Dose Oral Toxicity Study of RBX-127 in Sprague-Dawley Rats with a 14-Day R

Study ID: NPR-TOX-2025-027

Test Article: RBX-127 (Small-Molecule CB2 Partial Agonist)

Species: Sprague-Dawley Rat • Route: Oral gavage

Sponsor: Acme Biopharma, Inc.

1234 Innovation Way, Suite 500, Atlanta, GA 30322, USA

Study Conducted at: Northbridge Preclinical Research, LLC

890 Research Park Blvd, Building 4, Durham, NC 27703, USA

Study Dates: Dosing: April 14, 2025 – May 12, 2025; Recovery: May 13, 2025 – May 26, 2025

Report Date: August 08, 2025

This nonclinical laboratory study was conducted in accordance with the principles of Good Laboratory Practice

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1. Abbreviations and Definitions

AE: Adverse Event
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
AUC: Area under the concentration–time curve
Cmax: Maximum observed concentration
CV: Coefficient of variation
GLP: Good Laboratory Practice
IND: Investigational New Drug Application
NOAEL: No-Observed-Adverse-Effect Level
OECD: Organisation for Economic Co-operation and Development
QAU: Quality Assurance Unit
TK: Toxicokinetics

2. Study Synopsis

This GLP-compliant, 28-day repeat-dose toxicity study evaluated the safety profile of RBX-127 (Small-Molecule CB2 Partial Agonist) administered by Oral gavage to Sprague-Dawley Rat at dose levels of 0, 50, 150, and 500 mg/kg/day (n=10/sex/group). Additional recovery cohorts (control and high-dose; n=5/sex/group) were included to assess reversibility following a 14-day drug-free period.

Mortality did not occur at any dose level. RBX-127 was generally well tolerated at 50 and 150 mg/kg/day. At 500 mg/kg/day, treatment-related clinical signs included intermittent piloerection and decreased activity, occurring primarily during the first week and diminishing over time.

Mean body weight gain and food consumption were minimally reduced (≤5%) at 500 mg/kg/day compared with control. Ophthalmic examinations were unremarkable. Electrocardiography (subset) did not show RBX-127–related effects.

Clinical pathology revealed mild, nonadverse increases in ALT and AST (≤1.3× control) at 500 mg/kg/day, without bilirubin elevation or histopathological corroboration. Hematology and urinalysis changes were within physiological variability.

Toxicokinetics demonstrated dose-proportional exposure from 50 to 150 mg/kg/day, with less than dose-proportional increases at 500 mg/kg/day, suggestive of saturation of absorption. Accumulation over 28 days was modest (≤1.3×).

Macroscopic and microscopic pathology identified minimal–mild, reversible centrilobular hepatocellular hypertrophy in some animals at 500 mg/kg/day, consistent with adaptive enzyme induction. No degenerative or necrotic lesions were observed. Findings were fully resolved following the 14-day recovery period.

Under the conditions of this study, the NOAEL was 150 mg/kg/day (males and females). These data support continued clinical development and are suitable for inclusion in Module 4 of the IND.

Key Personnel:
- Study Director: Jordan A. Kim, PhD
- Principal Investigator (Pathology): Priya S. Mehta, DVM, DACVP
- Bioanalytical Lead: Samuel T. Wu, MS
- Toxicokinetics Lead: Erin L. Carter, PhD
- QA Unit Head: Monica R. Shah, MS
- Sponsor Representative: Benjamin Siciliano, PhD

NOAEL: 150 mg/kg/day (males and females)

3. Compliance and Study Administration

This nonclinical laboratory study was conducted in accordance with the principles of Good Laboratory Practice (GLP) as set forth in 21 CFR Part 58 and OECD Principles of GLP, unless stated otherwise in documented deviations.
Quality Assurance inspections were conducted throughout the study.

4. Study Objectives

- Characterize potential target organ toxicities of RBX-127 following daily oral administration for 28 days.
- Determine the NOAEL and assess the reversibility of any RBX-127–related effects following a 14-day recovery.
- Characterize systemic exposure and dose proportionality via TK sampling on Days 1 and 28.

5. Study Design

This was a randomized, controlled, parallel-group study. Animals (10/sex/group) were assigned to 4 dose groups (0, 50, 150, 500 mg/kg/day). A subset (5/sex/group) in the control and high-dose groups was designated for recovery. Dosing occurred once daily by oral gavage for 28 consecutive days.

Animals were clinically observed twice daily for viability and at least once daily for detailed clinical signs. Weekly detailed examinations included functional observational battery. Body weight and food consumption were recorded weekly. Clinical pathology (hematology, clinical chemistry, urinalysis) was performed pretest and at study termination (main phase) and at the end of the recovery period for designated animals.

Toxicokinetic blood samples were collected on Days 1 and 28 at pre-dose and up to 8 hours post-dose (0.5, 1, 2, 4, 6, 8 h). Plasma concentrations of RBX-127 were quantified using a validated LC–MS/MS method.

6. Materials and Methods

Animals: Sprague-Dawley rats (Crl:CD(SD)), 7–9 weeks old at initiation, were obtained from a reputable vendor and acclimated for ≥7 days. Animals were housed in an AAALAC-accredited facility with controlled temperature (20–24°C), humidity (40–70%), and a 12:12 h light:dark cycle.

