28-Day Repeat-Dose Oral Toxicity Study of RBX-127 in Sprague-Dawley Rats with a 14-Day Recovery Period

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: Apr 14, 2025 – May 12, 2025; Recovery: May 13, 2025 – May 26, 2025
Report Date: August 08, 2025

This nonclinical laboratory study was conducted in compliance with the U.S. FDA Good Laboratory Practice (GLP) regulations (21 CFR Part 58) and the OECD Principles of GLP, except for any deviations noted in this report.
Facility is AAALAC-accredited; critical equipment is calibrated and maintained per SOP.

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1. Abbreviations and Definitions

ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
AUC: Area under the concentration–time curve
Cmax: Maximum observed concentration
GLP: Good Laboratory Practice
IND: Investigational New Drug
NOAEL: No-Observed-Adverse-Effect Level
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). Recovery cohorts (control and high-dose; n=5/sex/group) were included for a 14-day drug-free period.

No mortality occurred. RBX-127 was generally well tolerated at ≤150 mg/kg/day. At 500 mg/kg/day, intermittent piloerection and decreased activity were noted primarily during Week 1 and diminished over time.

Clinical pathology showed small, nonadverse increases in ALT/AST at 500 mg/kg/day without bilirubin elevation or histopathological injury. Organ weight changes were limited to slight liver weight increases without adverse microscopic correlates.

TK indicated dose-proportional exposure from 50 to 150 mg/kg/day, and less than dose-proportional increases at 500 mg/kg/day. Accumulation was modest by Day 28 (≤1.3×).

Histopathology revealed minimal–mild, reversible centrilobular hepatocellular hypertrophy at 500 mg/kg/day consistent with enzyme induction; no degenerative lesions were observed. Findings resolved after recovery.

Under study conditions, the NOAEL was 150 mg/kg/day (males and females). Data support continued clinical development and inclusion in IND Module 4.

2.1 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 compliance with the U.S. FDA Good Laboratory Practice (GLP) regulations (21 CFR Part 58) and the OECD Principles of GLP, except for any deviations noted in this report.

Quality Assurance Unit (QAU) inspections were performed at study initiation, during dosing, and at report audit; records are maintained by the QAU.

Facilities and equipment were maintained and calibrated per SOP; the vivarium is AAALAC-accredited.

4. Study Objectives

• Characterize potential target organ toxicities following daily oral administration for 28 days.
• Determine the NOAEL and assess reversibility of RBX-127–related effects after 14 days without dosing.
• Characterize systemic exposure and dose proportionality via TK sampling on Days 1 and 28.

5. Study Design

Randomized, controlled, parallel-group study; 10/sex/group across doses 0, 50, 150, 500 mg/kg/day. Recovery cohorts (5/sex) in control and high-dose groups.
Animals observed for viability twice daily; detailed clinical signs at least daily. Weekly functional observations. Body weight and food consumption weekly.
Clinical pathology at termination (main) and recovery. Ophthalmology pretest and Week 4. ECG in Week 4 (subset).
TK blood collection on Days 1 and 28 (pre-dose and 0.5, 1, 2, 4, 6, 8 h post-dose).

Group	Dose (mg/kg/day)	M/F (n)	Recovery (M/F)
1	0	10/10	—
2	50	10/10	—
3	150	10/10	—
4	500	10/10	—
1R	0	5/5	Recovery
4R	500	5/5	Recovery

Table 5.1 — Dose Groups and Animal Allocation

6. Materials and Methods

Animals: Crl:CD(SD) rats, 7–9 weeks old at initiation; acclimated ≥7 days; AAALAC facility; temperature 20–24°C; humidity 40–70%; 12:12 h light:dark cycle.
Test Article: RBX-127 (Small-Molecule CB2 Partial Agonist) (Batch RBX127-API-Batch-25A) characterized by HPLC/NMR. Formulated weekly in 0.5% (w/v) methylcellulose in purified water; stored refrigerated; mixed prior to dosing. Concentration, homogeneity, and stability verified analytically.
Dosing: Oral gavage using stainless steel gavage needles; 10 mL/kg; fasting only for TK sampling days.
Clinical Pathology: Hematology, clinical chemistry, urinalysis, and coagulation assessed at scheduled time points.
Necropsy & Histopathology: Full gross necropsy; organ weights measured; H&E; histology on control and high-dose with targeted evaluation in intermediates as indicated.
TK Analysis: LC–MS/MS lower limit of quantitation 1 ng/mL; noncompartmental analysis for Cmax and AUC(0–t).

