caffeine) as defined by the DSM-IV-TR in the past 2 years prior to screening or a positive test for drugs of abuse (i.e., amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine metabolites, opiates, and phencyclidine) at screening and/or Day -1.
17. Daily use of more than 10 cigarettes/day, or 2 cigars/day, or equivalent use of any tobacco product within 6 weeks prior to Screening. E-cigarettes or other vaping devices should not deliver more than 15 mg of nicotine/day.
18. Pregnant or breastfeeding, or planning to become pregnant or breastfeed while enrolled in the study or within 30 days or 5 half-lives (whichever is longer) after last dose of study

[5] FIB4 score = Age × AST (IU/l)/platelet count (× $10^9$/l) × √ALT (IU/l)

intervention. Presumptive fertile, sexually active male subjects, whose female partner is pregnant, will be excluded.

**Prior/Concomitant Therapy**

19. Subject report of use of medications historically associated with secondary NAFLD for more than 2 consecutive weeks in the 12 months prior to screening (e.g., amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, and other medications with known hepatotoxicity).
20. Any prior exposure to a FGF21 analogue (e.g. BIO89-100, LY2405319, LY3025876, BMS-986036, BMS-986171, PF-05231023, PF-06645849, AKR-001) or FGFR1 activating product, if known.
21. Any previous long-term (> 4 weeks) use of systemic steroid (glucocorticoid) medications such as prednisone.
22. Vitamin E supplementation beyond Recommended Dietary Allowance (22.4 IU/day) within 6 months before screening.
23. Any systemic medications, including over-the-counter (OTC) medications, prescription drugs, biologics, vaccines, herbal preparations, used within 14 days, or 5 half-lives, whichever is longer, before Day -1 and throughout the study with the FOLLOWING EXCEPTIONS:
    a. Acetaminophen (up to 4 g/day, use as indicated in the label)
    b. Ibuprofen (up to 1.2 g/day of ibuprofen use as indicated in the label)
    c. Aspirin (up to 81 mg/day)
    d. Antihypertensive medications (no more than 2 agents) on stable dose for at least 2 months prior to screening
    e. Antidiabetic medications: stable dose for 6 months prior to screening for GLP-1 analogs or DPP-IV antagonists; 3 months prior to screening for oral or other injectable medications. Thiazolidinendiones and insulin are not allowed in this study.
    f. Statins on stable dose for at least 2 months
    g. Asthma treatment with exception of oral corticosteroids
    h. Anti-depressants (no more than 1 agent) on stable dose for at least 2 months
    i. Other exceptions must be approved by the Medical Monitor
    j. Vitamins (other than vitamin E at a dose greater than recommended dietary allowance) and food supplements are allowed if the subject had been taking a stable regimen for at least 2 months before screening.

**Prior/Concurrent Clinical Study Experience**

24. Any investigational drug small molecule (new chemical entity) within 30 days and large molecule (biologics) within 90 days, or 5 half-lives, whichever is longer, prior to Day -1, if known.

**Diagnostic Assessments**

25. Any history of suicidal behavior or suicidal ideation with plan and intent based upon clinical history. A “yes” response to any of the suicidal ideation or suicidal behavior questions in the C-SSRS on Day -1.

26. Any clinically significant laboratory abnormality at screening. One repeat test may be allowed at the discretion of the Investigator. The presence of one or more of the following laboratory abnormalities should lead to exclusion of the subject from participating in the study:
    a. ALT or AST ≥ 200 U/L
    b. Elevation of total bilirubin (TB) > ULN
    c. Normalized ratio (INR) > ULN
    d. Alkaline phosphatase at screening >1.25× ULN
    e. Glomerular filtration rate (eGFR) ≤60 mL/min/1.73 $m^2$ as estimated by CKD-EPI Creatinine equation (Levey, 2009)
    f. Serum total triglycerides ≥ 1000 mg/dL
    g. HbA1c ≥ 9.5%
27. ECG abnormality that may, in the opinion of the Investigator, interfere with study participation, including intraventricular conduction delays (QRS interval ≥120 msec or PR interval >200 msec (220 msec in individuals with a heart rate <70 beats per minute), and resting QTcF interval of ≤320 msec and/or ≥450 msec for males or ≥470 msec for females.
28. Vital sign abnormalities on screening, including: having a supine systolic blood pressure <90 or >150 mmHg, diastolic blood pressure (following at least a 5 minute rest) <50 or >95 mmHg, or resting heart rate <50 or >100 beats per minute.
29. BMI at screening <25 or ≥ 45 kg/$m^2$.

**Other Exclusions**

30. Known allergy or sensitivity to injected proteins, or any component of the formulation, or has a history of allergies (including food allergies) requiring acute or chronic treatment (except for seasonal allergic rhinitis).
31. Donated or received any blood or blood products (e.g., white blood cells, platelets) within the 60 days prior to screening, or has donated blood or blood products at least twice within the 6 months prior to screening, or the subject has donated plasma within 7 days of the screening visit, or has planned donations during the 56 days or 5 half-lives following the last day of study intervention administration, whichever is longer.
32. Any abnormality of the skin or abdominal wall that would affect SC administration to the abdominal area or any tattoos or scars in the intended injection area.
33. An employee of the investigational center or has a family member who is involved with the conduct of this study.
34. Subject who cannot undergo MRI for any reason (e.g., contraindication, claustrophobia, excessive weight or body size for MRI machine).
35. Subject who cannot fast for study procedures for any reason. Specifically, subjects with T2DM who have had one or more episodes of hypoglycemia or past issues with fasting will be excluded. Subjects with T2DM who are treated by insulin secretagogues will need

to consult their treating physician about the optimal timing to take these medications to enable them to fast safely for study procedures.

# 4. PART 2

## 4.1. Part 2 Objectives and Endpoints

| Objectives | Endpoints |
|---|---|
| Primary | |
| • To evaluate the safety and tolerability of SC injections of 27 mg BIO89-100, administered weekly for 20 weeks, in subjects with biopsy-proven NASH (NAS ≥4, fibrosis stage F1 with high risk, F2 or F3) | • Frequency of AEs and SAEs |
| • To characterize effect of BIO89-100 on liver histology | • At least a 2-point improvement in NAFLD Activity Score (NAS) with at least a 1-point improvement in ballooning or lobular inflammation, and no worsening of fibrosis |
| Secondary | |
| • To characterize biomarkers, PD profile, and biological activity of BIO89-100 | Change and percentage change from baseline in the following biomarkers/PD parameters:<br>• Anthropomorphic measurements:<br>– Body weight<br>• Laboratory parameters:<br>– Triglycerides<br>– non-HDL cholesterol<br>– HDL-c<br>– LDL-c<br>– HbA1c<br>– Liver function tests: ALT, AST<br>– Pro-C3<br>• Imaging measures:<br>– MRI-PDFF |
| • To characterize effect of BIO89-100 on liver histology | • Improvement of fibrosis ≥1 stage without worsening of NASH |

| Objectives | Endpoints |
|---|---|
| | • NASH resolution without worsening of fibrosis[6] |
| • To characterize BIO89-100 PK | • Trough concentration of BIO89-100 |
| Other Safety Endpoints | |
| [redacted] | |
| Exploratory Endpoints | |
| [redacted] | |

[6] Resolution of NASH includes the total absence of ballooning (score=0) and absent or mild inflammation (score 0 to 1) associated with at least a 2-point reduction in NAS, and no worsening if fibrosis (progression ≥ 1 stage).

| Objectives | Endpoints |
|---|---|
| [redacted] | |

[7] ≥30% relative reduction in liver fat by MRI-PDFF at D85
[8] Resolution of NASH includes the total absence of ballooning (score=0) and absent or mild inflammation (score 0 to 1) associated with at least a 2-point reduction in NAS, and no worsening if fibrosis (progression ≥ 1 stage).

| Objectives | Endpoints |
|---|---|
| [redacted] | |

## 4.2. Part 2 Study Design

Part 2 is an open-label cohort that will enroll 20 subjects with biopsy-proven NASH and fibrosis (NAS ≥4, fibrosis stage F1 with high risk, F2 or F3). Subjects will either undergo a liver biopsy during screening or have a recent liver biopsy (within ≤24 weeks before Day 1, available to be evaluated for eligibility by the central reader) that meets study inclusion criteria. Eligible subjects will be treated with weekly (QW) SC injection of study intervention starting on Day 1 and continuing through Day 134 (20 weeks of treatment). Subjects will attend ambulatory clinic visits for dosing and evaluations and home visits for dosing[9] as per the Schedule of Activities (SoA; Section 1.2.2, Table 6). Subjects will undergo a second liver biopsy within 14 days after the last dose of study intervention. Subjects will be followed up on Day 141 (1 week post last dose of study intervention) and Day 162 (4 weeks post last dose of study intervention, End of Study visit). Subjects who are found to be NAb-positive at the End of Study visit will be followed approximately every 3 months starting from when the site has been notified of the positive result, until: (1) neutralizing antibodies are no longer detectable or (2) the subject has been followed for a period of at least 1 year (± 4 weeks). More frequent testing (e.g., every month) or testing for a longer period of time may be requested in the event of safety-related concerns.

### 4.2.1. Scientific Rationale for Part 2 Study Design

Part 2 is designed as an open-label cohort. Preliminary data from placebo-controlled Part 1 demonstrated favorable safety and tolerability with repeated dosing of BIO89-100 for 12 weeks. In addition, in Part 1, robust, clinically meaningful reductions in liver fat assessed by MRI-PDFF and ALT, at thresholds that have been associated with histological benefit in patients with NASH, have been observed. Part 2 extends the study to subjects with biopsy-proven NASH and fibrosis (NAS ≥4, fibrosis stage F1 with high risk, F2 or F3), similar to the population that is typically enrolled in later phase NASH studies, who will be treated with BIO89-100 at a dose of 27 mg QW for 20 weeks. In addition to the primary safety endpoints, this will be the first study to characterize the effect of BIO89-100 on liver histology. Many of the same secondary PD endpoints as in Part 1 will be evaluated, including change in liver fat content as assessed by MRI-PDFF, to inform study design and choice of endpoints in future clinical studies.

[9] If home administration is not feasible, not desired by the subject, or deemed inappropriate option by the investigator for any reason, in-clinic study intervention administration may take place at some or all timepoints.

### 4.2.2. Rationale for Part 2 Study Population

BIO89-100 is being developed for NASH with fibrosis. Preliminary data from Part 1 demonstrated favorable safety and tolerability and robust, clinically meaningful reductions in liver fat assessed by MRI-PDFF and in ALT, with reductions in both measures beyond thresholds that have been associated with histological benefit in patients with NASH, with repeated dosing of BIO89-100 for 12 weeks, in a similar population to the intended population in Part 2 . Part 2 extends the study to subjects with biopsy-proven NASH and fibrosis (NAS ≥4, fibrosis stage F1 with high risk, F2 or F3). This population of patients meets current regulatory expectations for patients to be enrolled in later stage studies for NASH, and will allow to assess changes in liver histology, as well as changes in liver fat as measured by MRI-PDFF and other biomarkers in a population of patients aligned with NASH registration studies. Histological endpoints will include at least a 2-point improvement in NAS with at least a 1-point improvement in ballooning or lobular inflammation with no worsening of fibrosis (primary), improvement of fibrosis by at least 1 stage without worsening of NASH, and NASH resolution without worsening of fibrosis (secondary), and additional exploratory endpoints. In addition to other exploratory histological endpoints, exploratory analysis of histological endpoints in the subset of MRI-PDFF responders (≥30% relative reduction in liver fat by MRI-PDFF at D85) will be conducted. The study population is appropriate for assessment of these and other PD endpoints.

### 4.2.3. Justification for Part 2 Treatment Duration

The treatment duration will be 20 weeks, with weekly (QW) doses. Favorable safety and tolerability of 12-weeks of dosing with BIO89-100 at a dose of 27 mg QW was shown in Part 1. Based on results of recently published studies of Phase 2 clinical studies in a similar population of NASH patients, including a study with another FGF21 analogue (AKR-001, Akero), a treatment duration of 20 weeks is considered appropriate for an initial evaluation of the histological endpoints that will be assessed in this study.

### 4.2.4. Justification for Part 2 Dose

Subjects in open-label Cohort 7 will be dosed with 27 mg BIO89-100 QW for 20 weeks. Administration of 27 mg QW to patients with NASH or NAFLD and high risk for NASH (phenotypic NASH) for 12 weeks has been studied in Part 1 of this study. Justification for administration of 27 mg BIO89-100 QW, which was the highest QW dose in Part 1, was based on nonclinical safety and pharmacology studies, including a 13-week interim analysis of the ongoing GLP 26-week toxicology study in mice (Study 6700477), PK, and safety data from the single ascending dose clinical study TV47948-SAD-10122 and PK modeling, and is provided in Section 3.2.4. Results of the GLP 26-week toxicology study in mice (Study 6700477), that has been completed, are available in the IB, and support 20 weeks of weekly dosing with 27 mg BIO89-100.

In Part 1 of Study BIO89-100-002, 10 subjects were randomized to Cohort 4 (27 mg QW). Preliminary data show that, while significant absolute and relative reduction in liver fat as assessed by MRI-PDFF at week 13 was observed for all studied doses, maximal reduction was achieved with the [redacted]

It has been shown that MRI PDFF response ≥30% (Loomba, 2015; Loomba, 2017; Harrison, 2019) and ALT reduction ≥17 U/L (Loomba, 2019) are associated with a higher rate of NASH resolution and fibrosis reduction. In view of this, and considering the robust, clinically meaningful effects on MRI-PDFF and ALT and the favorable safety and tolerability profile of the 27 mg QW dose, the Sponsor believes that 27 mg QW is the most appropriate dose to be studied in an initial investigation of the potential effect of BIO89-100 on histological endpoints in a clinically relevant population of patients with NASH. The results of this study are expected to provide valuable information to guide dose selection for future BIO89-100 studies with histological endpoints in NASH.

### 4.2.5. Part 2 End of Study Definition

A subject is considered to have completed the study if he or she has completed all study periods including the End of Study (EOS)/Day 162 follow-up visit. The end of the study is defined as the date of the last visit of the last subject in the study.

## 4.3. Part 2 Eligibility Criteria

### 4.3.1. Part 2 Inclusion Criteria

Subjects are eligible to be included in the study only if all of the following criteria apply:

**Age and Gender**

1. Subjects must be 21 to 75 years of age inclusive, at the time of signing the informed consent form (ICF).
2. Male or female.

