SYNAPSE THERAPEUTICS INC.

STUDY PROTOCOL

Single Ascending Dose Study of SYN-409 in Healthy Adult Volunteers

NX-PK-2026-004
Phase I Clinical Trial
IND Number: 184,902
Date: August 2, 2026

Pharmacokinetics, Safety, and Tolerability of SYN-409: A Double-Blind, Randomized, Placebo-Controlled Trial

Sponsor:
Synapse Therapeutics Inc.
Clinical Development Division, South San Francisco, CA

Contract Research Organization (CRO):
Nexa Clinical Solutions Inc.
Operations Headquarters, Boston, MA

Investigational Product:	SYN-409 (Highly Selective Small-Molecule Inhibitor)
Study Phase:	Phase I
Protocol Version:	Version 1.2
Date of Protocol:	August 2, 2026
Target Population:	Healthy Male and Female Adult Volunteers (Ages 18–55)

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1 Protocol Synopsis & Objectives

Protocol Abstract & Study Rationale

This Phase I study is designed to evaluate the safety, tolerability, and pharmacokinetic (PK) profile of single ascending oral doses of SYN-409 in healthy adult subjects. SYN-409 is a novel, highly selective, small-molecule inhibitor of the intracellular kinase pathway designed for the treatment of inflammatory diseases. Preclinical models in rodents and non-human primates have demonstrated dose-dependent efficacy with no significant target-related toxicities. This trial represents the first-in-human (FIH) clinical evaluation of the compound.

1.1 Background and Scientific Rationale

Intracellular kinase dysregulation plays a pivotal role in the pathogenesis of chronic autoimmune and inflammatory diseases. Preclinical characterization of SYN-409, developed by Synapse Therapeutics, indicates high potency (IC_{50} = 4.2 nM) and excellent selectivity against a panel of 450 human kinases. In animal safety studies, SYN-409 demonstrated clean cardiovascular, respiratory, and central nervous system safety margins at exposures up to 50-fold higher than the projected therapeutic range.

The pharmacokinetic profile in animals was characterized by rapid absorption (t_{max} between 1.0 and 2.5 hours), moderate volume of distribution ($V_d/F \approx 1.5$ L/kg), and a terminal elimination half-life supporting once-daily dosing. The scaling predictions based on physiologically based pharmacokinetic (PBPK) models suggest that the target therapeutic exposure in humans will be achieved at doses between 100 mg and 300 mg [Sterling and Harrison, 2025]. This trial is required to characterize the human PK profile and confirm dose proportionality over a broad range of doses.

1.2 Study Objectives

The clinical and scientific objectives of this protocol are defined hierarchically as follows:

1.2.1 Primary Objectives

- To evaluate the safety and tolerability of single ascending oral doses of SYN-409 in healthy male and female adult subjects.
- To characterize the single-dose pharmacokinetics of SYN-409 in plasma and urine following oral administration.

1.2.2 Secondary Objectives

- To assess the dose-proportionality of SYN-409 exposure (C_{max} and AUC) over the studied dose range (10 mg to 600 mg).
- To determine the excretion profile of parent compound and major metabolites in urine.

1.2.3 Exploratory Objectives

- To assess the relationship between plasma concentrations of SYN-409 and potential pharmacodynamic (PD) biomarkers (e.g., inhibition of target phosphorylation in peripheral blood mononuclear cells).

2 Study Design & Methodology

2.1 Overall Study Design

This is a Phase I, randomized, double-blind, placebo-controlled, single ascending dose (SAD) study in healthy adult subjects. The study will be conducted in five sequential cohorts, evaluating doses of 10 mg, 30 mg, 100 mg, 300 mg, and 600 mg administered orally as a liquid suspension under fasting conditions.

Each cohort will consist of 10 subjects, randomized in an 8:2 ratio to receive either active SYN-409 (n = 8) or matching placebo (n = 2). Dose escalation will proceed sequentially, pending safety and tolerability review of the cumulative data from the preceding cohort by the Safety Review Committee (SRC). Figure 1 illustrates the progression of subjects through the clinical trial.

