Rapid *ex vivo Demodex* killing by APT-001 in an epilation assay

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***Demodex* blepharitis remains a difficult chronic condition, and benchmark therapy has only recently shifted toward targeted acaricidal agents after decades of hygiene-focused or empiric treatment approaches (1–3). We report findings from protocol `APTB0002` for an aggregated final-formulation APT-001 dataset, a 1.8% spinosad ophthalmic ointment, comprising 109 mites collected from 24 contributing subjects in Guatemala City, Guatemala between July–September 2025, together with a vehicle placebo-control dataset (24 mites collected from 9 subjects at Loh Ophthalmology in Miami, Florida between February–March 2025) and a TP-03 (XDEMVY®) comparator dataset representing lotilaner ophthalmic solution 0.25% (36 mites; patient counts unavailable). Median time to death (TTD) was 41.0 minutes (Q1–Q3, 33.0–57.0) for APT-001, versus 846.5 minutes for vehicle placebo control and 550.0 minutes for TP-03 (XDEMVY). All observed APT-001 demodicidal events occurred before the earliest event in either comparator cohort, yielding complete temporal separation on Kaplan-Meier analysis; both log-rank and Wilcoxon testing were significant at $p < 0.001$ for APT-001 versus vehicle placebo control and for APT-001 versus TP-03 (XDEMVY), whereas standard Cox proportional hazards models did not return finite hazard-ratio estimates because of separation. These data indicate rapid *ex vivo* miticidal activity of APT-001 against *Demodex* mites and support continued clinical study.**

***Demodex* blepharitis | APT-001 | spinosad | *ex vivo* assay | ophthalmic ointment**

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Introduction

Demodex spp. mites are strongly implicated in chronic blepharitis characterized by cylindrical dandruff, lid margin inflammation, and ocular discomfort, particularly in older adults and in patients with characteristic lash debris (1, 4). For most of the modern treatment era, management has relied on eyelid hygiene and off-label or empiric approaches such as tea tree oil scrubs rather than a durable targeted pharmacologic strategy (1, 5). XDEMVY (lotilaner ophthalmic solution 0.25%; Tarsus Pharmaceuticals, Inc.) now provides an FDA-approved benchmark for *Demodex* blepharitis and therefore a clinically relevant context for evaluating emerging miticidal programs (2, 3).

Spinosad is a naturally derived, fermented antiparasitic mixture whose activity is linked to insect nicotinic acetylcholine receptor binding with secondary effects on GABA signaling (6, 7). That mechanism made spinosad an attractive candidate for ocular *Demodex* control. The present manuscript reports the aggregated final-formulation APT-001 workbook cohort from protocol `APTB0002` and contextualizes those findings against a vehicle placebo-control dataset and a TP-03 (XDEMVY) comparator dataset retained in the master workbook.

Materials and Methods

Study design and ethics. The study was conducted as a prospective, open-label *ex vivo Demodex* survival assay under protocol `APTB0002`, in which eyelash samples were collected after informed consent from adults with clinically suspected *Demodex* blepharitis at participating ophthalmology sites. Protocol `APTB0002` and the informed-consent materials were reviewed and approved by WCG IRB before study initiation. Because the study articles were applied only to epilated lashes and not to participants themselves, the protocol classified the work as minimal-risk exploratory research. The primary endpoint was *Demodex* time to death (TTD) after treatment exposure, with death defined as cessation of body or leg movement under light microscopy.

Study sites and data provenance. The vehicle placebo-control dataset was generated at Loh Ophthalmology in Miami, Florida, with retained workbook dates spanning February–March 2025. The aggregated APT-001 dataset was generated at Clinica de Ojos Dr. Gutierrez and Imagenes Diagnosticas Oculares in Guatemala City, Guatemala, with retained workbook dates spanning July–September 2025. This manuscript analyzes the retained aggregated APT-001 workbook cohort. The TP-03 (XDEMVY) comparator dataset was created from Tarsus Pharmaceuticals' published reports and reflects a reconstructed public-data comparator derived from the Tarsus Pharmaceuticals Form S-1 registration statement filed September 25, 2020 (8).