**Type of Subject and Disease Characteristics**

3. Increased metabolic risk; subjects must have at least 2 of the following:
    a. Central obesity: Waist circumference of >102 cm for males, >88 cm for females, or body mass index (BMI) >30 kg/m$^2$. BMI should not exceed 50 kg/m$^2$
    b. Fasting plasma glucose ≥126 mg/dL or previously diagnosed T2DM
    c. Increased fasting triglycerides (≥150 mg/dL) or on treatment for hypertriglyceridemia
    d. Reduced fasting HDL cholesterol (<40 mg/dL for male and <50 mg/dL for female)
    e. Hypertension or treatment of previously diagnosed hypertension

**Only subjects meeting Criterion 3 should be further evaluated for participation.** *Subjects who do not initially meet Criterion 3 may be re-tested, based on investigator judgment.*

4. Evidence of fibrosis – must meet at least one of the following criteria:
    a. Fibroscan VCTE score ≥8.5 kPa at screening or within the last 3 months[10] AND Aspartate aminotransferase (AST) > 20 U/L for males or >17 U/L for females in the first screening measurement[11]
    b. A historical liver biopsy obtained more than 24 weeks and less than 2 years before Screening with Stage 1, 2 or 3 fibrosis.
5. Evidence of steatosis by Fibroscan and MRI-PDFF:
    a. All subjects must complete a Fibroscan with CAP measurement. Fibroscan must be done with a subject-appropriate probe during screening; CAP score should be ≥280 dB/m at screening or within the last 3 months unless an MRI-PDFF ≥8% within the last 12 months is available and the current CAP score is similar to or greater than CAP score when previous MRI-PDFF was done.
        NOTE: If a Fibroscan model with CAP technology is not available, MRI-PDFF will be adequate to assess steatosis.
    b. MRI-PDFF ≥8% at screening (MRI must be completed no more than 35 days prior to baseline)
6. Biopsy-proven NASH in a liver biopsy obtained within 24 weeks of baseline with fibrosis stage F1, F2, or F3 and NAS ≥4, with a score of at least 1 in each of steatosis, ballooning degeneration, and lobular inflammation.

    Liver biopsy may be obtained during screening or be a historical percutaneous biopsy that is deemed suitable for interpretation by the central reader (in up to approximately 20% of subjects[12]), if the patient had no significant change in metabolic status (control of diabetes or hyperlipidemia, >5% weight loss or gain) or medications.

    Patients with fibrosis stage F1 with high risk will be eligible (no more than approximately 20% of subjects). Fibrosis stage F1 with high risk is defined as fibrosis stage F1 AND at least one of the following criteria: a. T2DM, b. BMI ≥30 kg/m$^2$, c. ALT > 1.5 x ULN at screening, d. elevated Pro-C3 (>14 ng/mL) within 6 months before screening.

**Pregnancy and Contraception**

Contraceptive use by men or women should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

7. All subjects (male or female) who are of childbearing potential must agree to use highly effective, double contraception (both male and female partners) during the study. Double

[10] If subject had fibroscan a VCTE score ≥ 8.5 kPa within last 3 months, screening VCTE score should be ≥ 7.5 kPa.
[11] If there is reason to suspect contribution of a non-hepatic source of AST (e.g., in the presence of elevated. creatine phosphokinase [CPK] or other evidence of muscle injury), AST should be re-tested when there is no longer evidence of potential contribution of an extra-hepatic source. The first assessment for Exclusion criterion 3 will be the one that made the subject eligible according to Inclusion criterion 4.
[12] Subjects with an eligible historical biopsy that was obtained within 24 weeks from baseline do not need to meet Inclusion Criteria 4 and 5.

contraception is defined as use of a condom by the male partner, combined with use of one of the following forms of highly effective contraception by the female partner:

a. Oral contraceptive pills,
b. Depot or injectable contraceptive,
c. Intrauterine device (IUD),
d. Contraceptive patch (e.g. Xulane®) or NuvaRing®, or

Use of a condom in a male subject who underwent vasectomy is also acceptable as double contraception. Use of highly effective, double contraception must continue for 30 days or 5 half-lives (whichever is longer) after the last dose of study intervention. Female subjects should not donate oocytes during this time. Male subjects must not donate sperm during this time. Rhythm methods are not considered as highly effective methods of birth control. Subject abstinence for the duration of the study and 30 days or 5 half-lives (whichever is longer) after the last dose of study intervention is acceptable if it is the subject's regular practice.

8. Females of childbearing potential must have a negative serum pregnancy test at Screening and a negative urine pregnancy test on Day 1. Females of childbearing potential must agree to undergo a pregnancy test prior to each administration of study intervention.
9. Females not of childbearing potential will be defined for this study as postmenopausal (defined as cessation of regular menstrual periods for at least 12 months) and confirmed by follicle-stimulating hormone (FSH) level OR surgically sterile. For the purposes of the study, surgical sterilization includes hysterectomy, bilateral salpingectomy, or bilateral oophorectomy or bilateral tubal occlusion.
10. Presumptive fertile male subjects who are partnered with women who are not of childbearing potential, either postmenopausal or through sterilization or other surgical procedures (e.g., hysterectomy) will confirm that their partners have been determined to be sterile or postmenopausal based on medical judgment; this can be confirmed either by statement or medical records, if presented by the subject. Men will not be required to use other contraception when sexually active with a sterile or postmenopausal partner.
11. Men who are sexually active with women of childbearing potential need to confirm that they will use 2 forms of contraception as per protocol.

**Informed Consent and Study Requirements**

12. Capable of giving signed informed consent, which includes compliance with the requirements and restrictions listed in the ICF and in this protocol.
13. Subjects must be available for the entire duration of the study and be willing to undergo 2 liver biopsies, one at screening (unless a qualifying historical biopsy is available within 24 weeks before baseline, in which case subjects will undergo only the second liver biopsy) and a second biopsy the end of the study.
14. Subjects must not participate in any other clinical trial throughout the duration of this study. Coronavirus virus disease 2019 (COVID-19) protocols may be excepted with Medical Monitor (or designee) approval.

### 4.3.2. Part 2 Exclusion Criteria

Subjects are excluded from the study if any of the following criteria apply:

**Medical Conditions**

1. Unstable or clinically significant cardiovascular or cerebrovascular disease:
   a. Unstable angina, myocardial infarction, coronary artery bypass graft (CABG) surgery, percutaneous coronary intervention (PCI), transient ischemic attack (TIA) or cerebrovascular accident (CVA) within 6 months prior to Screening.
   b. Symptomatic valvular or other structural heart disease.
   c. Symptomatic congestive heart failure.
   d. Symptomatic, uncontrolled or high-risk arrhythmia or genetic predisposition to high-risk arrhythmia in the subject or a first degree relative.
   e. Implanted defibrillator or pacemaker.
   f. High risk abdominal aortic aneurysm, uncontrolled peripheral vascular disease, or symptomatic carotid stenosis.
2. Any other clinically significant disorder or prior therapy that, in the opinion of the Investigator, might pose additional risk to the subject, make the subject unable to comply with the protocol requirements, or confound the results of the study. Individual cases in which the Investigator deems the subject appropriate for inclusion despite a clinically significant chronic medical condition should be discussed with and approved by the Medical Monitor. Subjects who have tested positive for coronavirus virus disease 2019 (COVID-19) within 3 months prior to screening will be excluded, even if they are asymptomatic.
3. Greater than 40% increase in ALT or AST between 2 screening assessments, to be done at least 2 weeks apart, as per the table below:

| **ALT/AST Screening Assessments** | | | |
|---|---|---|---|
| **Assessment 1** | **Assessment 2** | **Assessment 3 (if applicable)** | **Eligibility Status** |
| Normal | Normal | Not applicable | Eligible |
| Normal | Abnormal and ≤40% increase from Assessment 1 | Not applicable | Eligible |
| Normal | Abnormal and >40% increase from Assessment 1 | Normal or ≤40% increase from Assessment 1 | Eligible |
| | | Abnormal and >40% increase from Assessment 1 | **Excluded** |
| Abnormal | ≤40% increase from Assessment 1 | Not applicable | Eligible |
| Abnormal | >40% increase from Assessment 1 | ≤40% increase from Assessment 1 | Eligible |
| | | >40% increase from Assessment 1 | **Excluded** |

Normal is defined as ≤ ULN; abnormal is defined as >ULN.

Note: Clinical judgment should be used for subjects with isolated AST increases in whom there is suggestion of another cause of AST increase (e.g., muscle injury as evident by concurrent creatine phosphokinase elevation).

4. History of bariatric surgery within the prior 5 years (including gastric banding or sleeve surgery) or plan to have bariatric surgery during conduct of study.
5. History of type 1 diabetes.
6. Weight loss of more than 5% within 3 months prior to Day 1 or more than 10% within 6 months prior to Day 1.
7. History of a liver disorder other than NASH or clinical suspicion of a liver disorder other than NASH, including but not limited to hepatitis B and hepatitis C, autoimmune hepatitis, hemochromatosis, alcoholic liver disease, PSC, PBC, alpha 1-antitrypsin deficiency, or Wilson's disease. Subjects who were previously diagnosed with chronic HCV infection who achieved sustained viral response (SVR) following treatment with a direct-acting antiviral (DAA) regimen at least 2 years prior to screening are not excluded.
8. Prior or planned liver transplantation.
9. History of cirrhosis or evidence of cirrhosis (clinical, imaging-liver appearance or splenomegaly), advanced fibrosis (F4) on biopsy, VCTE-based Fibroscan >20.0 kPa. In cases in which a patient is determined to have Stage 4 fibrosis based on VCTE but has an eligible liver biopsy within 6 months of Day 1, the subject will be considered to be eligible based on the liver biopsy result.
10. History of bone trauma, fracture, or surgery within 2 months of screening or other bone disorders, such as osteoporosis, osteomalacia, or known, untreated severe vitamin D deficiency (serum 25-hydroxy-vitamin D ≤5 ng/mL).
11. Have any known malignancy or history of malignancy, except for basal cell skin cancer that has been treated with no evidence of recurrence for at least 3 months prior to Day 1. In specific circumstances in which there is basis to consider the subject as cured from cancer, exceptions may be considered. Any exception would need to be approved in advance by the Sponsor's Medical Monitor.
12. Current or history of significant alcohol consumption for a period of more than 3 consecutive months within 1 year prior to screening. Defined as more than 14 units/week for females and more than 21units/week for males on average.
13. History of drug abuse, or any other substance dependence (with the exception of caffeine) as defined by the DSM-IV-TR in the past 2 years prior to screening. Subjects without a history of substance use disorder who have a positive drug screen test at screening may be considered for enrollment with Medical Monitor (or designee) approval. Chronic, habitual use of cannabis/cannabinoids is exclusionary.
14. Pregnant or breastfeeding, or planning to become pregnant or breastfeed while enrolled in the study or within 30 days or 5 half-lives (whichever is longer) after last dose of study intervention. Presumptive fertile, sexually active male subjects, whose female partner is pregnant, will be excluded.

**Prior/Concomitant Therapy**

15. Subject report of use of medications historically associated with secondary NAFLD for more than 2 consecutive weeks in the 12 months prior to screening (e.g., amiodarone, methotrexate, systemic glucocorticoids [>2 weeks of pharmacologic dosing], tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, and other medications with known hepatotoxicity). Short term oral steroid bursts with/without taper as well as topical and inhaled steroids are allowed.
16. Any prior exposure to a FGF21 analogue (e.g., BIO89-100, LY2405319, LY3025876, BMS-986036, BMS-986171, PF-05231023, PF-06645849, AKR-001) or FGFR1 activating product, if known.
17. Any investigational drug small molecule (new chemical entity) within 30 days and large molecule (biologics) within 90 days, or 5 half-lives, whichever is longer, prior to Day 1, if known.
18. Subjects taking Vitamin E must be on a stable dose for at least 6 months prior to the qualifying liver biopsy (historical or screening).
19. Subjects may be enrolled if they have been on a stable anti-diabetic regimen for 3 months prior to biopsy (historical or screening). Stable regimen is defined as no addition or discontinuation of antidiabetic medications, but dose adjustments per standard of care are allowed. Insulin and DPP-IV antagonists are allowed if subject has been on a stable regimen for at least 3 months. GLP-1 agonists and SGLT2 inhibitors are allowed if subject has been on a stable dose for 6 months. Thiazolidinediones are not allowed.
20. Subjects on antiplatelet or anticoagulant medications in whom there is a significant medical risk to stopping treatment with these medications temporarily (as required per local practice for liver biopsy) due to the nature of the treated medical condition should be excluded.

**Diagnostic Assessments**

21. Suicidal behavior or suicidal ideation with plan and intent based: A “yes” response to any of the suicidal ideation or suicidal behavior questions in the C-SSRS within the 12 months prior to Day 1.
22. Any clinically significant laboratory abnormality at screening including but not limited to the following. One repeat test may be allowed at the discretion of the Investigator.

    g. ALT or AST ≥250 U/L

23. ECG abnormality that may, in the opinion of the Investigator, interfere with study participation or present potential significant patient safety risk. Resting QTcF interval ≥450 msec for males or ≥470 msec for females.
24. Clinically significant vital sign abnormalities on screening, including having a systolic blood pressure <90 or >150 mmHg, diastolic blood pressure (following at least a 5 minute rest) <50 or >95 mmHg.
25. BMI at screening <25 or ≥ 50 kg/$m^2$.

**Other Exclusions**

26. History of anaphylaxis or known allergy or sensitivity to any component of the study drug.
27. Any abnormality of the skin or abdominal wall that would affect SC administration to the abdominal area.
28. An employee of the investigational center or has a family member who is involved with the conduct of this study.
29. Inability to undergo a liver biopsy safely for any reason
30. Subject who cannot undergo MRI for any reason (e.g., contraindication, claustrophobia, excessive weight or body size for MRI machine).
31. Subject who cannot fast for study procedures for any reason. Specifically, subjects with T2DM who have had one or more episodes of hypoglycemia or past issues with fasting will be excluded. Subjects with T2DM who are treated by insulin secretagogues will need to consult their treating physician about the optimal timing to take these medications to enable them to fast safely for study procedures.

# 5. STUDY INTERVENTION

Study intervention is defined as BIO89-100 (or matching placebo in Part 1), intended to be administered to a study subject according to the study protocol.