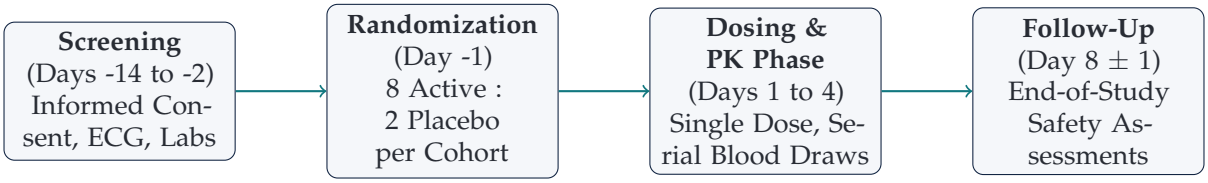

Figure 1: Overview of study design flowchart per cohort. Subject progression through screening, baseline randomization, treatment phase with intensive PK profiling, and safety follow-up.

2.2 Study Cohorts and Dosing Schedule

Dose levels have been selected based on preclinical toxicology and PK scaling studies [Sterling and Harrison, 2025]. The starting dose of 10 mg is estimated to be well below the anticipated pharmacologically active dose and provides a safety margin of at least 100-fold relative to the animal no-observed-adverse-effect-level (NOAEL).

The dosing schedule is summarized below:

- **Cohort 1:** Single dose of 10 mg active or placebo.
- **Cohort 2:** Single dose of 30 mg active or placebo.
- **Cohort 3:** Single dose of 100 mg active or placebo.
- **Cohort 4:** Single dose of 300 mg active or placebo.
- **Cohort 5:** Single dose of 600 mg active or placebo.

Dose escalation will be halted if two or more subjects in a cohort experience a drug-related severe adverse event (SAE) or if grade 3 laboratory abnormalities are reported.

2.3 Subject Eligibility Criteria

Subjects must meet all of the following criteria to be eligible for enrollment in this study:

2.3.1 Inclusion Criteria

[1] Healthy male or female volunteers aged 18 to 55 years, inclusive.

[2] Body Mass Index (BMI) between 18.5 and 30.0 kg/m^2, inclusive, and a total body weight $\geq$ 50 kg.

[3] Medically healthy as determined by pre-study physical examination, 12-lead ECG, vital signs, and clinical laboratory evaluations.

[4] Agreement to use highly effective contraception methods throughout the study and for 30 days post-dose.

2.3.2 Exclusion Criteria

[1] History of significant cardiovascular, renal, hepatic, or gastrointestinal disease that might interfere with absorption, distribution, metabolism, or excretion of the drug.

[2] Positive screen for hepatitis B, hepatitis C, or human immunodeficiency virus (HIV).

[3] Regular consumption of alcohol exceeding 14 units per week, or active drug abuse.

[4] Participation in another clinical study within 30 days prior to screening.

3 Sample Collection & Bioanalytical Methods

3.1 Pharmacokinetic Blood Sampling Schedule

Serial blood samples for PK determination will be collected via indwelling intravenous cannula or direct venipuncture. A total of 16 PK blood samples will be collected from each subject over a 72-hour post-dose period.

Table 1 outlines the exact timing and tolerance windows for each blood collection point relative to the time of oral dosing ($T_0 = 0.0$ hours).

Table 1: PK Blood Collection Schedule and Sampling Windows

Time Point	Target Time (h)	Window (min)	Rationale
Pre-dose	-0.50 (within 30m prior)	–	Establish baseline level
Post-dose	0.25	$\pm$ 2	Capture initial absorption phase
Post-dose	0.50	$\pm$ 2	Capture absorption phase
Post-dose	1.00	$\pm$ 5	Capture near-peak levels
Post-dose	1.50	$\pm$ 5	Capture peak concentration range
Post-dose	2.00	$\pm$ 5	Expected $t_{\max}$ range
Post-dose	3.00	$\pm$ 10	Capture early distribution phase
Post-dose	4.00	$\pm$ 10	Capture distribution phase
Post-dose	6.00	$\pm$ 10	Capture early elimination phase
Post-dose	8.00	$\pm$ 15	Capture elimination phase
Post-dose	12.00	$\pm$ 15	Capture elimination phase
Post-dose	24.00	$\pm$ 30	Confirm 24h trough concentration
Post-dose	36.00	$\pm$ 45	Extended elimination phase
Post-dose	48.00	$\pm$ 60	Extended elimination phase
Post-dose	72.00	$\pm$ 120	Determine terminal elimination phase

3.2 Urine Sampling Schedule

Urine samples will be collected cumulatively over the following intervals post-dose: pre-dose (-2 to 0 hours), 0–4 hours, 4–8 hours, 8–12 hours, 12–24 hours, and 24–48 hours. Total urine volume will be recorded for each interval, and a 10 mL aliquot will be retained and frozen at -80°C for assaying parent drug and metabolite concentrations.