Assay procedures. Per protocol, epilated lashes were placed on glass slides, the slides were maintained near body temperature during microscopy, treatment was applied at the slide edge using a micropipette, and survival was assessed by microscopy with video capture until all visible mites had ceased movement. Survival time was defined

from first treatment contact to cessation of motion, and the present manuscript is aligned to the protocol-defined primary-endpoint framework emphasizing visible mites followed for formal TTD assessment in the *ex vivo* assay.

Manuscript cohorts. The manuscript was intentionally restricted to the aggregated APT-001 workbook cohort from protocol `APTB0002`, represented in the master workbook by five retained solution labels grouped as APT-001 for analysis: `EPS-979-055B`, `EPS-979-059`, `EPS-1144-171-C`, `EPS-1144-171-A`, and `1108-ARC-3`. The main analysis therefore centers on APT-001, the vehicle placebo-control dataset, and the TP-03 (XDEMVY; lotilaner ophthalmic solution 0.25%) comparator dataset from Tarsus Pharmaceuticals; patient-count metadata were not available for the TP-03 cohort.

Statistical analysis. All statistical analyses and figure generation were performed in R. TTD distributions were summarized by mite count, contributing subjects where available, mean, standard deviation, median, interquartile range, and minimum-to-maximum range, and Kaplan-Meier curves were generated for the three manuscript cohorts. Pairwise comparisons were performed with log-rank testing, and Wilcoxon rank-sum testing was used as a secondary nonparametric assessment of distributional separation. Standard Cox proportional hazards models were attempted in accordance with the protocol, but APT-001 was temporally separated from both comparator cohorts, preventing finite hazard-ratio estimation.

Results

Cohort-level survival summary. APT-001 comprised 109 analyzed mite observations from 24 contributing subjects, whereas the vehicle placebo-control dataset comprised 24 mite observations from 9 subjects and the TP-03 (XDEMVY) comparator cohort comprised 36 mite observations with no retained patient-count metadata. APT-001 demonstrated markedly shorter *Demodex* TTD than either comparator cohort. Median TTD was 41.0 minutes for APT-001, versus 846.5 minutes for vehicle placebo control and 550.0 minutes for TP-03 (XDEMVY); the maximum observed TTD for APT-001 was 106.0 minutes, whereas the earliest observed TP-03 (XDEMVY) event occurred at 240 minutes and the earliest vehicle placebo-control event occurred at 360 minutes. The full distribution of individual mite observations showed the same separation, with APT-001 clustered at substantially earlier death times than either comparator cohort (Figure 1).

Survival analysis. Kaplan-Meier analysis showed complete temporal separation between APT-001 and both comparator cohorts (Figure 2). Every observed APT-001 event occurred before the earliest vehicle placebo-control or TP-03 (XDEMVY) event, producing a rapid early decline in survival probability for APT-001 and markedly right-shifted curves for the comparator cohorts. Pairwise testing corroborated that separation: both log-rank and Wilcoxon testing were significant at $p < 0.001$ for APT-001 versus vehicle placebo control and for APT-001 versus TP-03 (XDEMVY). Standard Cox proportional hazards modeling did not yield finite hazard-ratio estimates because the event times were completely separated.

Table 1. *Ex vivo Demodex* time-to-death summary for the three manuscript cohorts. Subject counts were unavailable for the TP-03 (XDEMVY) cohort. Q1, first quartile; Q3, third quartile; SD, standard deviation.

Cohort	Mites, n	Subjects, n	Median TTD, min (Q1–Q3)	Mean TTD, min (+/- SD)	Range, min (min-max)
APT-001	109	24	41.0 (33.0–57.0)	46.2 (+/- 19.9)	16.0–106.0
Vehicle control	24	9	846.5 (593.8–1086.0)	869.7 (+/- 354.1)	360.0–1536.0
TP-03 (XDEMVY)	36	NA	550.0 (360.0–615.0)	573.5 (+/- 279.8)	240.0–1260.0