Test Article: RBX-127 API lot RBX127-API-Batch-25A was characterized by HPLC and NMR. Dose formulations were prepared weekly in 0.5% methylcellulose, stored refrigerated, and stirred before dosing. Concentration, homogeneity, and stability were confirmed analytically.

Dosing: Oral gavage using stainless steel gavage needles; dosing volume 10 mL/kg based on most recent body weight. Animals fasted overnight before TK sampling only.

Clinical Observations: Cage-side and detailed clinical signs assessed per SOP. Ophthalmology was performed pretest and Week 4 by a veterinary ophthalmologist. ECG (subset) was recorded in Week 4 under light isoflurane anesthesia.

Clinical Pathology: Hematology (RBC, WBC, HGB, HCT, PLT), clinical chemistry (ALT, AST, ALP, BUN, CRE, GLU, TP, ALB), and urinalysis (pH, SG, protein, glucose, ketones) were assessed. Coagulation (PT, aPTT) at terminal sacrifice.

Necropsy & Histopathology: Complete gross necropsy performed on all animals; organ weights measured (liver, kidneys, adrenals, heart, lungs, spleen, brain, testes/ovaries). Tissues were fixed in 10% neutral buffered formalin. Paraffin sections stained with H&E were examined microscopically in control and high-dose groups, with targeted evaluation in intermediate dose groups if indicated.

TK Analysis: Plasma samples were analyzed for RBX-127 using LC–MS/MS with a lower limit of quantitation of 1 ng/mL. Noncompartmental analysis (NCA) was used to derive Cmax and AUC(0–t).

7. Results

Mortality and Clinical Signs: No deaths occurred. At 500 mg/kg/day, intermittent piloerection and decreased spontaneous activity were noted primarily in Week 1, diminishing thereafter. No abnormal posture, gait, or convulsions were observed. No RBX-127–related ophthalmic or ECG findings were identified.

Body Weight and Food Consumption: Mean body weight gain was comparable to control at 50 and 150 mg/kg/day. At 500 mg/kg/day, marginally lower body weight gain (≤5%) and food consumption (≤4%) were observed without statistical significance and without clinical impact.

Clinical Pathology: Small, nonadverse increases in ALT and AST (≤1.3× control) were noted at 500 mg/kg/day with no bilirubin change. Hematology values, including RBC and WBC, were within normal ranges; minor shifts were not dose-related. Urinalysis was unremarkable.

Organ Weights: Slight increases in absolute and relative liver weights (≤10%) were observed at 500 mg/kg/day, with no adverse histopathological correlate.

TK Results: Systemic exposure increased roughly proportionally from 50 to 150 mg/kg/day (Cmax and AUC). At 500 mg/kg/day, exposure increases were less than dose-proportional. Accumulation ratios between Day 1 and Day 28 were ≤1.3.

Gross Pathology & Histopathology: Minimal–mild, reversible centrilobular hepatocellular hypertrophy was observed at 500 mg/kg/day without degeneration or necrosis. No adverse findings in kidneys, heart, or CNS. Recovery groups showed complete resolution.

8. Discussion

RBX-127 was generally well tolerated. The principal RBX-127–related findings at 500 mg/kg/day consisted of mild clinical signs early in dosing, small shifts in liver-associated enzymes, and minimal–mild hepatocellular hypertrophy consistent with enzyme induction. The absence of degenerative changes indicates adaptive, nonadverse hepatic responses.

Deviations from dose proportionality at the high dose suggest saturable absorption or first-pass metabolism. The exposure and effect profile supports selection of 150 mg/kg/day as the NOAEL in both sexes. All RBX-127–related findings were reversible following 14 days without dosing.

9. Conclusion

Under the conditions of this GLP study, daily oral administration of RBX-127 to Sprague-Dawley rats for 28 days was generally well tolerated up to 500 mg/kg/day. The NOAEL was 150 mg/kg/day (males and females).

The data support continued clinical development and provide an adequate nonclinical safety margin for initial clinical dosing, subject to integration with other IND components.

10. Protocol Deviations

- Formulation sampling on Day 14 for Group 3 occurred ~70 minutes after target time due to equipment maintenance. Impact: None; concentrations within specification.
- One ECG time point for Animal 308F was not recorded due to instrument artifact. Impact: None; replicate ECG in Week 4 was within normal limits.

11. References

FDA. 21 CFR Part 58 — Good Laboratory Practice for Nonclinical Laboratory Studies.
OECD. OECD Principles of Good Laboratory Practice and Compliance Monitoring.
ICH M3(R2). Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals.

Appendix A. Individual Animal Body Weight (Excerpt)

See main report for full tables. (Omitted in fallback format)

Appendix B. Bioanalytical Method Summary (LC–MS/MS)

Method summary: Protein precipitation; LC–MS/MS; LLOQ 1 ng/mL; accuracy/precision within acceptance.

Signature Page

I attest to the accuracy of this final report to the best of my knowledge.

Study Director: Jordan A. Kim, PhD
Electronic Signature: /s/ Jordan A. Kim, PhD
Date: August 08, 2025

QAU Statement: Audits of critical phases and report review were conducted. This report accurately reflects raw data.
QAU Head: Monica R. Shah, MS
Electronic Signature: /s/ Monica R. Shah, MS
Date: August 08, 2025

Sponsor Representative: Benjamin Siciliano, PhD
Electronic Signature: /s/ Benjamin Siciliano, PhD
Date: August 08, 2025