3.3 Bioanalytical Methodology

Plasma and urine concentrations of SYN-409 will be determined using a validated high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method [Zhao et al., 2025].

3.3.1 Sample Processing

Blood samples will be collected in 4 mL K_2EDTA tubes and immediately inverted 8–10 times. Tubes will be centrifuged at 1500 g for 10 minutes at 4°C within 30 minutes of collection. The resulting plasma will be split into two aliquots (primary and back-up) and stored in polypropylene tubes at −80°C.

3.3.2 LC-MS/MS Assay Specifications

- **Calibration Range:** 0.500 to 1000 ng/mL in plasma; 2.00 to 5000 ng/mL in urine.
- **Lower Limit of Quantification (LLOQ):** 0.500 ng/mL.
- **Internal Standard:** Deuterated SYN-409 (d_4-SYN-409).
- **Precision and Accuracy:** Within-run and between-run coefficient of variation (CV) $< 15\%$ ($< 20\%$ at LLOQ), and accuracy within 85% to 115% of nominal values.

4 Pharmacokinetics & Statistical Modeling

4.1 Pharmacokinetic Parameter Estimation

Pharmacokinetic analysis of plasma and urine concentrations of SYN-409 will be performed using non-compartmental analysis (NCA) methods [Gibaldi and Perrier, 2007]. Calculations will be executed using Phoenix WinNonlin® version 8.3 or validated PK packages in R (version 4.3.2).

The following PK parameters will be estimated from individual concentration-time data:

- $C_{\max}$**:** Maximum observed plasma concentration, obtained directly from the raw data.
- $t_{\max}$**:** Time to reach maximum plasma concentration, obtained directly from the raw data.
- λ_z**:** Terminal elimination rate constant, estimated by linear regression of the terminal log-linear phase of the concentration-time curve.
- $t_{1/2}$**:** Terminal elimination half-life, calculated as:

$$t_{1/2} = \frac{\ln(2)}{\lambda_z} \tag{1}$$

- $\mathbf{AUC}_{0-t}$**:** Area under the plasma concentration-time curve from time zero to the last quantifiable concentration, calculated using the linear-up log-down trapezoidal rule.
- $\mathbf{AUC}_{0-\infty}$**:** Area under the plasma concentration-time curve extrapolated to infinity, calculated as:

$$\mathrm{AUC}_{0-\infty} = \mathrm{AUC}_{0-t} + \frac{C_t}{\lambda_z} \tag{2}$$

  where C_t is the last quantifiable concentration.
- $\mathbf{CL}/F$**:** Apparent total clearance after oral administration, calculated as Dose / $\mathrm{AUC}_{0-\infty}$.
- V_z/F**:** Apparent volume of distribution during the terminal elimination phase, calculated as $\mathrm{CL}/F/\lambda_z$.

4.2 Dose Proportionality Analysis

To assess the dose proportionality of SYN-409 over the 10 mg to 600 mg dose range, a power model will be fitted to the primary parameters ($C_{\max}$ and $\mathrm{AUC}_{0-\infty}$):

$$\ln(\text{Parameter}) = \beta_0 + \beta_1 \cdot \ln(\text{Dose}) + \varepsilon \tag{3}$$

where β_1 represents the dose-proportionality coefficient. True dose proportionality is concluded if the 90% confidence interval for β_1 falls entirely within the interval $[0.90, 1.10]$.

4.3 Expected PK Profiles

Figure 2 displays the expected pharmacokinetic profiles for two dose levels of SYN-409 based on preclinical scaling studies and physiological parameters.