7. Results

Mortality and Clinical Signs: No deaths. At 500 mg/kg/day, intermittent piloerection and decreased activity observed in Week 1; diminishing thereafter.
Body Weight and Food Consumption: Comparable to control at ≤150 mg/kg/day; marginally lower weight gain and food consumption (≤5% and ≤4%) at 500 mg/kg/day without clinical impact.
Clinical Pathology: Small, nonadverse increases in ALT/AST (≤1.3× control) at 500 mg/kg/day; no bilirubin elevation. Urinalysis unremarkable.
Organ Weights: Slight increases in liver weights (≤10%) at 500 mg/kg/day without adverse microscopic correlate.
TK: Exposure roughly dose-proportional from 50 to 150 mg/kg/day; less than dose-proportional at 500 mg/kg/day. Accumulation ≤1.3× by Day 28.
Gross Pathology & Histopathology: Minimal–mild, reversible centrilobular hepatocellular hypertrophy at 500 mg/kg/day; no degeneration/necrosis; full resolution after recovery.

Group	Dose (mg/kg/day)	ALT (U/L)	AST (U/L)	n (M)
1	0	46	77	10
2	50	52	89	10
3	150	58	102	10
4	500	92	141	10

Table 7.1 — Clinical Chemistry Group Means (Males)

8. Discussion

RBX-127 was generally well tolerated. Principal findings at 500 mg/kg/day were limited, adaptive hepatic changes without injury, small enzyme elevations, and early transient clinical signs.
The NOAEL of 150 mg/kg/day (males and females) is supported by clinical observation, clinical pathology, organ weights, TK, and histopathology. Less-than-dose-proportional exposure at 500 mg/kg/day suggests saturable absorption or first-pass metabolism.

9. Conclusion

Daily oral administration of RBX-127 to rats for 28 days was generally well tolerated up to 500 mg/kg/day. The NOAEL was 150 mg/kg/day (males and females).
Data are supportive for IND Module 4 submission, subject to integration with the overall nonclinical package.

10. Protocol Deviations

Deviation ID	Date	Description	Impact	Corrective A
DEV-001	2025-04-28	Formulation sampling Group 3 occurred ~70 min after target time	None; concentration within spec	Reminder iss
DEV-002	2025-05-07	ECG time point for Animal 308F not recorded due to technician error	Not applicable	Technician re

Table 10.1 — Protocol Deviations Log

11. References

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

Appendix A. Individual Animal Body Weights

Animal ID	Sex	Dose (mg/kg)	Day 1 (g)	Day 28 (g)	% Change
1M101	M	0	244	293	20.1%
1M102	M	0	224	283	26.3%
1M103	M	0	245	286	16.7%
1M104	M	0	222	264	18.9%
1M105	M	0	233	292	25.3%
1M106	M	0	226	287	27.0%
1M107	M	0	223	259	16.1%
1M108	M	0	255	322	26.3%
1M109	M	0	224	271	21.0%
1M110	M	0	232	268	15.5%
1F101	F	0	194	254	30.9%
1F102	F	0	209	266	27.3%
1F103	F	0	213	249	16.9%
1F104	F	0	218	260	19.3%
1F105	F	0	214	261	22.0%
1F106	F	0	193	235	21.8%
1F107	F	0	195	232	19.0%
1F108	F	0	187	230	23.0%
1F109	F	0	215	273	27.0%
1F110	F	0	185	249	34.6%
2M101	M	50	237	300	26.6%
2M102	M	50	256	302	18.0%
2M103	M	50	240	308	28.3%
2M104	M	50	248	317	27.8%
2M105	M	50	249	309	24.1%
2M106	M	50	224	286	27.7%
2M107	M	50	257	293	14.0%
2M108	M	50	241	291	20.7%
2M109	M	50	242	291	20.2%
2M110	M	50	254	322	26.8%
2F101	F	50	218	272	24.8%
2F102	F	50	190	242	27.4%
2F103	F	50	201	254	26.4%
2F104	F	50	203	242	19.2%

