# SYNOPSIS

**Name of Sponsor:** Alnylam Pharmaceuticals, Inc.

**Name of Investigational Product:** ALN-AAT

**Name of Active Ingredient:** ALN-61444

**Title of Study:** A Phase 1/2, Randomized, Single-blind, Placebo-controlled, Single-ascending and Multiple-dose, Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics Study of Subcutaneously Administered ALN-AAT in Healthy Adult Subjects and Patients with ZZ Type Alpha-1 Antitrypsin Deficiency Liver Disease

**Study center(s):** Parts A and B of the study will take place in up to 2 clinical study centers in the United Kingdom (UK); Part C is expected to be undertaken in multiple centers in multiple countries.

**Duration of Study Participation (including Screening):**
- **Part A:** up to 160 days; with additional time for alpha-1 antitrypsin (AAT) monitoring, if necessary (every Q 28 days)
- **Part B:** up to 244 days; with additional time for AAT monitoring, if necessary (Q28 days)
- **Part C:** up to 404 days; with additional time for AAT monitoring, if necessary (Q28 days)

**Phase of development:** 1/2

**Objectives:**

**Primary:**
- To evaluate the safety and tolerability of single or multiple doses of ALN-AAT when administered to healthy adult subjects and patients with homozygous ZZ type AAT deficiency liver disease (PiZZ patients)

**Secondary:**
- To characterize the pharmacokinetics (PK) of ALN-AAT in healthy adult subjects and PiZZ patients
- To assess the effect of ALN-AAT on serum levels of AAT protein in healthy adult subjects and PiZZ patients

**Exploratory:**
- To assess the effect of ALN-AAT treatment on levels of AAT polymers or globules in liver in PiZZ patients
- To assess liver fibrosis in PiZZ patients via noninvasive methods, including blood-based biochemical assays (Fibrotest™ and enhanced liver fibrosis test) and exploratory imaging methods
- To assess the impact of ALN-AAT on patient quality of life as assessed by the European Quality of Life (QOL) 5 dimensions
- To assess the impact of ALN-AAT on patient-reported respiratory symptoms as assessed by the Chronic Obstructive Pulmonary Disease Assessment Test
- To evaluate additional parameters measured in stored biological samples which may indicate ALN-AAT activity or confirm the mechanism of action of ALN-AAT in healthy adult subjects and PiZZ patients
Methodology: This is a multicenter, randomized, single-blind, placebo-controlled study of ALN-AAT administered subcutaneously (SC) to healthy adult subjects and adult PiZZ patients. The study is designed to evaluate the safety, tolerability, PK, and pharmacodynamics (PD) of ALN-AAT. A Safety Review Committee (SRC) will perform ongoing reviews of safety, tolerability, and available PD data collected in all study phases (Parts A, B, and C) with the primary purpose of protecting the safety of subjects/patients participating in this clinical study. The study will be conducted in 3 sequential phases:

- Part A: single ascending dose (SAD) phase in healthy adult subjects
- Part B: multiple ascending dose (MAD) phase in healthy adult subjects
- Part C: parallel group multiple-dose (MD) phase in adult PiZZ patients with mild to moderate liver fibrosis

Part A: Healthy subjects will be enrolled in 1 of 4 ascending-dose cohorts. Each cohort will be composed of 4 subjects randomized 3:1 to receive a single dose of ALN-AAT or placebo, respectively. Subjects will be screened from 90 to 2 days before study drug administration. Eligible subjects will be admitted to the clinical study center on Day -1 to determine continued eligibility and for predose assessments. Subjects in each cohort will be randomized on Day 0 and will receive a single dose of study drug (ALN-AAT or placebo). Subjects will be discharged from the clinical study center on Day 1 after completing the 24-hour postdose follow-up assessments.

Subjects will return to the clinical study center on an outpatient basis for safety, tolerability, PK, and PD monitoring at specified time points through the last postdose follow-up visit (Day 70). For subjects with AAT levels at the last postdose follow-up study visit (Day 70) that are <80% of the mean pretreatment value, monitoring visits will occur every 28±7 days until AAT levels return to ≥80% of the mean pretreatment value.