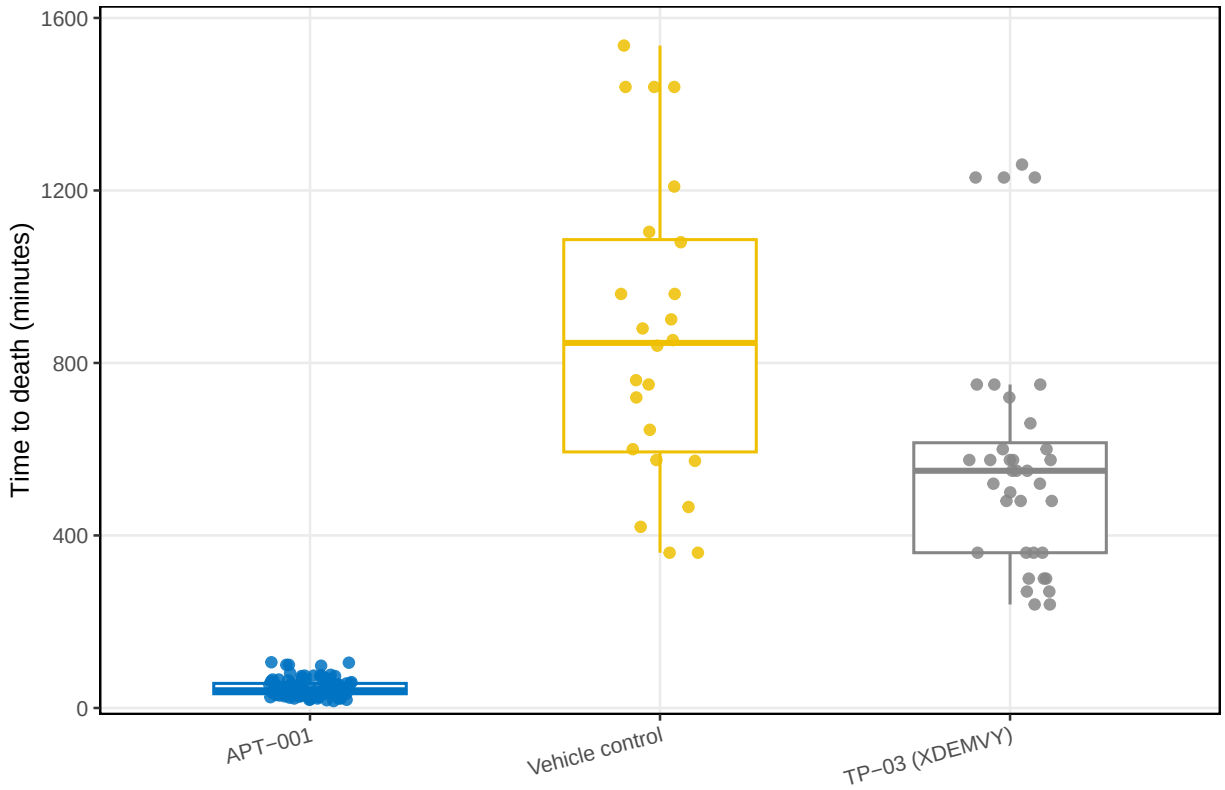

Fig. 1. Observed *ex vivo* time-to-death distributions for the three manuscript cohorts. Points represent individual mite observations retained in the workbook analysis dataset.

Table 2. Pairwise statistical comparisons for the manuscript cohorts. Standard Cox proportional hazards fits were attempted per protocol; complete temporal separation prevented finite hazard-ratio estimation in the *ex vivo* dataset.

Comparison	Log-rank p	Wilcoxon p	Cox PH note
Vehicle control vs APT-001	< 0.001	< 0.001	Non-finite HR from complete separation.
TP-03 (XDEMVY) vs APT-001	< 0.001	< 0.001	Non-finite HR from complete separation.