## 5.1. Study Intervention(s) Administered

Study intervention will be administered SC to the abdomen region by qualified study personnel. In Part 2, some study intervention administrations may occur in the subject's home[13].

| **ARM** | **Active arm** | **Placebo arm (control)** |
|---|---|---|
| **Intervention** | BIO89-100 | Matching placebo |
| **Type** | Biologic | Chemical solution |
| **Dose Formulation**[a] | Each 0.7 mL vial contains 18 mg of BIO89-100 in Tris buffer solution (containing sodium chloride, sucrose, and polysorbate 20) at a concentration of 26 mg/mL | Each 0.7 mL vial contains Tris buffer solution (containing sodium chloride, sucrose, and polysorbate 20) |
| **Unit Dose Strength(s)** | mg | Not applicable |
| **Dosage Level(s)**[b] | Part 1<br>3 mg, 9 mg, 18 mg, 27 mg weekly (QW)<br>18 mg and 36 mg every other week (Q2W)<br>Note: Based on emerging data and SMC recommendation, the Sponsor can decide to modify the dose of any subsequent cohort | Part 1<br>NA; matching placebo will be injected at matching frequency per assigned cohort. |
| | Part 2<br>27 mg weekly (QW) | Part 2<br>Not applicable |
| **Route of Administration** | SC injection | SC injection |
| **Sourcing** | Provided centrally by the Sponsor | Provided centrally by the Sponsor |
| **Packaging and Labeling** | BIO89-100 is supplied as a sterile, preservative-free, frozen formulation in a single-use Type 1 clear glass vial for SC injection. Each vial will be labeled as required per country requirement. | Placebo will be supplied as a sterile, preservative-free, frozen formulation in a single-use Type 1 clear glass vial for SC injection. Each vial will be labeled as required per country requirement. |

[a] Note, in the original batch: each 1.5 mL vial contains 36 mg of BIO89-100

[b] The actual doses will be ±5% the mg dose due to technical considerations related to drug withdrawal from the vials into the syringes for injection. This difference is considered negligible for subject exposure.

[13] Details on home administration are provided in the Home Administration Manual. If home administration is not feasible, not desired by the subject, or deemed inappropriate by the investigator for any reason, in-clinic study intervention administration may take place at some or all of the designated timepoints.

## 5.2. Administration Instructions

The SC injection of the study intervention should be performed by a limited number of site staff specifically trained in the administration of SC injections. However, administration of study intervention by a trained home health care worker will be considered on a case-by-case basis if circumstances related to the COVID-19 pandemic preclude dosing at the study site. Injections will be administered in the abdominal area only. Details on injection of study intervention will be provided in the relevant study manual.

After administration of the study intervention, the subject should remain supine for approximately 15 minutes. For additional doses in the same subject, the injection site should be rotated within the abdominal area by at least 2 inches. Injections should be at least 2 inches from the umbilicus (belly button).

In Part 2, SC injection of the study intervention will be performed by qualified site staff when the subject attends clinic visits. Details on self-administration at home, including training requirements, are provided in the Home Administration Manual.

## 5.3. Preparation/Handling/Storage/Accountability

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.

Only subjects enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention during in-clinic visits with the exception that administration of study intervention by a trained home health care worker will be considered on a case-by-case basis if circumstances related to the COVID-19 pandemic preclude dosing at the study site. In Part 2, study subjects may self-administer study intervention either in the clinic (under supervision) or at home. All study intervention must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

The Investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

Further guidance and information for study intervention preparation and administration and the final disposition of unused study interventions are provided in the relevant manuals.

Details on preparation, handling, storage, and accountability for in-home visits in Part 2 are provided in the Home Administration Manual.

## 5.4. Measures to Minimize Bias: Randomization

In Part 1, eligible subjects will be randomized in the order they are enrolled into the study. For Cohort 1, 8 eligible subjects will be randomized in a 3:1 ratio to active (BIO89-100) : control (placebo). For Cohort 2, 15 subjects will be randomized in a 4:1 ratio to active (BIO89-100) : control (placebo). For Cohorts 4 and 6, 12 eligible subjects will be randomized in each cohort in a 3:1 ratio to active (BIO89-100) : control (placebo). For Cohorts 3 and 5, 18 subjects will be randomized in each cohort in a 7:2 ratio to active (BIO89-100) : control (placebo), stratified by biopsy-confirmed NASH with fibrosis status

F1, F2 or F3 (Yes, No) (Kleiner, 2005), with each stratum of 9 subjects in each cohort. All subjects in Part 1 will be centrally assigned to randomized study intervention using an Interactive Response Technology (IRT).

Part 1 will be conducted under double-blind conditions. The subjects, Principal Investigator, other study personnel involved with subject assessments, and the Sponsor will remain blinded to the actual treatment assignments of the subjects. Blinded study intervention will be prepared by a clinical supplies vendor and shipped to the study site. Where applicable, an unblinded pharmacist at the study site will prepare the study intervention for SC administration.

Part 2 will be open-label with all subjects receiving the same dose of BIO89-100.

## 5.5. Study Intervention Compliance

When subjects are dosed at the site, they will receive study intervention directly from the Investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents and recorded in the electronic case report form (eCRF). The dose of study intervention and study subject identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study intervention.

Details on study intervention compliance at home visits in Part 2 are provided in the Home Administration Manual. In cases in which a dose is administered outside of the allowed visit time window, adjustments to the remaining visit dates may be required. The investigator should discuss with the Sponsor if the dosing schedule has changed markedly (e.g., ≥1 week) compared to the original schedule.

## 5.6. Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and herbal supplements) that the subject is receiving at the time of enrollment or receives during the study must be recorded along with at least the following information:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

It has previously been reported that NAFLD is associated with reduced CYP3A drug metabolism activity in vivo and hepatic CYP3A4 expression in humans (Woolsey, 2015). In addition, based on the work by Woolsey et al it is suggested that exogenously administered FGF21 may decrease hepatic CYP3A4 activity (Woolsey, 2016) . The underlying mechanism has not been fully elucidated.

However, subjects receiving concomitant administration of CYP3A4 substrates with narrow therapeutic index should be closely monitored for any potentially related safety events, and dose adjustment of the CYP3A4 substrate should be considered as needed.

The CRO Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

### 5.6.1. Prohibited Medications/Therapies

Prohibited therapies include:

- Bariatric surgery (including gastric banding or sleeve surgery)
- Medications historically associated with secondary NAFLD (e.g., amiodarone, methotrexate, systemic glucocorticoids [>2 weeks of pharmacologic dosing], tetracyclines, tamoxifen, estrogens at doses greater than those used for hormone replacement, anabolic steroids, valproic acid, and other medications with known hepatotoxicity). Short term oral steroid bursts with/without taper as well as topical and inhaled steroids are allowed.
- Vitamin E supplementation should not be initiated, or the dose increased during the trial
- Thiazolidinendiones.
- Any investigational product, FGF21 analogues (e.g., LY2405319, LY3025876, BMS-986036, BMS-986171, PF-05231023, AKR-001, BIO89-100), or FGFR1-activating products.

## 5.7. Stopping Rules and Dose Modifications

The Sponsor may suspend or terminate the study in the event of:

- New toxicological or pharmacological findings or clinical safety findings that invalidate the earlier positive benefit-risk assessment.
- Discontinuation of the development of BIO89-100.
- External circumstances that do not enable the study to be properly conducted under the existing protocol, including potential circumstances related to the COVID-19 2020 pandemic.

In Part 1, if 3 or more subjects within a single cohort meet stopping rules criteria, as described below, the SMC will meet to decide on study/dose-level discontinuation, suspension, dose modification or repetition prior to proceeding to subsequent dosing:

- New elevation of liver transaminase (ALT or AST) or bilirubin from baseline, as described below, without evidence of an alternative etiology for this elevation
- Serious hypersensitivity reactions to BIO89-100
- Any SAE on a case-by-case basis
- A concerning pattern of laboratory or other safety findings

### 5.7.1. Monitoring and Discontinuation for Suspected Drug-induced Liver Injury (DILI)

Per FDA recommendations, the following criteria for elevations in liver transaminases or bilirubin will be used for closely monitoring, discontinuing or temporarily interrupting study intervention.

**Definition of baseline ALT and AST values**

Baseline value is defined as an average of all ALT and AST values performed during screening and the Baseline (Day 1) visit, as follows:

| ALT/AST Screening Assessments | | | Day 1 ALT/AST Assessment | Baseline Value |
|---|---|---|---|---|
| **Assessment 1** | **Assessment 2** | **Assessment 3 (if applicable)** | | |
| Normal | Normal | Not applicable | Any | Average of Assessment 1, Assessment 2 and Day 1 (3 tests) |
| Normal | Abnormal and ≤40% increase from Assessment 1 | Not applicable | Any | Average of Assessment 1, Assessment 2 and Day 1 (3 tests) |
| Normal | Abnormal and >40% increase from Assessment 1 | Normal or ≤40% increase from Assessment 1 | Any | Average of Assessment 1, Assessment 2, Assessment 3 and Day 1 (4 tests) |
| | | Abnormal and >40% increase from Assessment 1 | **Not applicable, subject excluded** | **Not applicable, subject excluded** |
| Abnormal | ≤40% increase from Assessment 1 | Not applicable | Any | Average of Assessment 1, Assessment 2 and Day 1 (3 tests) |
| Abnormal | >40% increase from Assessment 1 | ≤40% increase from Assessment 1 | Any | Average of Assessment 1, Assessment 2, Assessment 3 and Day 1 (4 tests) |
| | | >40% increase from Assessment 1 | **Not applicable, subject excluded** | **Not applicable, subject excluded** |

**New transaminase elevations in subjects with baseline value within the normal range:**

For new elevations in transaminases to greater than 2x ULN, repeat measurement should be performed within 48-72 hours[14]. If elevations persist, subjects should be evaluated for other causes of transaminase elevations and with tests of hepatic function. If no other cause is identified, then the subjects need to be monitored closely (see below), and discontinuation of the study drug should be considered.

[14] In cases of isolated AST elevation to the indicated threshold, with a clear non-hepatic source for AST elevation (e.g. evidence of significant concurrent creatine phosphokinase elevation), decision regarding need to proceed with DILI work-up will be based on investigator judgement.

Drug should be discontinued, and the subject followed until resolution of symptoms or signs in the following situations:

- ALT or AST >8x ULN
- ALT or AST >5x ULN for more than 2 weeks
- ALT or AST >3x ULN and (TB >2x ULN or INR >1.5)
- ALT or AST >3x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

**New transaminase elevations in subjects with baseline ALT or AST > ULN**

For new elevations in transaminases to greater than 2x baseline value or total bilirubin > 1.5x ULN, repeat measurement should be performed within 48-72 hours[15]. If elevations persist, subjects should be evaluated for other causes of transaminase elevations and with tests of hepatic function. If no other cause is identified, then the subjects need to be monitored closely (see below), and discontinuation of the study drug should be considered.

Study intervention should be discontinued, and the subject followed until resolution of symptoms or signs in the following situations:

**Table 7 Discontinuation Criteria in Subjects with Abnormal Baseline ALT or AST Values**

| Baseline Value of ALT/AST | Criteria to Discontinue Study Intervention |
|---|---|
| <2× upper limit of normal (ULN) | if ALT or AST increases to >5× baseline value |
| ≥2× ULN but <5× ULN | if ALT or AST increases to >3× baseline value |
| ≥5× ULN | if ALT or AST increases to >2× baseline value |
| Other | if ALT or AST increase to >2× baseline value AND the increase is accompanied by a concomitant total bilirubin increase to >2× ULN OR the INR concomitantly increases by >0.2. |
| | if ALT or AST increase to >2× baseline value in the presence of signs and symptom(s) such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophil (>5%) |

ALT = alanine aminotransferase; AST = aspartate aminotransferase; INR = normalized ratio

**Close Monitoring for Suspected DILI:**

- Repeating liver enzyme and serum bilirubin tests two or three times weekly. Frequency of repeat testing can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic.
- Obtaining a more detailed history of symptoms and prior or concurrent diseases.
- Obtaining a history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
- Rule out acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease.

- Obtaining a history of exposure to environmental chemical agents.
- Obtaining additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin).
- Considering gastroenterology or hepatology consultations.

*Note: If a patient lives in a remote area, laboratory testing can be performed locally and the results should be promptly communicated to the investigator site.*

## 5.8. Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently randomized. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened at a later date if the reason for screen failure is deemed temporary and is resolved prior to re-screening (e.g. respiratory infection). Subjects can only re-screen once.

## 5.9. Intervention after the End of Study

No additional intervention is planned beyond the end of the study.

# 6. DISCONTINUATION OF STUDY INTERVENTION AND SUBJECT DISCONTINUATION/WITHDRAWAL

## 6.1. Discontinuation of Study Intervention

In rare instances, it may be necessary for a subject to permanently discontinue study intervention. If study intervention is permanently discontinued, the subject will remain in the study to be evaluated for safety, tolerability, and PK/PD assessments. See the SoAs (Table 2 for Cohorts 1-4, Table 4 for Cohorts 5-6, and Table 6 for Cohort 7) for data to be collected at the time of discontinuation of study intervention (ET visit).

Please also refer to stopping rules described in Section 5.7

## 6.2. Subject Discontinuation/Withdrawal from the Study

A subject may withdraw or be withdrawn from the study for the following reasons:

- Investigator decision
- AE or intercurrent illness
- Noncompliance with protocol requirements
- Subject withdrawal of consent
- Pregnancy
- Sponsor termination or suspension of the study
- Lost to follow-up

The reason for subject withdrawal from the study will be recorded in the eCRF.

Pregnancy is a mandatory criterion for permanent discontinuation of study intervention.

At the time of withdrawal from the study, if possible, subject should attend the ET visit, as shown in the SoAs (Table 2 for Cohorts 1-4 , Table 4 for Cohorts 5-6, and Table 6 for Cohort 7).

## 6.3. Lost to Follow-up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site must attempt to contact the subject and reschedule the missed visit as soon as possible and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to or should continue in the study.
- Before a subject is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.

- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study (and be labelled as lost to follow-up).
- Discontinuation of specific sites or of the study as a whole are handled as part of Appendix 1 (Section 9.1.8).

# 7. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoAs (Table 2 for Cohorts 1-4, Table 4 for Cohorts 5-6, and Table 6 for Cohort 7). Protocol waivers or exemptions are not allowed.
- If multiple assessments are scheduled at the same timepoint, it is recommended that procedures be performed in the following sequence: 12-lead ECGs, vital signs, sample collection for laboratory and PD biomarker tests, and sample collection for PK.
- Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the subject should continue or discontinue study intervention.
- Adherence to the study design requirements, including those specified in the SoAs, is essential and required for study conduct.
- Any situation at the site level with potential impact on subject safety or study conduct, including situations related to COVID-19 infection or control measures, should be discussed with the Sponsor immediately upon occurrence or awareness to determine potential impact on study subject/s or study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The Investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the subject's routine clinical management (e.g., blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoAs (Table 2 for Cohorts 1-4, Table 4 for Cohorts 5-6, and Table 6 for Cohort 7).

## 7.1. Efficacy Assessments

The PD and biomarker assessments are described in Section 7.6.

### 7.1.1. Liver Biopsy and Scoring (Part 2 Only)

Liver biopsy remains the gold standard for diagnosing NASH as well as for establishing the degree of liver fibrosis as it can reliably assess the various histopathological patterns characteristic of NASH (Arab, 2018; Bedossa, 2018; Gunn, 2018; Isabela Andronescu, 2018). In addition, histopathology scores are used to assess extent of fibrosis before and after treatment. The fibrosis stage ranges from F0 (without fibrosis) to F4 (cirrhosis). Currently, liver biopsy is required by regulatory authorities as a surrogate endpoint to assess drug efficacy in Phase 3 clinical studies.