The following are planned dose levels for Part A; however, the actual dose administered will be determined after SRC review of data from the previous cohorts (eg, if a safety signal is observed upon dose escalation, then upon SRC recommendation, a “de-escalation” cohort may be initiated):

- Cohort 1: 0.3 mg/kg
- Cohort 2: 1.0 mg/kg
- Cohort 3: 3.0 mg/kg
- Cohort 4: 6.0 mg/kg
- Cohort 5 (Optional)
- Cohort 6 (Optional)

The maximum dose administered will not exceed 10.0 mg/kg.

Part B: Healthy subjects will be enrolled into 1 of 2 ascending, multiple-dose cohorts. Each cohort will be composed of 6 subjects randomized 4:2 to receive a total of 4 doses of ALN-AAT or placebo, respectively, administered every 4 weeks (Q4W). A dosing interval of Q4W is planned; however, a minimum interval of 2 weeks (7 total doses over a similar 12-week period) may be recommended by the SRC for all cohorts or additional cohorts based upon review of safety and PD data from Part A. Subjects will be screened from 90 to 2 days before dose study drug administration. Eligible subjects will be admitted to the clinical study center on Day -1 to determine continued eligibility and for predose assessments. Subjects in each cohort will be randomized on Day 0 and will receive an initial dose of study drug. Subjects will be discharged from the clinical study center on Day 1 following the completion of the 24-hour postdose follow-up assessments.
Subjects will return to the clinical study center for study drug administration Q4W for the remaining doses of study drug, and will be observed at the clinical study center for at least 6 hours following each dose. Subjects will be admitted to the clinical study center on Day 83, 1 day prior to the final dose. Subjects will be discharged from the clinical study center on Day 85 following the completion of the 24-hour postdose follow-up assessments.

Subjects will return to the clinical study center on an outpatient basis for safety, tolerability, PK, and PD monitoring at specified time points through the last postdose follow-up visit (Day 154). For subjects with AAT levels at the last postdose follow-up visit (Day 154) that are <80% of the mean pretreatment value, monitoring visits will occur every 28±7 days until AAT levels return to ≥80% of the mean pretreatment value.

Dose levels for both Part B planned cohorts will be selected after SRC review of safety, tolerability, and available PD data covering at least 21 days after the last dose administered to the last subject in the highest planned dosing cohort in Part A. The doses administered in cohorts in Part B will be no greater than the highest dose determined to be safe and well tolerated in Part A. Part B may begin while optional cohorts in Part A are ongoing. The following are anticipated dose levels for Part B cohorts; however the actual doses administered will be determined after SRC review of the data from Part A:

- Cohort 1: 1.0 mg/kg
- Cohort 2: 3.0 mg/kg
- Cohort 3 (Optional)
- Cohort 4 (Optional)

**Part C:** PiZZ patients with mild to moderate liver fibrosis will be enrolled in 1 of 2 parallel-dose cohorts. Each cohort will be composed of 6 patients randomized 4:2 to receive a total of 6 doses of ALN-AAT or placebo, respectively, administered Q4W. A dosing interval of Q4W is planned; however, a minimum interval of 2 weeks (12 doses over a 22-week period) may be recommended by the SRC for all cohorts or a single additional cohort.

Patients will be screened up to 180 days before study drug administration. Screening evaluation will include a liver biopsy to verify the presence of liver pathology consistent with a diagnosis of ZZ liver disease and confirm an Ishak fibrosis score of 1 to 4, inclusive. Patients in each cohort will be randomized on Day 0 and will receive an initial dose of study drug. Patients must be observed for at least 6 hours postdose, and must remain at or near the clinical study center so that all evaluations up to 24 hours postdose can be completed.

Patients will return to the clinical study center on an outpatient basis for safety, tolerability, PK, and PD monitoring at specified time points through the last postdose follow-up visit. For patients with AAT levels at the last postdose follow-up study visit that are <80% of the mean pretreatment value, monitoring visits will occur every 28±7 days until AAT levels return to ≥80% of the mean pretreatment value.