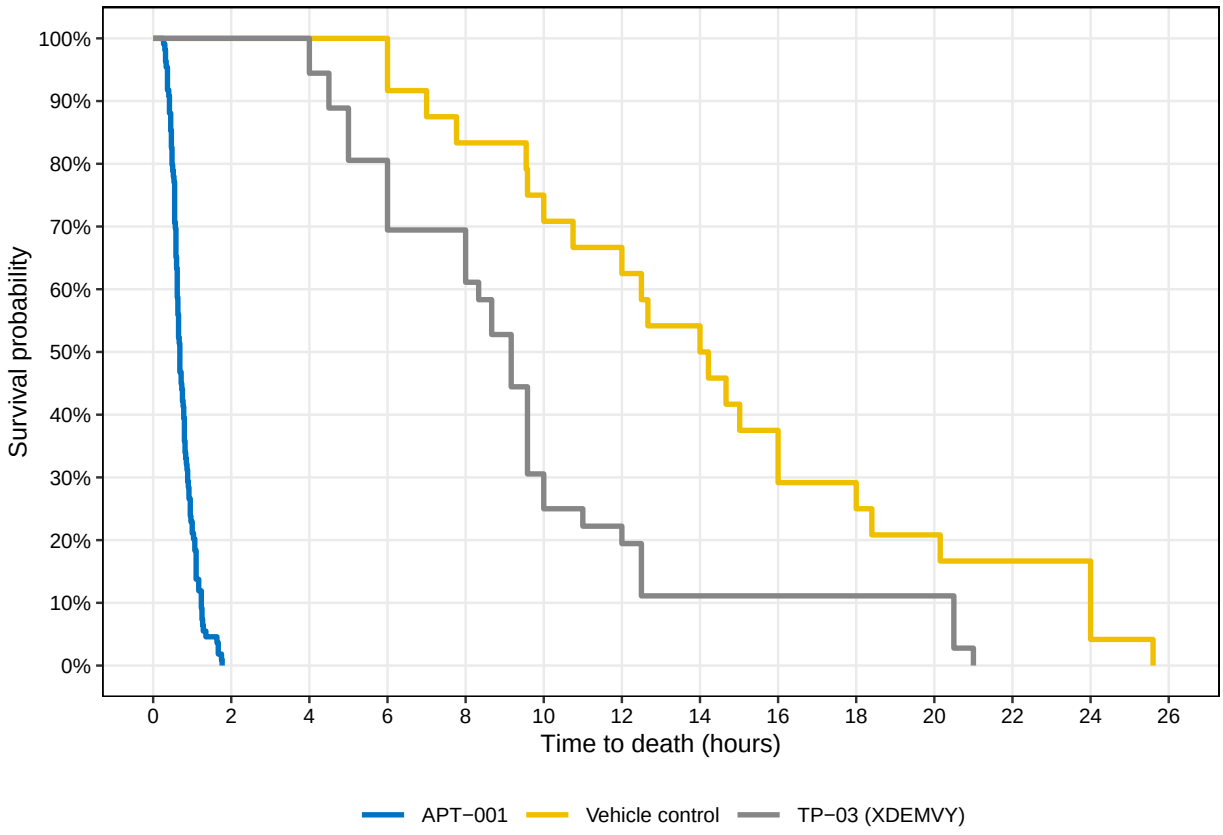

Fig. 2. Kaplan-Meier curves for the three manuscript cohorts. APT-001 showed complete temporal separation from both the vehicle placebo-control dataset and the TP-03 (XDEMVY) comparator cohort.

Discussion

This analysis shows that APT-001 achieved substantially faster *ex vivo Demodex* killing than both the vehicle placebo-control dataset and the TP-03 (XDEMVY) comparator cohort. Because all observed APT-001 events occurred within 106.0 minutes, whereas the earliest observed TP-03 (XDEMVY) and vehicle placebo-control events occurred only after 240 and 360 minutes, respectively, the separation between cohorts was large, early, and internally consistent across descriptive summaries, Kaplan-Meier analysis, and nonparametric testing. That pattern is compatible with meaningful miticidal activity beyond vehicle effects alone.

The TP-03 comparison is best interpreted as contextual rather than head-to-head. XDEMVY (lotilaner ophthalmic solution) 0.25% is an FDA-approved ectoparasiticide indicated for the treatment of *Demodex* blepharitis (2, 3), and the TP-03 dataset therefore provides a practical benchmark for positioning the *ex vivo* activity of APT-001 within the current therapeutic landscape. At the same time, the present dataset remains an *ex vivo* survival assay rather than a clinical efficacy study, the APT-001 and vehicle placebo-control datasets were generated at different sites and during different collection periods, the TP-03 comparator was reconstructed from publicly disclosed Tarsus materials (8), and patient-count metadata were not retained for the TP-03 cohort. Even with those constraints, the observed separation was sufficiently large that the principal signal did not depend on a fragile modeling assumption.

Taken together, these findings support continued clinical study of APT-001 for *Demodex* blepharitis. APT-001 is already being evaluated in the VISTA-1 clinical study (NCT06720896) (9), and the present *ex vivo* results provide a mechanistic bridge between formulation-level performance and subsequent patient-level testing.

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COMPETING FINANCIAL INTERESTS

Several authors are employees of Aperta Biosciences, LLC, the sponsor of the APT-001 program.

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