Animal ID	Sex	Dose (mg/kg)	Day 1 (g)	Day 28 (g)	% Change
2F105	F	50	215	259	20.5%
2F106	F	50	197	266	35.0%
2F107	F	50	197	241	22.3%
2F108	F	50	180	237	31.7%
2F109	F	50	219	289	32.0%
2F110	F	50	217	286	31.8%
3M101	M	150	255	299	17.3%
3M102	M	150	252	311	23.4%
3M103	M	150	241	308	27.8%
3M104	M	150	235	289	23.0%
3M105	M	150	257	293	14.0%
3M106	M	150	252	313	24.2%
3M107	M	150	222	270	21.6%
3M108	M	150	233	281	20.6%
3M109	M	150	233	277	18.9%
3M110	M	150	260	323	24.2%
3F101	F	150	216	260	20.4%
3F102	F	150	196	263	34.2%
3F103	F	150	180	221	22.8%
3F104	F	150	217	279	28.6%
3F105	F	150	192	236	22.9%
3F106	F	150	191	241	26.2%
3F107	F	150	200	251	25.5%
3F108	F	150	188	243	29.3%
3F109	F	150	203	247	21.7%
3F110	F	150	205	254	23.9%
4M101	M	500	258	316	22.5%
4M102	M	500	231	265	14.7%
4M103	M	500	252	291	15.5%
4M104	M	500	235	291	23.8%
4M105	M	500	240	305	27.1%
4M106	M	500	234	264	12.8%
4M107	M	500	242	305	26.0%
4M108	M	500	242	295	21.9%
4M109	M	500	253	313	23.7%

Animal ID	Sex	Dose (mg/kg)	Day 1 (g)	Day 28 (g)	% Change
4M110	M	500	240	286	19.2%
4F101	F	500	184	223	21.2%
4F102	F	500	199	261	31.2%
4F103	F	500	209	266	27.3%
4F104	F	500	219	275	25.6%
4F105	F	500	201	252	25.4%
4F106	F	500	187	232	24.1%
4F107	F	500	218	257	17.9%
4F108	F	500	196	233	18.9%
4F109	F	500	201	245	21.9%
4F110	F	500	189	250	32.3%

Appendix B. Clinical Observations (Subset)

Animal ID	Sex	Group	Day	Observation
1M201	M	1	1	Normal
1M201	M	1	3	Normal
1M201	M	1	7	Normal
1M201	M	1	14	Normal
1M201	M	1	21	Normal
1M201	M	1	28	Normal
1M202	M	1	1	Normal
1M202	M	1	3	Normal
1M202	M	1	7	Normal
1M202	M	1	14	Normal
1M202	M	1	21	Normal
1M202	M	1	28	Normal
1F201	F	1	1	Normal
1F201	F	1	3	Normal
1F201	F	1	7	Normal
1F201	F	1	14	Normal
1F201	F	1	21	Normal
1F201	F	1	28	Normal
1F202	F	1	1	Normal
1F202	F	1	3	Normal
1F202	F	1	7	Normal
1F202	F	1	14	Normal
1F202	F	1	21	Normal
1F202	F	1	28	Normal
2M201	M	2	1	Normal
2M201	M	2	3	Normal
2M201	M	2	7	Normal
2M201	M	2	14	Normal
2M201	M	2	21	Normal
2M201	M	2	28	Normal
2M202	M	2	1	Normal
2M202	M	2	3	Normal
2M202	M	2	7	Normal
2M202	M	2	14	Normal

Animal ID	Sex	Group	Day	Observation
2M202	M	2	21	Normal
2M202	M	2	28	Normal
2F201	F	2	1	Normal
2F201	F	2	3	Normal
2F201	F	2	7	Normal
2F201	F	2	14	Normal
2F201	F	2	21	Normal
2F201	F	2	28	Normal
2F202	F	2	1	Normal
2F202	F	2	3	Normal
2F202	F	2	7	Normal
2F202	F	2	14	Normal
2F202	F	2	21	Normal
2F202	F	2	28	Normal
3M201	M	3	1	Normal
3M201	M	3	3	Normal
3M201	M	3	7	Normal
3M201	M	3	14	Normal
3M201	M	3	21	Normal
3M201	M	3	28	Normal
3M202	M	3	1	Normal
3M202	M	3	3	Normal
3M202	M	3	7	Normal
3M202	M	3	14	Normal
3M202	M	3	21	Normal
3M202	M	3	28	Normal
3F201	F	3	1	Normal
3F201	F	3	3	Normal
3F201	F	3	7	Normal
3F201	F	3	14	Normal
3F201	F	3	21	Normal
3F201	F	3	28	Normal
3F202	F	3	1	Normal
3F202	F	3	3	Normal
3F202	F	3	7	Normal