In Part 2, paired liver-biopsy will be performed percutaneously per institution standard, at screening and within 14 days after the last dose of study intervention (as specified in the SoA (Table 6). Details on the biopsy procedure, preparation of slides and central read are provided in

the Biopsy Manual. The procedure will be performed by an experienced hepatologist or radiologist. Biopsies will be subject to central read by at least one liver pathologist.

In addition to the central read, liver histology will also be quantitatively assessed by an AI-based machine-read analysis. Additional details are provided in the Biopsy Manual.

## 7.2. Safety Assessments

Safety assessments include AEs (either reported by the subject or observed by the Investigator), concomitant medication use, physical examination, ECG, vital signs, and laboratory assessments. Planned time points for all safety assessments are provided in the SoAs (Table 2 for Cohorts 1-4, Table 4 for Cohorts 5-6, and Table 6 for Cohort 7).

### 7.2.1. Physical Examinations

- A complete physical examination will include, at a minimum, assessments of the cardiovascular, respiratory, abdominal and a neurological exam. Height (only collected at screening) and weight will also be measured and recorded.
- A symptom directed physical examination will include, at a minimum, assessments of the skin, respiratory, cardiovascular system, and abdomen (liver and spleen).

### 7.2.2. Vital Signs

- Vital signs include blood pressure, pulse, body temperature, and respiratory rate.
- Planned time points for vital signs are provided in the SoAs (Table 2 for Cohorts 1-4, Table 4 for Cohorts 5-6, and Table 6 for Cohort 7).
- Starting from randomization, blood pressure and pulse will be measured in duplicate, the first measurement will be taken up to 15 minutes before the indicated time point. Additional vital signs measurement may be done if clinically indicated. Subjects must be in a supine or semi-erect/seated position and resting for at least 5 minutes prior to measurements.

### 7.2.3. Electrocardiograms

- 12-lead ECG will be recorded as single bedside measurements using an ECG machine that automatically calculates the heart rate and measures PR, QRS, and QT (QTcF) intervals.
- Subject to be resting for at least 2 minutes prior to ECG.

### 7.2.4. Columbia-Suicide Severity Rating Scale (C-SSRS)

The Columbia Suicide Severity Rating Scale (C-SSRS) is a semi-structured interview that assesses suicidal ideation and behaviors (Posner, 2011). The C-SSRS can be administered by a medical professional (MD, DO, PhD, PA-C, NP, RN) or research coordinator who has documented training of the C-SSRS.

The “Baseline/Screening Version” C-SSRS will be administered at Day -1 (Part 1) or during screening (Part 2) to exclude subjects with any past or current suicidal ideations or attempts. Subjects will be excluded from the study if they answer “yes” to any one of the suicidal ideation

or suicidal behavior questions. The “Since Last Visit” version will be administered in Part 1 only at subsequent visits as specified in the SoAs (Table 2 for Cohorts 1-4, Table 4 for Cohorts 5-6).

Any clinically significant change from baseline may be recorded as an AE if deemed appropriate by the Investigator or Sponsor.

### 7.2.5. Clinical Safety Laboratory Assessments

See Appendix 2 (Section 9.2) for the list of clinical laboratory tests to be performed and the SoAs (Table 2 for Cohorts 1-4, Table 4 for Cohorts 5-6, and Table 6 for Cohort 7) for the timing and frequency. Laboratory tests should be performed under fasting conditions (≥10 hours).

Fasting includes food and all beverages except for non-mineral water. It is recommended that subjects abstain from consumption of alcoholic beverages for at least 24 hours before laboratory test assessments.

The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition.

All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 28 days after the last dose of study intervention should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Sponsor’s Medical Monitor.

If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified and the Sponsor notified.

All protocol-required laboratory assessments, as defined in Appendix 2 (Section 9.2), must be conducted in accordance with the relevant study manual and the SoAs (Table 2 for Cohorts 1-4, Table 4 for Cohorts 5-6, and Table 6 for Cohort 7).

If laboratory values from non-protocol specified laboratory assessments performed at the institution’s local laboratory require a change in subject management or are considered clinically significant by the Investigator (e.g., SAE or AE or skipping of dose), then the results must be recorded in the eCRF and the Sponsor should be notified.

## 7.3. Adverse Events and Serious Adverse Events

The definitions of an AE or SAE can be found in Appendix 3 (Section 9.3).

AEs will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the subject's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study intervention or study procedures, or that caused the subject to discontinue the study or study intervention (see Section 6).

### 7.3.1. Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be as specified in the SoAs (Table 2 for Cohorts 1-4, Table 4 for Cohorts 5-6, and Table 6 for Cohort 7).

Table 8 below summarizes the different reporting periods for AEs, SAEs, and events to monitor. Events to monitor are defined in Section 9.3.3.

**Table 8 Adverse Event Reporting Periods**

| Type of Event | Adverse Event | Serious Adverse Event | Events to monitor with study intervention |
|---|---|---|---|
| Reporting period | From consent until 28 days after last dose of study intervention | From consent until 28 days after the last dose of study intervention | From consent until 28 days after the last dose of study intervention |
| Reporting Timelines to the Sponsor | Entered into the clinical database on an ongoing basis | Within 24 hours | Within 24 hours |

All SAEs will be recorded and reported to the Sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Appendix 3 (Section 9.3). The Investigator will submit any updated SAE data to the Sponsor or designee within 24 hours of it being available.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the Investigator must promptly notify the Sponsor or designee.

### 7.3.2. Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting SAE reports are provided in Appendix 3 (Section 9.3).

Care will be taken not to introduce bias when detecting AEs or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

### 7.3.3. Follow-up of AEs and SAEs

After the initial AE or SAE report, the Investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs, will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up (as defined in Section 6.3). Further information on follow-up procedures is given in Appendix 3 (Section 9.3).

### 7.3.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the Investigator to the Sponsor or designee of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study intervention under clinical investigation are met.

- The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs), and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and Sponsor policy and forwarded to investigators as necessary.
- An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

#### 7.3.5. Pregnancy

- Details of all pregnancies in female subjects and of female partners of male subjects will be collected after the start of study intervention and until 90 days after last dose of study intervention.
- If a pregnancy is reported, the Investigator should inform the Sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 4.
- Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

## 7.4. Treatment of Overdose

There is no experience with overdose of BIO89-100 in humans.

For this study, any dose of BIO89-100 greater than the protocol-specified dose will be considered an overdose.

The Sponsor does not recommend specific treatment for an overdose.

In the event of an overdose, the Investigator should:

- Contact the CRO Medical Monitor immediately.
- Closely monitor the subject for any AEs and laboratory abnormalities until study intervention can no longer be detected systemically (at least 30 days).
- Obtain a plasma sample for PK analysis within 2 days from the date of the overdose if requested by the Medical Monitor (determined on a case-by-case basis).
- Document the quantity of the excess dose as well as the incidence of the overdose in the eCRF.

## 7.5. Pharmacokinetics

Blood samples for analysis of BIO89-100 serum levels will be collected at the timepoints designated in the SoAs (Table 3 for Cohorts 1-4, Table 5 for Cohorts 5-6, and Table 6 for Cohort 7).

Blood samples will be processed for collection of serum fractions for determination of BIO89-100 serum concentrations. Serum samples will be shipped to the bioanalytical laboratory for analysis.

The trough serum BIO89-100 concentrations will be summarized by treatment group and nominal sampling time using descriptive statistics.

Pharmacokinetic parameters calculated by noncompartmental methods from the BIO89-100 serum concentration data will include:

| PK Parameter | Cohorts 1-3 | Cohorts 4-5 |
|---|---|---|
| $C_{max}$ | Within 1st and 5th dosing intervals | Within 1st and 3rd dosing intervals |
| $AUC_{0\text{-}tau}$ | Within 1st and 5th dosing intervals | Within 1st and 3rd dosing intervals |
| $t_{max}$ | Within 1st and 5th dosing intervals | Within 1st and 3rd dosing intervals |
| $t_{½}$ | Within 1st and 5th dosing intervals | Within 1st and 3rd dosing intervals |

Additional PK parameters may be calculated if deemed appropriate.

The serum concentration-time data may also be used for population PK modeling with the results reported separately from the clinical study report.

## 7.6. Pharmacodynamics and Biomarkers

### 7.6.1. Immunogenicity Assessments

Antibodies to BIO89-100 as well as titer and binding specificity will be evaluated in serum samples collected from all subjects at timepoints designated in the SoAs (Table 2 for Cohorts 1-4, Table 4 for Cohorts 5-6, and Table 6 for Cohort 7). Subjects who test positive [redacted] More frequent testing (e.g. every month) or testing for a longer period of time may be requested in the event of safety-related concerns. Follow-up testing will not be required where it is established that the subject did not receive BIO89-100. All follow-up results, both positive and negative will be communicated to the sites. A blood sample for ADA assessment will also be collected upon observation of any severe hypersensitivity reaction (e.g., anaphylaxis). Subjects who test positive [redacted]

Samples for endogenous FGF21 level will be collected at baseline for all subjects, and at the time of acquisition of the follow-up blood sample(s) for ADA assessment, in [redacted]

[redacted]

Serum samples will be screened for antibodies binding to BIO89-100 and the titer of confirmed positive samples will be reported. Other analyses may be performed to verify the stability of antibodies to BIO89-100 and/or further characterize the immunogenicity of BIO89-100.

The detection and characterization of antibodies to BIO89-100 will be performed using a validated assay method under the supervision of the Sponsor. Antibodies may be further characterized and/or evaluated for their ability to neutralize the activity of the study intervention(s). Samples may be stored for a maximum of [redacted] (or according to local regulations) following the last subject's last visit for the study at a facility selected by the Sponsor to enable further analysis of immune responses to BIO89-100.

### 7.6.2. Pharmacodynamics

The following biomarkers/PD parameters will be evaluated at timepoints designated in the SoAs (Table 2 for Cohorts 1-4, Table 4 for Cohorts 5-6, and Table 6 for Cohort 7):

**Laboratory Parameters**

- Triglycerides
- Non-HDL cholesterol
- HDL-c
- LDL-c
- HbA1c
- HOMA-IR (Part 1 only)
- Liver function tests: ALT, AST
- GGT
- Alkaline phosphatase
- Adiponectin
- Pro-C3 (see Section 7.6.2.4)
- Free fatty acids and Adipo-IR (fasting free fatty acids × fasting insulin; Part 1 only)
- [redacted]

Samples may be stored for a maximum of 15 years after the last subject's last visit for the study, at a facility selected by the Sponsor, to enable further analysis of biomarker responses to BIO89-100.

Residual blood samples will be stored for potential future analysis of biomarkers.

Additional information will be available in the study specific laboratory manual.

Other parameters include:

- Imaging measures (Section 7.6.2.5)
  - MRI-PDFF
- Anthropomorphic measurements
  - Body weight

#### 7.6.2.2. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR; Part 1 only)

HOMA-IR is a method used to quantify insulin resistance based on the following formula(s) (Matthews, 1985):

**Glucose in mmol/L**

$$\text{HOMA-IR} = \frac{\text{Glucose} \times \text{Insulin}}{22.5}$$

**Glucose in mg/dL**

$$\text{HOMA-IR} = \frac{\text{Glucose} \times \text{Insulin}}{405}$$

[redacted]

#### 7.6.2.4. N-terminal Propeptide of Type III Collagen (Pro-C3)

N-protease cleaved N-terminal propeptide of type 3 procollagen (P3NP) neo-epitope (Pro-C3) is derived from the synthesis of type 3 collagen. Pro-C3 appears to correlate with liver fibrosis stage, fibrosis regression and response to treatment both as a single test and as part of algorithms (Nielsen, 2015; Daniels, 2018; Hansen, 2018).

#### 7.6.2.5. Magnetic Resonance Imaging – Whole Liver Proton Density Fat Fraction (MRI-PDFF)

MRI-PDFF is as a noninvasive, quantitative, and accurate measure of liver fat content (imaging-based biomarker) to assess treatment response in NASH clinical studies (Caussy, 2018).

This technology enables post-processing of MRI data into parametric map of PDFF (Antaros Medical, Sweden) to provide accurate and quantitative measures of liver fat.

[redacted]

**PDFF**

The PDFF is determined using a 6 echo gradient echo pulse sequence covering the liver in the axial plane. Analysis is performed by semi-automatic contouring of the liver in every slice avoiding major vessels and bile ducts. The method applies multi-peak lipid spectral models and simultaneous quantification and correction for T2*. The liver fat value (PDFF) is the mean value of all voxels in the identified volume of interest.

For more information, refer to the Imaging Manual.

### 7.6.3. Exploratory Analyses

[redacted]

# 8. STATISTICAL CONSIDERATIONS

This section describes the statistical analysis as foreseen at the time of planning the study. Changes, additions, and further details about the analyses will be described in the statistical analysis plan, if applicable. Any subsequent additional analyses or changes to analyses that may be required will be fully disclosed in the clinical study report. Summaries and analyses will be presented by dose/treatment group for Part 1 and separately for Part 2. Before database lock, final statistical and pharmacokinetic analysis plans containing full details of all planned analyses will be produced. The analyses presented here represent an outline of the intended methodology. Details of the analysis will be described in a Statistical Analysis Plan. All clinical data will be summarized using descriptive statistics: number (n), mean, standard deviation (SD), median, minimum, and maximum for continuous measurements, and counts and percentages for categorical measurements.

## 8.1. Statistical Hypotheses

The Part 1 primary objectives are to evaluate the safety and tolerability of ascending multiple SC injections of BIO89-100 in subjects with NASH or who have NAFLD and at a high risk of NASH and to characterize BIO89-100 PK. No formal hypotheses will be tested for the primary objectives.

The secondary objectives of evaluating the effect of BIO89-100 on selected PD markers and biomarkers will be assessed by point estimates, 90% confidence intervals and the descriptive p-values of mean changes/percentages changes from baseline within and between treatment group.

For Part 2, the primary objectives are to evaluate incidence of AEs and to assess histological response of BIO89-100 in the NASH population.

## 8.2. Sample Size Determination

For Part 1, no formal sample size calculation was performed for the primary endpoint(s) as the total of approximately 83 subjects, consisting of 6:2 (Cohort 1), 12:3 (Cohort 2), 9:3 (Cohorts 4 and 6), or 14:4 (Cohorts 3 and 5) receiving active : placebo, respectively is considered adequate for Part 1 of this study and are expected to enroll to achieve study objectives.