Dose selection in Part C will be based on safety and available PD data from Parts A and B. The dose and regimens studied in Part C will not exceed those found to be safe and well tolerated in Part B cohorts. No dose will be chosen that met the stopping rules in Part B. Day 84 laboratory and clinical safety data from at least 2 Part B cohorts will be evaluated by the SRC prior to initiation of Part C. The SRC will convene every 3 months during the course of Part C to evaluate accumulating safety data. Additional ad hoc meetings may occur as required. Decisions to stop the study or discontinue individual patients will be made according to predetermined stopping rules. Additionally, the SRC may recommend discontinuation of the study at their discretion.

**Optional Cohorts:** Two additional dose cohorts may be enrolled in Parts A or B, and 1 additional dose cohort may be enrolled in Part C to better define dose response and/or safety and tolerability,
based upon SRC review of accumulated safety, tolerability, and available PD data. Similarly, expansion of a cohort in Part B or Part C by up to 6 additional subjects or patients receiving active treatment may occur to better understand dose response and/or safety and tolerability.

**Number of subjects and patients (planned):** Up to 48 healthy subjects and up to 18 patients will be enrolled in the study, including optional cohorts in each part.

- Part A: Up to 24 healthy subjects
- Part B: Up to 24 healthy subjects
- Part C: Up to 18 PiZZ patients

**Diagnosis and eligibility criteria:** To be eligible to participate in the study, subjects/patients must satisfy the following eligibility criteria.

**Inclusion Criteria for All Subjects and Patients in Parts A, B, and C**

1. Male or female, aged 18 to 65 years, inclusive
2. 12-lead electrocardiogram (ECG) within normal limits or with no clinically significant abnormalities in the opinion of the Investigator
3. Body mass index \( \geq 18.0 \text{ kg/m}^2 \) and \( \leq 30 \text{ kg/m}^2 \)
4. Female subjects/patients of childbearing potential cannot be pregnant, cannot be breastfeeding, and must agree to use one of the acceptable methods of contraception listed below from the time of signing the informed consent until 3 months following administration of the last dose of study drug:
   - Documented placement of an intrauterine device (IUD) or intrauterine system (IUS) and the use of a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] used with spermicidal foam/gel/film/cream/suppository).
   - Oral contraceptives (combination estrogen/progesterone pills), injectable progesterone, or subdermal implants and the use of a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] used with spermicidal foam/gel/film/cream/suppository).
   - Documented tubal ligation (female sterilization). In addition, a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] used with spermicidal foam/gel/film/cream/suppository).
   - True abstinence (when this is in line with the preferred and usual lifestyle of the subject/patient). Periodic abstinence (eg, calendar, ovulation, symptothermal, post ovulation methods) and withdrawal are not acceptable methods of contraception. Abstinent subjects/patients have to agree to use one of the above-mentioned contraceptive methods if they start sexual relationships during the study and for up to 3 months after the last dose of study drug.
5. Male subjects/patients must agree to use acceptable methods of contraception if the male subject’s/patient’s partner could become pregnant from the time of the first dose of study drug until 3 months following administration of the last dose of study drug. One of the following acceptable methods of contraception must be utilized:
   - Surgical sterilization (vasectomy with documentation of azoospermia) and a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] used with spermicidal foam/gel/film/cream/suppository).
   - The subject’s/patient’s female partner uses oral contraceptives (combination estrogen/progestosterone pills), injectable progesterone, or subdermal implants and a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] used with spermicidal foam/gel/film/cream/suppository).
The subject’s/patient’s female partner uses medically prescribed topically-applied transdermal contraceptive patch and a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] used with spermicidal foam/gel/film/cream/suppository).

The subject’s/patient’s female partner has undergone documented tubal ligation (female sterilization). In addition, a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] with spermicidal foam/gel/film/cream/suppository) must be used.

The subject’s/patient’s female partner has undergone documented placement of an IUD or IUS. In addition, a barrier method (condom or occlusive cap [diaphragm or cervical/vault caps] with spermicidal foam/gel/film/cream/suppository) must be used.

True abstinence (when this is in line with the preferred and usual lifestyle of the subject/patient). Periodic abstinence (eg, calendar, ovulation, symptothermal, post ovulation methods) and withdrawal are not acceptable methods of contraception. Abstinent subjects/patients have to agree to use one of the above-mentioned contraceptive methods if they start sexual relationships during the study and for up to 3 months after the last dose of study drug.