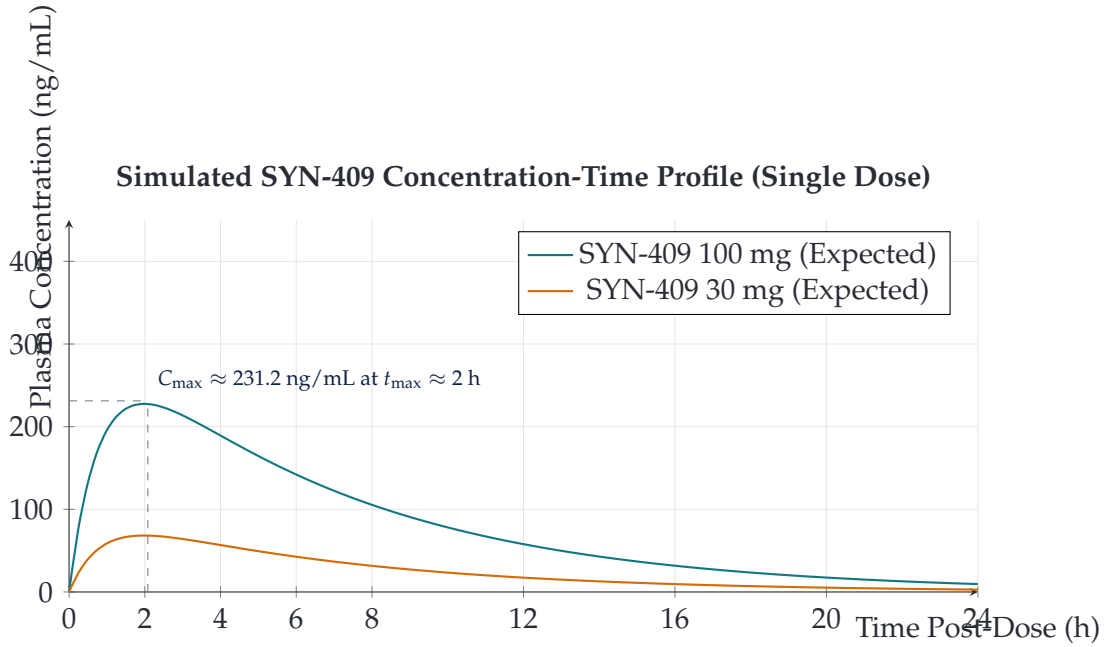

Figure 2: Predicted pharmacokinetic profile of SYN-409 based on preclinical scaling models. Continuous curves represent compartment model simulations.

5 Regulatory, Ethical & Study Monitoring

5.1 Ethical Conduct and Good Clinical Practice (GCP)

This study will be conducted in accordance with the International Council for Harmonisation (ICH) Guidance for Good Clinical Practice (E6 R2), the ethical principles of the Declaration of Helsinki, and applicable local clinical trial regulations.

All clinical staff will hold active GCP certifications. The study protocol, investigator brochure, and informed consent forms must be reviewed and approved by an independent Institutional Review Board (IRB) prior to screening any subjects.

5.2 Informed Consent Process

Before performing any study-related procedures, a clinical investigator will explain the study objectives, procedures, risks, and benefits to potential subjects. Subjects will be provided with an IRB-approved written informed consent form in simple, non-technical language.

Subjects will be given ample time to read the form and ask questions. A signed and dated informed consent form must be obtained from each subject before any screening procedures are initiated. Subjects may withdraw their consent at any time without penalty or loss of medical care.

5.3 Safety Monitoring Committee

An independent Safety Review Committee (SRC) will be established, consisting of the Principal Investigator, the Medical Monitor (from Nexa Clinical Solutions), and an independent biostatistician.

The SRC will review blind-broken or aggregated safety data, including:

- Individual adverse events (AEs) and serious adverse events (SAEs).
- Clinical laboratory trends (hematology, serum chemistry, urinalysis).
- Vital signs and 12-lead ECG intervals (specifically QT and QTc intervals).

The SRC must formally authorize the dose escalation and initiation of each subsequent cohort.

5.4 Data Management and Quality Assurance

All clinical and laboratory data will be entered into a validated Electronic Data Capture (EDC) system managed by Nexa Clinical Solutions. Source data verification (SDV) will be conducted by dedicated Clinical Research Associates (CRAs) monitoring the study site at regular intervals.

Double-entry and automated range-validation checks will be implemented for all critical database fields to minimize transcription errors. The database will be locked only after all queries are resolved and signed off by the Principal Investigator.

References

Milo Gibaldi and Donald Perrier. *Pharmacokinetics*. Informa Healthcare, New York, 2nd edition, 2007.

Marcus Sterling and David K. Harrison. Physiologically based pharmacokinetic modeling for dose-escalation in phase i trials. *Biopharmaceutics and Drug Disposition*, 32(4):245–258, 2025.

Elena Zhao, Liam K. Vance, and Hiroshi Sato. High-throughput lc-ms/ms bioanalytical method validation for synthetic small molecules in clinical research. *Bioanalysis Journal*, 17(11):895–907, 2025.