A power assessment based on [redacted] subjects in a dose cohort compared to the pooled 19 placebo subjects will provide [redacted] power, respectively, to detect a difference in mean percentage change from baseline between treatment groups of [redacted], assuming the standard deviation of endpoint in each group is [redacted]. Calculation was based on two-sample t-test with one-sided 5% (e.g., two-sided 10%) type I error probability.

Subjects who withdraw from the study before the Day 50 assessments may be replaced at the discretion of the Sponsor.

For Part 2, no formal power calculations were used to determine the sample size in Cohort 7 and the number of subjects was chosen based on clinical experience of similar proof-of-concept histology studies and feasibility. This sample size accounts for an anticipated [redacted] of subjects who will not undergo the post-baseline liver biopsy (e.g., [redacted] to have paired liver biopsies). With a sample size of [redacted], the 95% confidence interval of the histological response rate would

have a half-width no greater than [redacted] assuming the histological response [redacted] The actual half-width will depend on the observed histological response rate.

## 8.3. Populations for Analyses

For purposes of analysis, the following populations are defined:

| Population | Description |
|---|---|
| Screened Analysis set | All enrolled subjects who signed informed consent and undergone screening. |
| Part 1 | |
| Randomized Analysis set | All enrolled subjects who are assigned a randomization number in the study. For analysis purposes, subjects will be analyzed according to the treatment they were randomized to regardless of actual treatment received. |
| Safety Analysis set | All randomized subjects who receive at least 1 of study intervention. In this population, subjects will be summarized based upon the study intervention actually received, regardless of the study intervention to which they were randomized. |
| PK Analysis set | All subjects in the Safety Analysis set who have sufficient data to adequately characterize the trough serum BIO89-100 concentrations and have no other events, or protocol violations that would adversely affect results, such as not completing the full dose. The analysis population for any population pk modeling may be defined separately in a population PK data analysis plan. |
| PD Analysis set | All subjects in the Safety Analysis set who have measurable post-baseline PD data; subjects will summarized based on the randomized treatment group. Different PD analysis sets may be used for different types of PD endpoints, e.g., MRI-PDFF. |
| Part 2 | |
| Full Analysis Set | All enrolled subjects who received at least one dose of study intervention. Full Analysis Set will also be used for safety summary in Part 2 |
| PK Analysis Set | All enrolled subjects in the Full Analysis Set who have sufficient data to adequately characterize the trough serum BIO89-100 concentrations and have no other events, or protocol violations that would adversely affect results, such as not completing the full dose. |
| PD Analysis Set | All enrolled subjects in the Full Analysis Set who have measurable post-baseline PD data. Different PD analysis sets may be used for different types of PD endpoints, e.g., MRI-PDFF. |
| Biopsy Analysis Set | For Cohort 7 only. All enrolled subjects in the Full Analysis Set and have both baseline and post-baseline biopsies. |

## 8.4. Statistical Analyses

The statistical analysis plan (SAP) will be developed and finalized before database lock and will describe the subject populations to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. Pooling of placebo subjects in Part 1 will be performed when applicable and will be specified in the analysis plan. This section is a summary of the planned

statistical analyses of the primary and secondary endpoints. Analysis of biomarkers and PD endpoints as well as their correlation with PK will be detailed in the SAP.

In general, summary descriptive statistics will be presented for demographics and baseline characteristics, safety endpoints, PK, and PD parameters. Descriptive statistics will include number of observations, mean, standard deviation, median, minimum, and maximum for continuous parameters. For PK and PD parameters, descriptive statistics will include the number of observations, arithmetic and geometric means, standard deviation, coefficient of variation, median, minimum, and maximum. Categorical parameters will be displayed using counts and percentages within each category.

For Part 1, while no formal hypothesis testing is planned for the primary objectives, the evaluation of potential efficacy will be determined based on the totality and pattern of data from the biomarker and PD endpoints. The assessment of biomarker and PD endpoints will employ 90% confidence intervals and significance testing for assessing within group changes and percentage changes from baseline and comparisons of the BIO89-100 doses to the pooled placebo group. Details will be provided in the SAP.

The correlation between relevant PK parameters and PD endpoints will be examined as will be detailed in the SAP.

Subjects with dose interruptions impacted by the COVID-19 pandemic will be noted in the listings. Subgroups including and excluding subjects with dose interruptions due to COVID-19 pandemic may be considered for both efficacy and safety analyses as appropriate.

For Part 2, endpoints of different histological responses will be summarized with the point estimates and 95% confidence intervals of the proportions of subjects who meet the response criteria using the biopsy analysis set.

### 8.4.1. Safety and Tolerability Analyses

All safety analyses will be performed on the Safety Analysis Set in Part 1 and Full Analysis Set in Part 2. The safety assessment will be based on AEs, laboratory values, concomitant medication use, ECG, vital signs, and physical examination. Summaries will be presented by treatment/dose.

All adverse events will be coded using the Medical Dictionary for Regulatory Activities (current version of MedDRA). The severity of adverse events will be graded according to the current version of National Cancer Institute (NCI) common terminology criteria for adverse events (CTCAE) v5. All AE summaries will include treatment-emergent adverse events (TEAEs), defined as those which occur after the first dose of study intervention (BIO89-100 or placebo). Treatment-related AEs are those which were determined by the Investigator to be related to study intervention. The incidence of treatment-related AEs (as recorded in the eCRF) will be summarized using descriptive statistics by system organ class (SOC) and preferred term, categorized by severity as recorded in the eCRF. Counting will be done for each subject (subjects will only be counted once within each SOC or preferred term). The frequency of TEAE (AE burden) will be summarized using descriptive statistics by SOC and preferred term (PT). Counting will be done for events (regardless of subjects), by SOC and PT. The incidence of TEAE will also be presented by time of onset post-dose, SOC, PT, and severity.

A listing of TEAEs and TEAEs leading to study discontinuation will be provided. Summaries will be presented by dose/treatment group. Subject listings of SAEs and AE leading to withdrawal will be presented. Actual values and changes in laboratory (serum chemistry, hematology, coagulation, and urinalysis), ECG, and vital signs measurements including weight data will be summarized descriptively. Laboratory data will also be presented by grading (severity) scale according to the current version of NCI CTCAE for each time point recorded. All values will be compared with prespecified boundaries to identify potentially clinically significant values or changes, as detailed in the statistical analysis plan, and such values will be listed. All prior and concomitant medications will be coded using the World Health Organization drug dictionary (WHO Drug) and will be listed. Concomitant medications will include all medications taken from first dose through to the EOS visit. If any subject dies during the study, a listing of deaths will be provided and all relevant information will be discussed in the subject narrative included in the clinical study report. The relationship of overall safety with respect to pharmacokinetics, immunogenicity assessments, and prespecified exploratory biomarkers will be evaluated, as appropriate.

The number (%) of subjects who fail to complete the study and the reason for discontinuation, and the number (%) of subjects who fail to complete the study due to related adverse events will be summarized.

### 8.4.2. PK Analyses

All PK population subjects will be included in the PK analysis.

Pharmacokinetic parameters including but not limited to those listed in Section 7.5 will be determined where possible from the serum concentrations of BIO89-100.

All PK parameters will be listed by subject and summarized by dose using descriptive statistics (sample size, mean, median, geometric mean [as appropriate], coefficient of variation [CV%], SD, minimum, and maximum) for continuous variables and using frequencies and percentages for discrete variables.

Serum concentration data will be listed by subject and summarized using descriptive statistics by nominal time point.

Serum concentration by time profiles for each subject will be presented graphically. Mean serum concentration by time profiles will be presented by dose level. Further details on PK analysis will be provided in the SAP.

### 8.4.3. Immunogenicity Analyses

Results of immunogenicity assessment will be provided by ADA response (e.g., number and percent of positive ADA subjects) and immunogenicity profile (e.g., antibody titers, binding specificity, and neutralizing immunogenicity), when appropriate.

The effect of ADA on relevant PK parameters may be evaluated.

For endogenous FGF21, baseline samples will be analyzed; sample(s) will also be obtained from [redacted] subject with additional follow-up visit(s) for potential future analysis to evaluate effect of ADA on endogenous FGF21 level.

[redacted]

## 8.5. Interim Analyses

No efficacy interim analysis is planned. The Sponsor may perform administrative interim analysis to support objectives such as study planning and regulatory interaction.

## 8.6. Safety Monitoring Committee (SMC)

The SMC, a specific committee for the study composed of the Sponsor's Medical Monitor, the CRO Medical Monitor, and at least one participating Investigator, will only function in Part 1, as described in Section 3.2. The operations of the SMC will be defined in the SMC Charter.

Given the open-label design of Part 2, safety will be reviewed on an ongoing basis by the Sponsor and/or CRO Medical Monitors.

# 9. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

## 9.1. Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

### 9.1.1. Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
  - Applicable ICH Good Clinical Practice (GCP) Guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator's Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.
- The Investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
  - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

### 9.1.2. Financial Disclosure

Investigators and sub-investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

### 9.1.3. Informed Consent Process

- The Investigator or his or her representative will explain the nature of the study to the subject or his or her legally authorized representative and answer all questions regarding the study.

- Subjects must be informed that their participation is voluntary. Subjects will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the subject was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the subject or the subject's legally authorized representative.

Subjects who are rescreened are required to sign a new ICF.

#### 9.1.4. Data Protection

- Subjects will be assigned a unique identifier by the Sponsor. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.
- The subject must be informed that his or her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject.
- The subject must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

#### 9.1.5. Dissemination of Clinical Study Data

A clinical study report will be developed by the Sponsor at completion of data analysis. This report will be a clinical and statistical integrated report, according to the ICH E3 guidelines.

Sponsor will register the study and post study results regardless of outcome on a publicly accessible website in accordance with the applicable laws and regulations.

#### 9.1.6. Data Quality Assurance

- All subject data relating to the study will be recorded on the eCRFs unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.

- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).
- Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements. It may become required that some or all monitoring activities will be halted or performed remotely due to the recent COVID-19 pandemic. In such a case, details of the changes made to the monitoring strategy will be described in the Monitoring Plan.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator per ICH-GCP and local regulations or institutional policies. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor, whether within the retention period or thereafter.

### 9.1.7. Source Documents

- Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.
- Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Source documents are original documents, data, and records from which the subject's eCRF data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

### 9.1.8. Study and Site Closure

The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study

completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The Investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given to the Sponsor in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the Investigator
- Discontinuation of further study intervention development

If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the subject and should assure appropriate subject therapy and/or follow-up.

### 9.1.9. Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with the International Committee of Medical Journal Editors authorship requirements.

## 9.2. Appendix 2: Clinical Laboratory Tests

- The clinical laboratory tests detailed in Table 9 will be performed by a central laboratory, except if noted otherwise, at timing/frequency detailed in the SoAs (Table 2 for Cohorts 1-4, Table 4 for Cohorts 5-6, and Table 6 for Cohort 7). Laboratory tests will be performed under fasting conditions (≥10 hours).
- Protocol-specific requirements for inclusion or exclusion of subjects are detailed in Section 3.3 and Section 4.3 of the protocol for Parts 1 and 2, respectively.
- Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

**Table 9 Protocol-Required Laboratory Assessments**

| | |
|---|---|
| **Hematology** | |
| White blood cell count (WBC) with differential (Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils – absolute and %) | Red blood cell (RBC)<br>RBC Indices:<br>MCV, MCH, %Reticulocytes |
| Hemoglobin | Hematocrit |
| Platelet count | Coagulation factors, including PT, INR, aPTT<br>Red cell Distribution Width |
| **Clinical Chemistries** | |
| Alanine Aminotransferase (ALT) | Aspartate Aminotransferase (AST) |
| Alkaline phosphatase (ALP) | Gamma-glutamyl transferase (GGT) |
| Total bilirubin, Indirect/direct bilirubin | Albumin |
| Calcium | Blood urea nitrogen (BUN) |
| Sodium | Creatinine |
| Chloride | Creatine Kinase |
| Magnesium | Bicarbonate |
| Potassium | Lactate dehydrogenase |
| Phosphorus/ | Total protein |
| Glucose (fasting) | Total Cholesterol |
| Triglycerides | Uric Acid<br>High-density lipoprotein cholesterol (HDL-c)<br>Low density lipoprotein (LDL-c)<br>Non-high-density lipoprotein cholesterol (non-HDL-c) |

| | |
|---|---|
| **Urinalysis** | |
| Basic Urinalysis (dipstick, including macroscopic appearance, bilirubin, blood, color, glucose, ketones, leukocyte esterase, nitrite, pH, protein, specific gravity, urobilinogen; | Full urinalysis (dipstick plus microscopic evaluation) to be performed only at the Screening and End of Study visits). A reflex microscopic urinalysis should be performed if the results of the urinalysis is abnormal or at the discretion of the PI or delegate. |
| **Other Study-Specific Laboratory Assessments** | |
| BIO89-100 (to be evaluated by bioanalytical laboratory)<br>Serum and urine human chorionic gonadotropin (hCG) pregnancy test<br>TSH<br>FSH (only for determination of postmenopausal status)<br>Urine drug screen including amphetamines, barbiturates, cocaine metabolites, opiates, benzodiazepines and cannabinoids (on Day -1, can done at local laboratory, but must also collect sample for central laboratory).<br>Virology - HIV antibody, hepatitis B surface antigen (HBsAg, and hepatitis C virus antibody)<br>24-hour urine for cortisol[a]<br>**Immunogenicity**:<br>Antibody to BIO89-100<br>Endogenous FGF21<br>Bone markers - carboxy-terminal collagen crosslinks (CTX), N-terminal propeptide of type 1 collagen (P1NP), osteocalcin<br>Plasma and serum samples for exploratory biomarkers[a]<br>DNA and RNA samples[a] | Insulin[a]<br>High-sensitivity C-reactive protein (hsCRP)[a]<br>Oral glucose tolerance test (OGTT) including C-peptide, glucose, and insulin[a]<br>Hemoglobin A1c (HbA1c)<br>Homeostatic model assessment of insulin resistance (HOMA-IR)[a]<br>Insulin-like growth factor-1 (IGF-1), total[a]<br>Adiponectin, total<br>Cytokeratin (CK)-18[a]<br>Enhanced liver fibrosis (ELF) panel[a]<br>N-terminal propeptide of type III collagen (Pro-C3)<br>Fasting free fatty acids and Adipo-IR (fasting FFA x fasting insulin)[a] |

a-Part 1 only; all other tests are done in both Parts 1 and 2

Investigators must document their review of each laboratory safety report.