6. Willing to comply with protocol-required visit schedule and visit requirements, and able to provide written informed consent

7. Nonsmokers for at least 5 years before screening

Additional Inclusion Criteria for Subjects in Parts A and B

1. AAT levels within normal limits
2. Forced expiratory volume in 1 second (FEV₁) ≥85% of predicted, and FEV₁/forced vital capacity ratio ≥0.7

Additional Inclusion Criteria for Patients in Part C

1. Documented ZZ type AAT by phenotype or genotype
2. Liver histopathology consistent with ZZ liver disease, with fibrosis Ishak score of 1 to 4, inclusive, and positive for diastase-resistant periodic acid-Schiff-positive globules in hepatocytes, by liver biopsy performed within 6 months of the first dose of study drug. Note: documented results of liver biopsies performed outside of the study are acceptable for study inclusion, provided biopsy material is available for predose and postdose comparison.
3. Post-bronchodilator FEV₁ ≥70% of predicted and diffusing capacity of the lung for carbon monoxide ≥50% of predicted
4. If on any maintenance medication regimen other than augmentation therapy, likely, in the opinion of the Investigator, to be able to remain on a stable medication regimen for the duration of the study (no new medications within 30 days prior to first dose of study drug)

Exclusion Criteria for All Subjects and Patients in Parts A, B, and C

1. Any uncontrolled or serious disease, or any medical or surgical condition, that may interfere with participation in the clinical study and/or put the subject/patient at significant risk (according to Investigator’s judgment) if he/she participates in the clinical study, with the exception of AAT deficiency for patients in Part C
2. An underlying known disease, or surgical or medical condition that, in the opinion of the Investigator, might interfere with interpretation of the clinical study results, with the exception of AAT deficiency for patients in Part C
3. Active serious mental illness or psychiatric disorder, including but not limited to schizophrenia, bipolar disorder, or severe depression requiring current pharmacological intervention
4. Clinically significant illness within 7 days before the first dose of study drug
5. Systolic blood pressure ≤140 mmHg and a diastolic blood pressure of ≤90 mmHg after 10 minutes supine rest
6. Any clinical safety laboratory result considered clinically significant and unacceptable by the Investigator
7. Received an investigational agent within 90 days before the first dose of study drug or are active in the follow-up phase of another clinical study involving interventional treatment
8. Clinical laboratory evidence or clinical diagnosis of human immunodeficiency virus infection, hepatitis C virus infection, or chronic hepatitis B virus infection (as shown by hepatitis B surface antigen positivity)
9. Consume more than 14 (female) or 21 (male) units of alcohol per week (unit: 1 glass of wine [125 mL] = 1 measure of spirits = ½ pint of beer)
10. History or clinical evidence of alcohol abuse, within the 12 months before screening. Alcohol abuse is defined as regular weekly intake of more than 21 units for males and 14 units for females (using alcohol tracker at http://www.nhs.uk/Tools/Pages/NHSAlcoholtracker.aspx).
11. History or clinical evidence of drug abuse, within the 12 months before screening. Drug abuse is defined as compulsive, repetitive, and/or chronic use of drugs or other substances with or without problems related to their use and/or where stopping or a reduction in dose will lead to withdrawal symptoms.
12. History of intolerance to SC injection
13. Legal incapacity or limited legal capacity at screening
14. Any conditions which, in the opinion of the Investigator, would make the subject/patient unsuitable for enrollment or could interfere with the subject’s/patient’s participation in or completion of the study

Additional Exclusion Criteria for Subjects in Parts A and B
1. History of asthma or recurrent or chronic lung disease, excluding childhood asthma that has resolved
2. History of chronic liver disease from any cause
3. Alanine aminotransferase (ALT) and/or total bilirubin above the upper limit of normal (ULN; subjects with known Gilbert’s syndrome with unconjugated hyperbilirubinemia will be allowed)
4. Aspartate aminotransferase (AST), alkaline phosphatase (ALP), or gamma glutamyl transferase (GGT) > 2×ULN (no Investigator discretion); or, if AST, ALP, or GGT > ULN, but ≤2×ULN and considered clinically relevant by the Investigator
5. Complete blood count clinical laboratory results that are considered clinically relevant and unacceptable by the Investigator at screening and Day -1
6. Donated more than 500 mL of blood within 90 days before the first dose of study drug
7. Used prescription drugs within 14 days or 5 half-lives (whichever is longer) before the first dose of study drug, with the exception of medications necessary to treat an adverse event, hormone replacement therapy, and oral contraceptives, injectable progesterone, and subdermal implants for contraception
8. Used over-the-counter medication, excluding routine vitamins, within 7 days before the first dose of study drug, unless determined by the Investigator and Sponsor to be not clinically relevant
9. Positive screen for alcohol or drugs of abuse