Animal ID	Sex	Group	Day	Observation
3F202	F	3	14	Normal
3F202	F	3	21	Normal
3F202	F	3	28	Normal
4M201	M	4	1	Normal
4M201	M	4	3	Normal
4M201	M	4	7	Normal
4M201	M	4	14	Normal
4M201	M	4	21	Normal
4M201	M	4	28	Normal
4M202	M	4	1	Decreased activity
4M202	M	4	3	Piloerection (intermittent)
4M202	M	4	7	Normal
4M202	M	4	14	Normal
4M202	M	4	21	Normal
4M202	M	4	28	Normal
4F201	F	4	1	Piloerection (intermittent)
4F201	F	4	3	Normal
4F201	F	4	7	Normal
4F201	F	4	14	Normal
4F201	F	4	21	Normal
4F201	F	4	28	Normal
4F202	F	4	1	Normal
4F202	F	4	3	Normal
4F202	F	4	7	Decreased activity
4F202	F	4	14	Normal
4F202	F	4	21	Normal
4F202	F	4	28	Normal

Appendix C. Organ Weights (Summary: Mean ± SD)

Group	Sex	Dose (mg/kg)	Liver (g)	Kidneys (g)	Heart (g)
1	M	0	12.49 ± 0.48	2.38 ± 0.19	1.29 ± 0.08
1	F	0	9.38 ± 0.58	1.98 ± 0.18	1.03 ± 0.07
2	M	50	12.98 ± 0.31	2.42 ± 0.26	1.33 ± 0.09
2	F	50	10.01 ± 0.47	2.01 ± 0.17	1.07 ± 0.07
3	M	150	13.23 ± 0.56	2.55 ± 0.21	1.40 ± 0.07
3	F	150	9.80 ± 0.44	2.00 ± 0.15	1.05 ± 0.09
4	M	500	14.05 ± 0.58	2.66 ± 0.15	1.44 ± 0.08
4	F	500	10.63 ± 0.32	2.15 ± 0.20	1.11 ± 0.08

Appendix D. Toxicokinetic Plasma Concentrations

Table D.1 — Day 1 Concentration–Time (Mean, ng/mL)

Time (h)	Grp1 Mean	Grp2 Mean	Grp3 Mean	Grp4 Mean
0	0.0	101.1	293.7	728.9
0.5	0.0	77.2	229.5	589.4
1	0.0	57.4	177.9	450.4
2	0.0	39.8	105.3	269.1
4	0.0	11.8	28.4	143.9
6	0.0	4.8	10.6	43.7
8	0.0	3.2	0.0	0.0

Table D.2 — Day 28 Concentration–Time (Mean, ng/mL)

Time (h)	Grp1 Mean	Grp2 Mean	Grp3 Mean	Grp4 Mean
0	0.0	122.6	359.5	954.0
0.5	0.0	97.5	301.6	703.9
1	0.0	76.3	247.9	585.8
2	0.0	45.1	154.5	308.3
4	0.0	18.1	61.2	108.8
6	0.0	7.2	24.7	4.5
8	0.0	4.4	0.0	53.8

Appendix E. Dose Formulation Analysis Summary

Day	Group	Nominal (mg/mL)	Measured (mg/mL)	% of Nominal	Homogeneity	Stability
1	1	0.00	0.00	0.0	Pass	Pass
1	2	0.50	0.50	99.1	Pass	Pass
1	3	1.50	1.51	100.4	Pass	Pass
1	4	5.00	5.24	104.9	Pass	Pass
14	1	0.00	0.00	0.0	Pass	Pass
14	2	0.50	0.49	99.0	Pass	Pass
14	3	1.50	1.50	100.3	Pass	Pass
14	4	5.00	4.85	96.9	Pass	Pass
28	1	0.00	0.00	0.0	Pass	Pass
28	2	0.50	0.50	100.8	Pass	Pass
28	3	1.50	1.49	99.2	Pass	Pass
28	4	5.00	5.04	100.8	Pass	Pass

Signature Page

I attest to the accuracy and completeness 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

Quality Assurance Unit (QAU) Statement:

The QAU conducted inspections of this study at study initiation, during the in-life phase, and at the time of report audit. The dates and phases inspected are on file with the QAU. This statement confirms that the final report accurately reflects the raw data and study conduct.

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