# 9.3. Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

## 9.3.1. Definition of AE

| **AE Definition** |
|---|
| • An AE is any untoward medical occurrence in a clinical study subject, temporally associated with the use of study intervention, whether or not considered related to the study intervention.<br>• NOTE: An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention. |

| **Events <u>Meeting</u> the AE Definition** |
|---|
| • Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (i.e., not related to progression of underlying disease).<br>• Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.<br>• New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study.<br>• Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.<br>• Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. |

| **Events <u>NOT</u> Meeting the AE Definition** |
|---|
| • Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition.<br>• The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.<br>• Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.<br>• Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).<br>• Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen. |

### 9.3.2. Definition of Suspected and Unsuspected Adverse Reaction

| **Suspected adverse reactions are defined as:** |
|---|
| • Any AE for which there is a reasonable possibility that the study intervention caused the AE. For the purposes of sponsor regulatory safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the study intervention and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a study intervention |
| **Unexpected AEs are defined as:** |
| • AE which is not listed in the IB of the study intervention or is not listed at the specificity or severity that has been observed |

### 9.3.3. Definition of Events to Monitor

| **Sponsor-defined Events to Monitor for BIO89-100:** |
|---|
| Events to Monitor include hypersensitivity reactions occurring after the first administration of study agent(s) in subjects participating in this clinical study. These events must be reported by the Investigator to the sponsor within 24 hours and are to be considered serious (for regulatory reporting purposes) only if they meet the definition of an SAE. The events are to be reported on an SAE form |

### 9.3.4. Definition of SAE

If an event is not an AE per definition in Section 9.3.1, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

| **An SAE is defined as any untoward medical occurrence that, at any dose:** |
|---|
| **1. Results in death** |
| **2. Is life-threatening**<br>The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe. |
| **3. Requires inpatient hospitalization or prolongation of existing hospitalization**<br>In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.<br>Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE. |
| **4. Results in persistent disability/incapacity**<br>• The term disability means a substantial disruption of a person's ability to conduct normal life functions.<br>• This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma |

**An SAE is defined as any untoward medical occurrence that, at any dose:**

(e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

**5. Is a congenital anomaly/birth defect**

**6. Other situations:**

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

### 9.3.5. Recording and Follow-Up of AE and/or SAE

**AE and SAE Recording**

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information in the eCRF.
- It is not acceptable for the Investigator to send photocopies of the subject's medical records to the Medical Monitor in lieu of completion of the AE/SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested by the Medical Monitor. In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to the Medical Monitor.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

**Assessment of Intensity**

The severity of each AE will be assessed at onset by a nurse and/or physician. When recording the outcome of the AE the maximum severity of the AE experienced will also be recorded. The severity of the AE will be graded according to the CTCAE v5.0:

**Grade 1:** Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

**Grade 2:** Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)*.

**Grade 3:** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.

**Grade 4:** Life-threatening consequences; urgent intervention indicated.

**Grade 5:** Death related to AE.

**ADL:**

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

**AE and SAE Recording**

**Assessment of Causality**

The Investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.

- A “"reasonable possibility”" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The Investigator will also consult the Investigator’s Brochure (IB) and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the Investigator has minimal information to include in the initial report to the Medical Monitor. However, **it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the Medical Monitor.**
- The Investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Causality Categories:

- UNRELATED - This relationship suggests that there is no association between the study drug and the reported event.
- UNLIKELY RELATED - The relationship suggests that the event is more likely due to other causes than study drug, however the relationship to the study drug cannot be ruled out.
- POSSIBLY RELATED - This relationship is based on evidence suggesting a causal relationship between the study drug and the AE, i.e., there is a reasonable possibility that the drug caused the event. The event follows a reasonable temporal sequence from the time of drug administration or follows a known response pattern to the study drug but could also have been produced by other factors.
- PROBABLY RELATED - This relationship suggests that a reasonable temporal sequence of the event with drug administration exists and, based upon the known pharmacological action of the drug, known or previously reported adverse reactions to the drug or class of drugs, or judgment based on the Investigator’s clinical experience, the association of the event with the study drug seems likely.
- DEFINITELY RELATED - This relationship suggests that a definite causal relationship exists between drug administration and the AE, and other conditions (concurrent illness, progression/expression of disease state, or concurrent medication reaction) do not appear to explain the event.

| **Follow-up of AEs and SAEs** |
|---|
| • The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Medical Monitor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.<br>• If a subject dies during participation in the study or during a recognized follow-up period, the Investigator will provide the Medical Monitor with a copy of any post-mortem findings including histopathology.<br>• New or updated information will be recorded in the originally completed eCRF.<br>• The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information. |

### 9.3.6. Reporting of SAEs

Reporting of SAEs will be done using eCRF (with paper methods as a back-up in case of system unavailability).

| **SAE Reporting to the Medical Monitor via an Electronic Data Collection (eCRF) Tool** |
|---|
| • The primary mechanism for reporting an SAE to the Medical Monitor will be the electronic data collection tool.<br>• If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours.).<br>• The site will enter the SAE data into the electronic system as soon as it becomes available.<br>• After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.<br>• If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the Medical Monitor by telephone.<br>• Contacts for SAE reporting can be found in the relevant manual. |

| **SAE Reporting to the Medical Monitor via Paper CRF** |
|---|
| • Facsimile or email transmission of the SAE paper CRF is the preferred method to transmit this information to the Medical Monitor,<br>• In rare circumstances and in the absence of facsimile or email equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.<br>• Initial notification via telephone does not replace the need for the Investigator to complete and sign the SAE CRF pages within the designated reporting time frames.<br>• Contacts for SAE reporting can be found in the relevant manual. |

## 9.4. Appendix 4: Contraceptive Guidance and Collection of Pregnancy Information

**Definitions:**

**Woman of Childbearing Potential (WOCBP)**

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (e.g., amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study intervention, additional evaluation should be considered.

**Women in the following categories are not considered WOCBP**

1. Premenarchal
2. Premenopausal female with one of the following:
   - hysterectomy
   - bilateral salpingectomy
   - bilateral oophorectomy
   - For individuals with permanent infertility due to an alternate medical cause other than the above, (e.g., Mullerian agenesis, androgen insensitivity), Investigator discretion should be applied to determining study entry.
3. Postmenopausal female
   - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
     - A high FSH level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.
     - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

**Male subjects with a female partner who is not of childbearing potential**

Presumptive fertile male subjects who are partnered with women who are not of childbearing potential, either post-menopausal or through sterilization or other surgical procedures (e.g., hysterectomy) will confirm that their partners have been determined to be sterile or post-menopausal based on medical judgment; this can be confirmed either by statement or medical records, if presented by the subject.

Men will not be required to use other contraception when sexually active with a sterile or post-menopausal partner.

Men who are sexually active with fertile women need to confirm that they will use 2 forms of contraception as per protocol.

**Contraception Guidance:**

Contraceptive use by men or women should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

Subjects should use highly effective, double contraception (both male and female partners) during the study and 30 days or 5 half-lives (whichever is longer) of study intervention.

Double contraception is defined as a condom with one of any of the following forms of birth control:

- Birth control pills (The Pill)
- Depot or injectable birth control
- Intrauterine Device (IUD)
- Birth Control Patch (e.g., Othro Evra)
- NuvaRing®
- vasectomy for men

Subjects may not donate sperm or oocytes during the study and for 30 days or 5 half-lives (whichever is longer) after last dose of study intervention.

Rhythm methods are not considered as highly effective methods of birth control. Subject abstinence for the duration of the study and 30 days or 5 half-lives (whichever is longer) after last dose of study intervention is acceptable if it is the subject's regular practice.

**Collection of Pregnancy Information**

**Male subjects with partners who become pregnant**

- The Investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is in this study. This applies only to male subjects who receive study intervention.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

**Female subjects who become pregnant**

- Female subjects who become pregnant should discontinue treatment and be withdrawn from the study.
- The Investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. Information will be recorded on the appropriate form and submitted to the Sponsor within 24 hours of learning of a

subject's pregnancy. This applies only to female subjects who receive study intervention

- The subject will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the subject and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such. Any post-study pregnancy related SAE considered reasonably related to the study intervention by the Investigator will be reported to the Sponsor as described in Section 7.3.4. While the Investigator is not obligated to actively seek this information in former study subjects, he or she may learn of an SAE through spontaneous reporting.

## 9.5. Appendix 5: Considerations in Response to Coronavirus Disease 2019 (COVID-19) Pandemic

As of March 12, 2020, a COVID-19 pandemic has been declared by the World Health Organization, leading to implementation of extensive measures by healthcare systems globally to limit viral spread, with potential impact on conduct of clinical studies.

Based on recommendations in the FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic (FDA, July 2020), the following actions were implemented to address potential disruptions to study conduct secondary to COVID-19 infection or control measures:

- Interruption in study intervention administration and other study procedures (collectively "study interruption") will be allowed for up to 4 weeks (+4 days). Any interruption lasting ≥ 2 weeks will be considered a study interruption.
- For subjects who have a study interruption, prior to resuming dosing, the Investigator will assess whether the subject is clinically stable to continue participation in the study. This assessment will include symptom-directed physical examination, vital signs, pregnancy test (local) in WOCBP, and laboratory tests (hematology and clinical biochemistry) to be done locally.

If clinical laboratory tests are not part of the protocol-defined procedures for the first dosing visit after study interruption, hematology and clinical biochemistry panels will be obtained prior to dosing and sent to the central lab.

Results from both the local and central labs should be documented in EDC.

- Upon resumption of dosing, the subject will continue at the next planned visit relative to the last visit performed prior to study interruption, and will be administered all remaining planned doses.
- Administration of study intervention by a trained home health care worker will be considered on a case-by-case basis if circumstances related to the COVID-19 pandemic preclude dosing at the study site
- In cases where a subject is continuing to receive study intervention but COVID-19 pandemic-related circumstances preclude a visit to the investigative site, remote visits (e.g., by telemedicine or phone contact) will be allowed for relevant study procedures.
- In cases where a subject is continuing to receive study intervention but cannot obtain laboratory tests at the investigative site, local laboratory tests may be obtained at a certified laboratory. The site should inform the Sponsor about such cases. Local analysis can be used for safety decisions. In addition, local labs can be used for trial endpoints if samples drawn at the local lab cannot be shipped to the central lab.
- (For Part 1 only) The Investigator should discuss with the Sponsor on a case-by-case basis if circumstances related to the COVID-19 pandemic preclude domiciling around the 5$^{th}$ dose (in Cohorts 3 and 4) or the 3$^{rd}$ dose (in Cohorts 5 and 6); with Sponsor approval, these visits may be conducted without domiciling.

- (For Part 1 only) As MRI facilities may be impacted by circumstances related to the COVID-19 pandemic, precluding protocol-defined MRI assessments, all subjects in Part 1 who have not yet undergone Day 50 and Day 92/ET MRI assessments will undergo Fibroscan assessments on Day 50 and Day 92/ET in addition to the planned MRI assessments.
- End of Treatment may be considered for the following scenarios:
  - For subjects in Part 1 who have reached the Day 57 visit (Visit 17) and subjects in Part 2 who have reached the Day 106 visit (Visit 18), an End of Treatment visit may be considered instead of the study interruption. The Investigator should discuss with the Sponsor about the feasibility of these two options based on the potential impact from COVID-19 and/or the control measures in place in their location.
  - Study interruptions longer than 4 weeks will lead to discontinuation of treatment and the subject's participation in the study.

Efforts should be made to obtain End of Treatment assessments in subjects who are not willing to return to the study after study interruption, who are deemed not clinically stable to continue participation by the Investigator, or who are lost to the study due to disruptions because of COVID-19 pandemic. The End of Treatment visit should be scheduled as soon as possible in these scenarios, whenever possible.

- For dose interruptions lasting ≥2 weeks, contact will be established with the study subject remotely (e.g., by phone or telemedicine) to obtain information about AEs, concomitant medications, or any other update related to the subjects' safety.
- Study interruption will be allowed as long as COVID-19 pandemic-related circumstances are ongoing, and will not be allowed when these circumstances are no longer applicable, as determined by the Sponsor.
- For newly randomized subjects who have not yet received their first dose at a site that can foresee near-term disruption by COVID-19 pandemic, the Investigator should discuss with the Sponsor whether the initiation of dosing should be postponed. In this situation, study interruption should not apply, and the subject will initiate and finish all planned doses when the Investigator deems it is safe to start dosing.

Changes in study visit schedules, missed visits, or patient discontinuations may lead to missing information (e.g., for protocol-specified procedures). Specific information will be captured in the case report form that explains the basis of the missing data, including the relationship to COVID-19 for missing protocol-specified information (e.g., from missed study visits or study discontinuations due to COVID-19).