**Additional Exclusion Criteria for Patients in Part C**

1. History of chronic liver disease from any known cause other than ZZ type AAT deficiency
2. History of hepatic encephalopathy
3. History of gastrointestinal bleeding from esophageal or gastric varices complicating portal hypertension
4. Post-bronchodilator FEV₁ that has declined to <70% of predicted or by >15% relative to screening assessment
5. Patients receiving augmentation therapy for AAT deficiency or who have received augmentation therapy within 8 weeks of first dose of study drug
6. AST or ALT ≥3×ULN
7. Total bilirubin above the ULN (patients with known Gilbert’s syndrome with unconjugated hyperbilirubinemia will be allowed)
8. Serum albumin level ≤80% the lower limit of normal
9. Platelet count ≤100,000 per microliter
10. International normalized ratio or prothrombin time above the ULN of the reference range (as per the local laboratory reference range)
11. Any history of a bleeding disorder, or unable to abstain from medications that may interfere with normal blood clotting, such that the risk of bleeding following liver biopsy would be increased

**Investigational product, dosage and mode of administration:** ALN-AAT is a synthetic, chemically modified small interfering RNA targeting AAT messenger RNA with a covalently attached triantennary GalNAc ligand. ALN-AAT will be supplied as a sterile solution for SC injection at 200 mg/mL.

**Duration of treatment:**

- Part A: Single SC dose administration
- Part B: Four SC doses administered Q4W (last dose administered at 12 weeks)
- Part C: Six SC doses administered Q4W (last dose administered at 20 weeks)

**Reference therapy, dosage and mode of administration:** Subjects randomized to placebo will be administered sterile, preservative-free normal saline 0.9% solution for SC injection, which will be supplied by the clinical study center.

**Criteria for evaluation:**

**Safety:** Safety evaluation will include clinical laboratory tests (hematology, biochemistry, coagulation, and urinalysis), vital signs (oral body temperature, blood pressure, heart rate, and respiration rate), physical examinations, 12-lead ECGs, pulmonary function testing (spirometry), concomitant medications, and adverse event monitoring.

**Pharmacodynamics:** Formal PD evaluation will consist of measurement of serum AAT levels using a validated enzyme-linked immunosorbent assay.

**Pharmacokinetics:** Blood and urine samples will be collected for assessment of ALN-AAT PK parameters and possible metabolite analysis. Pharmacokinetic parameters may include, but are not limited to, maximum plasma concentration, time to reach maximum plasma concentration, area under the plasma concentration versus time curve, apparent terminal elimination half-life, fraction eliminated.
in the urine, and renal clearance.

**Exploratory:** Samples of DNA from whole blood will be obtained and archived from subjects and patients in all parts of the study for potential genotyping. Additional blood samples for possible analyses to elucidate ALN-AAT activity and/or mechanism of action will be collected. In Part C, liver biopsies to assess liver fibrosis, the presence of AAT polymers or globules, and potential exploratory markers of PiZZ liver disease will be collected, baseline exploratory noninvasive methods of liver fibrosis will be correlated with liver histology, and QOL/patient-reported outcome parameters will be assessed.

**Statistical methods:** Statistical analyses will be primarily descriptive; no formal hypotheses will be tested. All study data will be presented in by-subject data listings. Data from Parts A, B, and C will be generally analyzed separately. Summary tables will present results by cohort for each ALN-AAT dose and placebo, where the placebo subjects/patients will be combined across dose cohorts. Descriptive statistics will be presented for continuous variables, and frequencies and percentages will be presented for categorical and ordinal variables. Percentages will be based on the number of non-missing values in a dose group.