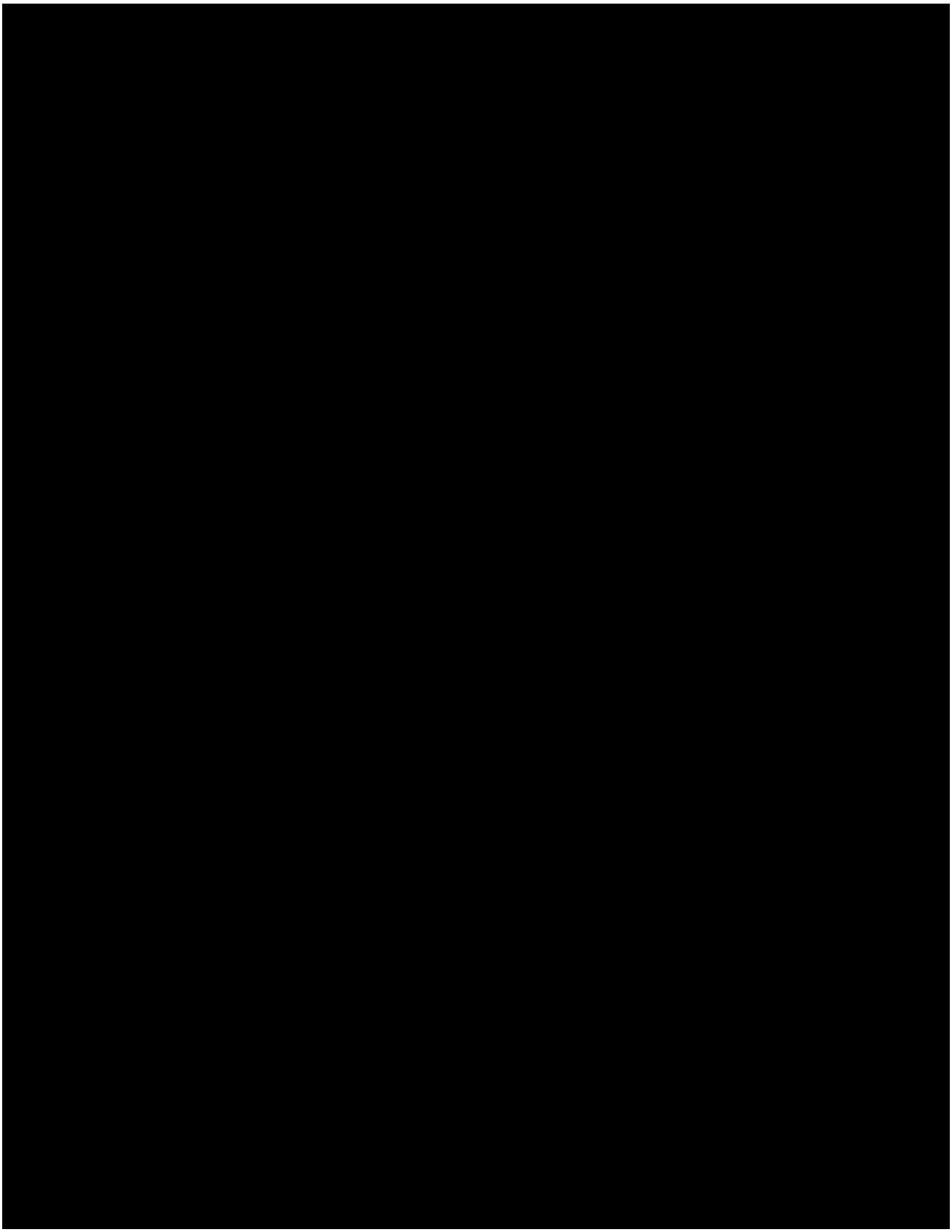
## 9.6. Appendix 6: Glossary

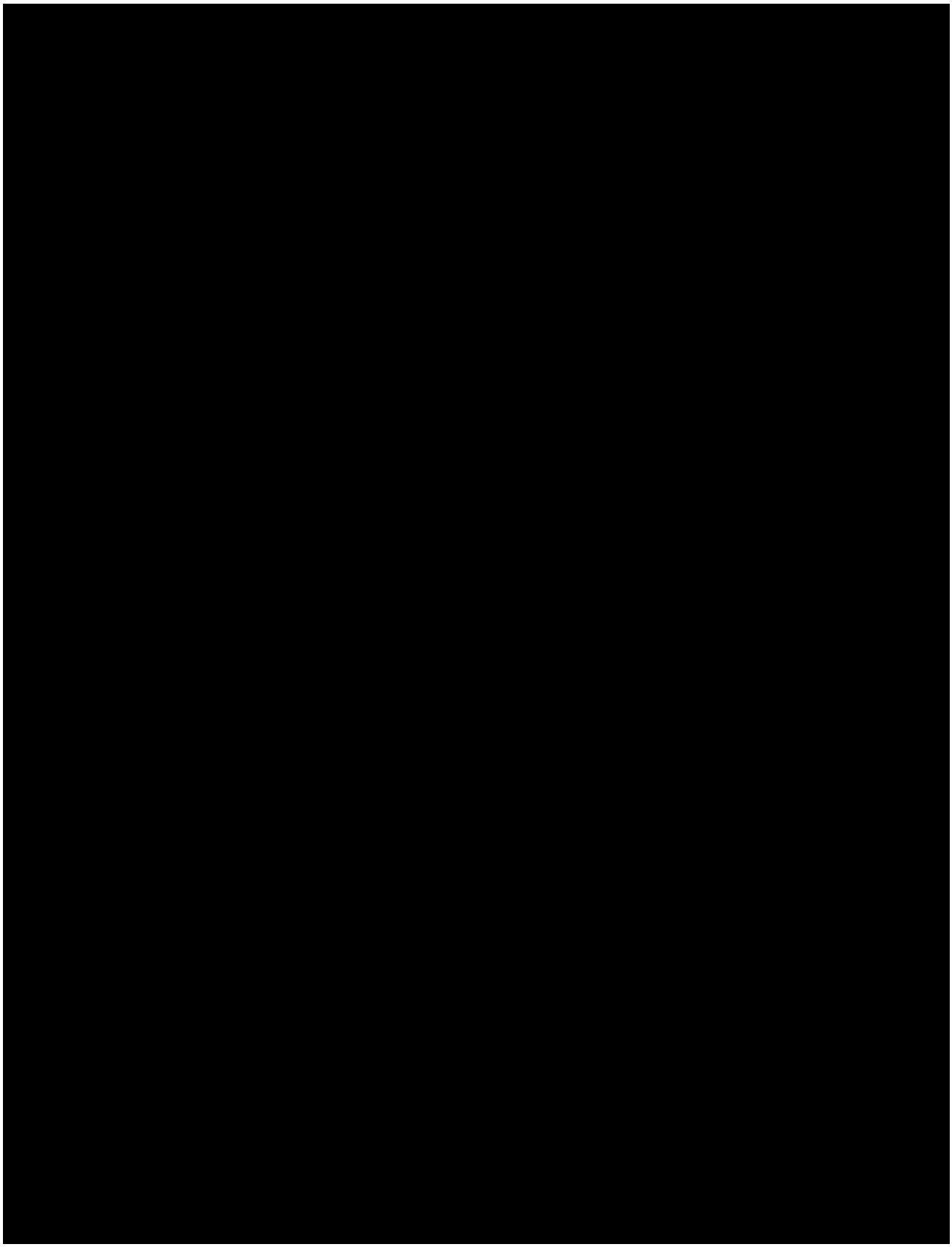
| Abbreviation Term | Description |
|---|---|
| AAA | Abdominal aortic aneurysm |
| ADA | Antidrug Antibodies |
| ADL | Activities of Daily Living |
| AE | Adverse events |
| AI | Artificial intelligence |
| ALP | Alkaline phosphatase |
| ALT | Alanine transaminase |
| AST | Aspartate transaminase |
| $AUC_{0\text{-}tau}$ | Area under the serum drug concentration by time curve within a dosing interval |
| BMD | Bone mineral density |
| BMI | Body mass index |
| BUN | Blood urea nitrogen |
| CAD | Coronary artery disease |
| CAP | Controlled Attenuation Parameter |
| CBC | Complete blood count |
| CFR | Code of Federal Regulations |
| CHF | Congestive heart failure |
| CIOMS | International Organizations of Medical Sciences |
| CK | Cytokeratin |
| $C_{max}$ | Maximal observed serum concentrations |
| COVID-19 | Coronavirus disease 2019 |
| CPK | Creatine phosphokinase |
| CRO | Clinical research organization |
| CTCAE | Common terminology criteria for adverse events |
| CTX | Carboxy-terminal collagen crosslinks |
| CV | Coefficient of variation |
| DAA | Direct-acting antiviral |
| DILI | Drug-induced liver injury |
| DNA | Deoxyribonucleic acid |
| DPP-IV | Dipeptidyl peptidase IV |
| ECG | Electrocardiogram |
| eCRF | Electronic case report form |
| eGFR | Estimated Glomerular filtration rate |
| ELF | Enhanced liver fibrosis |
| EOS | End of study |

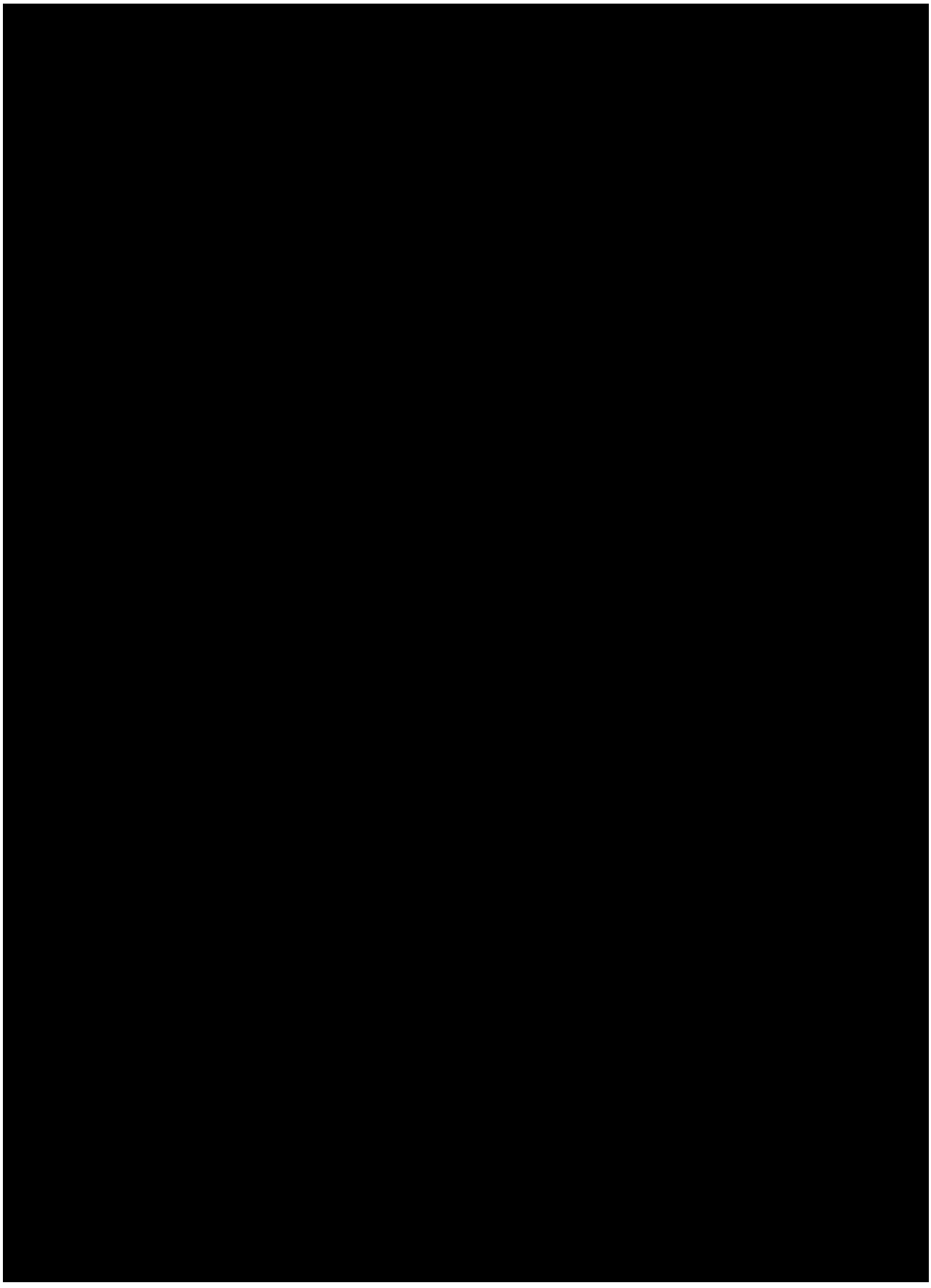
| Abbreviation Term | Description |
|---|---|
| ET | Early Termination |
| EU | European Union |
| FDA | Food and Drug Administration |
| FGFR | Fibroblast growth factor receptor |
| FIB4 | Fibrosis-4 |
| FSH | Follicle stimulating hormone |
| FU | Follow up |
| GCP | Good Clinical Practice |
| GGT | Gamma-glutamyl transferase |
| GI | Gastrointestinal |
| GLP | Good Laboratory Practice |
| GLP-1 | Glucagon-like peptide 1 |
| HA | Hyaluronic acid |
| HbA1c | Hemoglobin A1c |
| HCC | Hepatocellular carcinoma |
| hCG | human chorionic gonadotropin |
| HCV | Human coronavirus |
| HDL | High density lipoprotein |
| HDL-c | High density lipoprotein cholesterol |
| HIPAA | Health Insurance Portability and Accountability Act |
| HIV | Human Immunodeficiency Virus |
| HOMA-IR | Method used to quantify insulin resistance |
| HRT | Hormonal replacement therapy |
| hsCRP | High sensitivity C-reactive protein |
| IB | Investigator's brochure |
| ICF | Informed consent form |
| ICH | Council for Harmonisation |
| IEC | Independent Ethics Committee |
| IGF-1 | Insulin-like growth factor-1 |
| INR | Normalized ratio |
| IRB | Institutional Review Board |
| IRT | Interactive Response Technology |
| IUD | Intrauterine device |
| LDL | Low density lipoprotein |
| LDL-c | Low density lipoprotein cholesterol |
| MAD | Multiple ascending dose |
| MCH | Mean corpuscular hemoglobin |

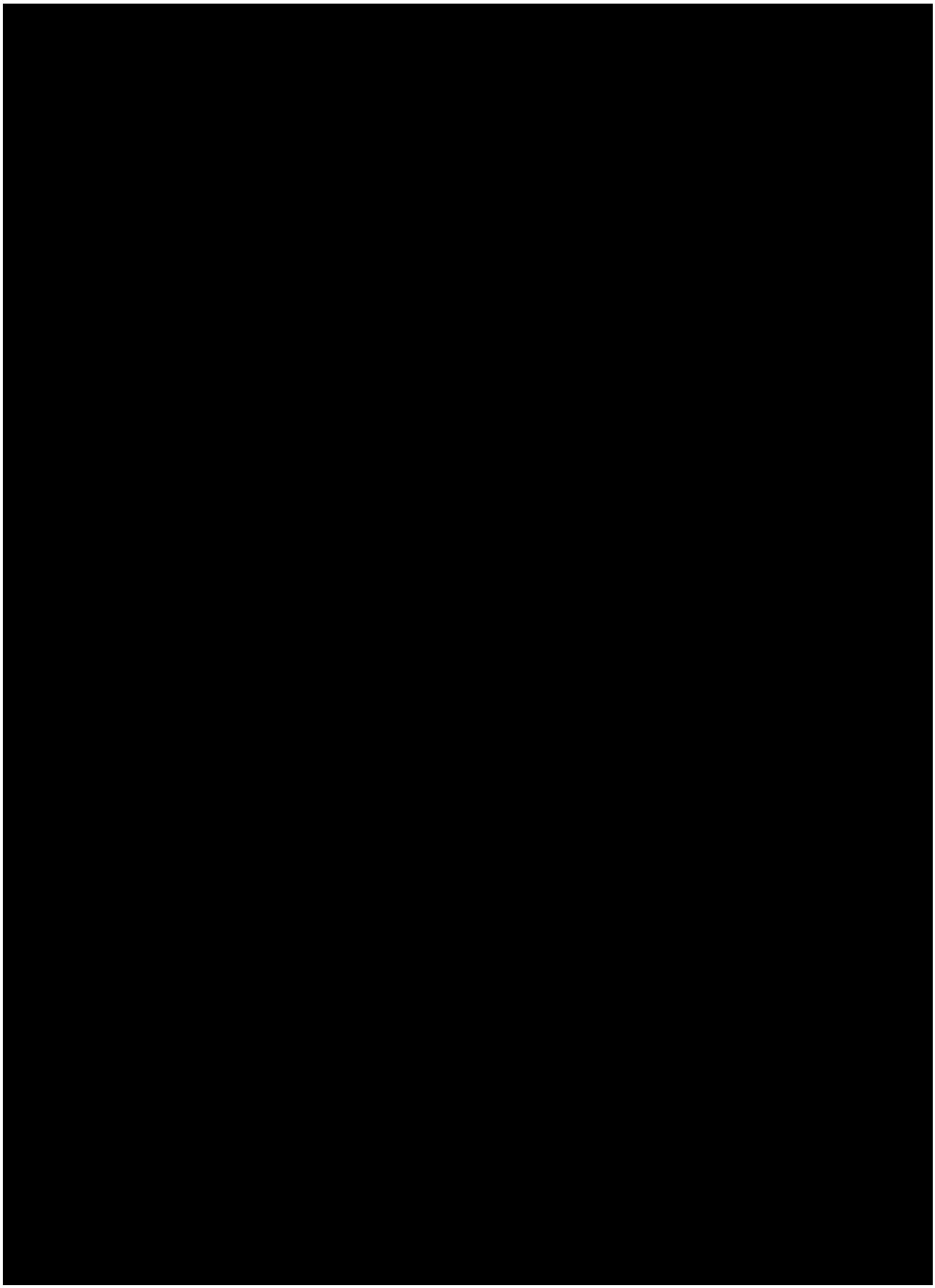
| Abbreviation Term | Description |
|---|---|
| MCV | Mean corpuscular volume |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MetS | Metabolic syndrome |
| MRI | Magnetic resonance imaging |
| n | Number |
| NAb | Neutralizing antibodies |
| NAFLD | Nonalcoholic fatty liver disease |
| NAS | NASH with NAFLD Activity |
| NASH | Nonalcoholic Steatohepatitis |
| NCI | National Cancer Institute |
| NOAEL | No observed adverse effect level |
| NYHA | New York Heart Association |
| OGTT | Oral glucose tolerance test |
| OTC | Over-the-counter |
| PBC | Primary biliary cirrhosis |
| P1NP | N-terminal propeptide of type 1 collagen |
| PD | Pharmacodynamic |
| PDFF | Proton density fat fraction |
| PEG | Polyethylene glycol |
| Pro-C3 | N-terminal propeptide of type III collagen |
| PK | Pharmacokinetic |
| PSC | Primary sclerosing cholangitis |
| PT | Preferred term |
| PVD | Peripheral vascular disease |
| QW | Weekly |
| Q2W | Every 2 weeks |
| QRS | Complex in ECG representing ventricular depolarization |
| QTcF | Fredericia corrected QT interval in ECG |
| RBC | Red blood cell |
| RNA | Ribonucleic acid |
| SAD | Single ascending doses |
| SAE | Serious adverse event |
| SAP | Statistical Analysis Plan |
| SAT | Subcutaneous abdominal fat |
| SC | Subcutaneous |
| SD | Standard deviation |
| SMC | Safety Monitoring Committee |

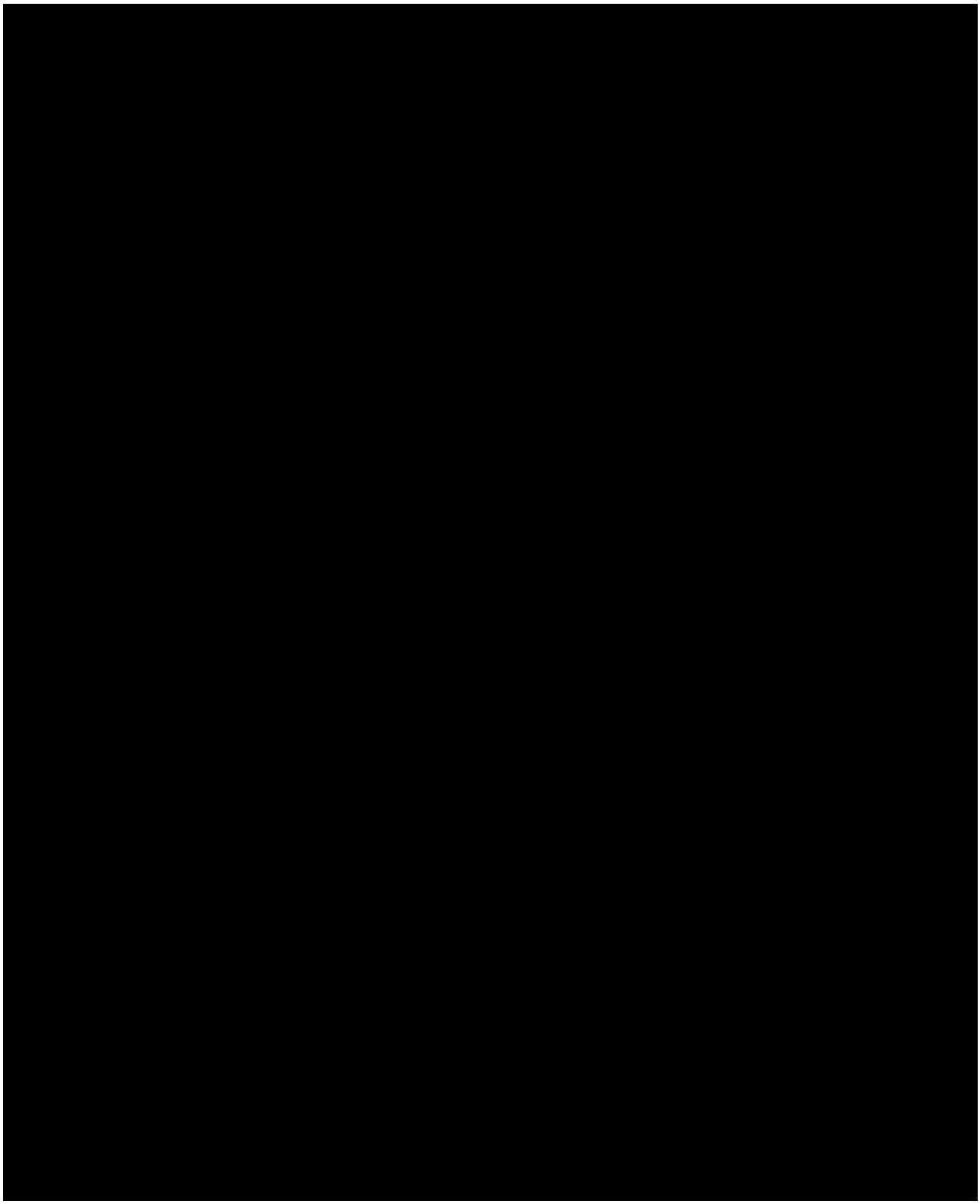
| Abbreviation Term | Description |
|---|---|
| SoA | Schedule of Activities |
| SOC | System organ class |
| SOP | Standard Operating Procedure |
| SVR | Sustained viral response |
| $t_{½}$ | Terminal elimination half-life |
| T2DM | Type 2 diabetes mellitus |
| TB | Total bilirubin |
| TEAE | Treatment-emergent adverse event |
| TIA | Transient ischemic attack |
| $t_{max}$ | Time to achieve $C_{max}$ |
| TSH | Thyroxine stimulating hormone |
| ULN | Upper limit of normal |
| US | United States |
| VAT | Visceral abdominal fat |
| VCTE | Vibration-controlled transient elastography |
| WBC | Blood cell count |
| WOCBP | Woman of Childbearing Potential |

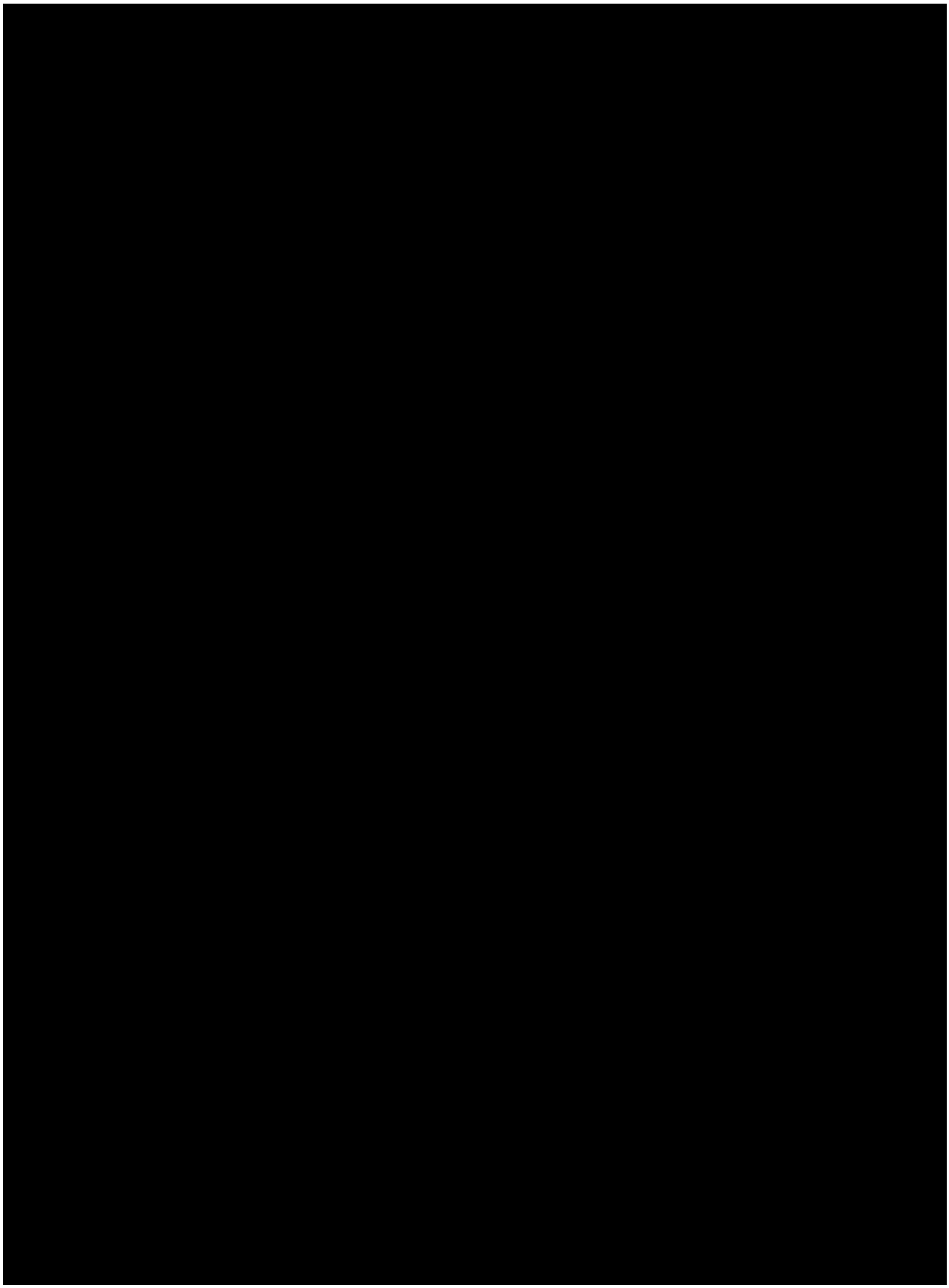


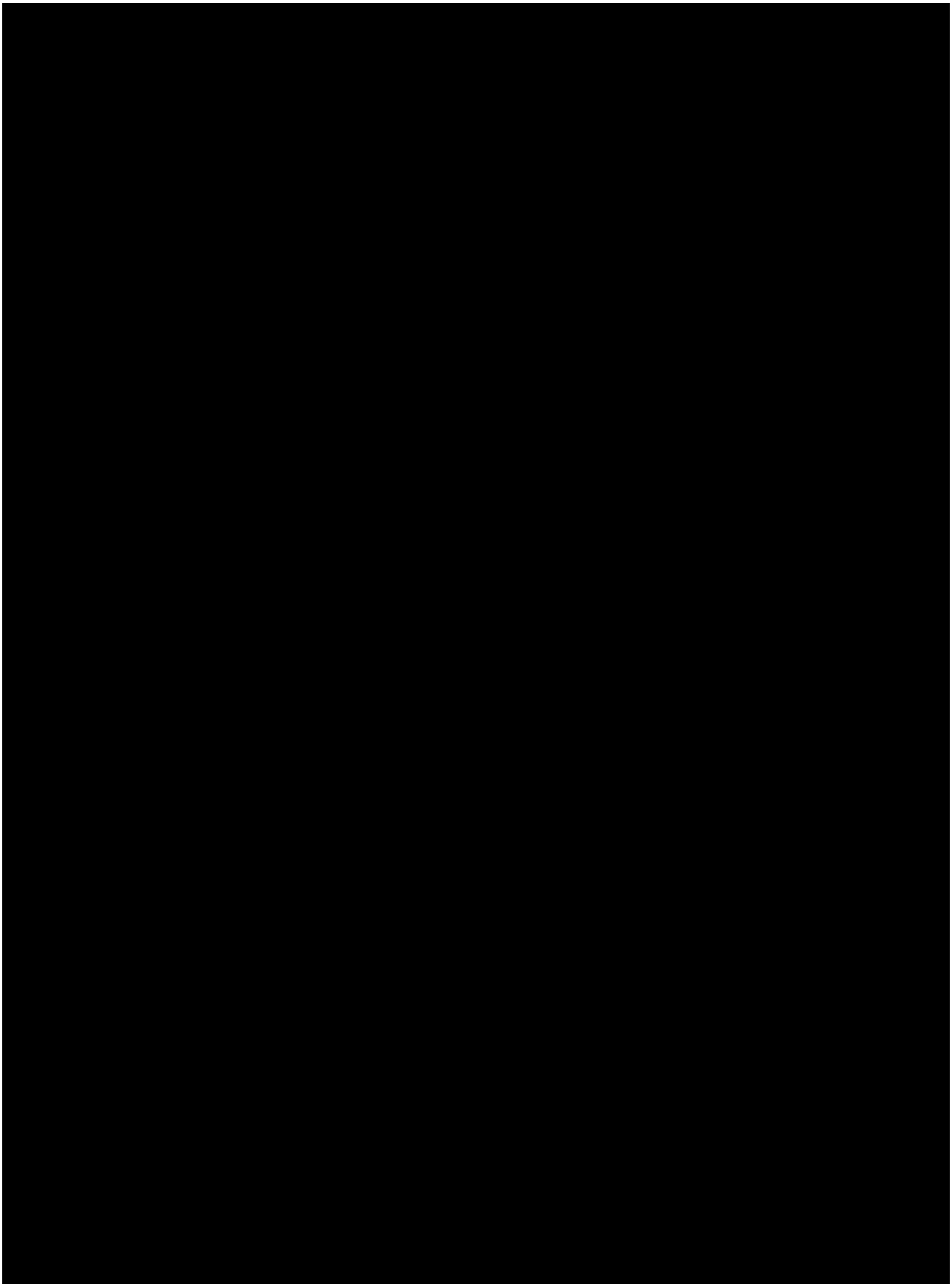












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